UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL – UFRGS

Fatores imunogenéticos e ambientais envolvidos no estabelecimento de doenças virais emergentes, reemergentes e negligenciadas no Brasil – Um enfoque na perspectiva *One Health*

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"Existem pelo menos três razões pelas quais devemos preservar a biodiversidade do planeta. A primeira delas é ética e estética. Como uma espécie dominante, que ocupa todas as regiões do mundo, temos uma responsabilidade moral de proteger nossos parentes biológicos. A partir dos estudos evolutivos genético-moleculares, aprendemos que todas as formas de vida constituem uma imensa família. Todos nós temos sentimentos gratificantes em contato com a natureza. Outra razão inclui os benefícios econômicos que já obtivemos desta diversidade, por meio de alimentos, remédios e produtos industriais. Apenas uma pequena parte das espécies foi considerada, até agora, com relação a esse aspecto. Finalmente, os ecossistemas naturais asseguram a estabilidade dos climas, das águas, solos e nutrientes, a proteção contra pestes e a presença de agentes polinizadores. A biosfera é um todo integrado: mudanças, mesmo no mais simples dos organismos, podem levar ao rompimento do equilíbrio."[1]

Professor Francisco Mauro Salzano

[1] Texto extraído do livro "DNA e eu com isso?" (Salzano, F., 2005, p. 88).

SUMÁRIO

LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

Abreviaturas

AIDS: *acquired immunodeficiency syndrome*/síndrome de imunodeficiência adquirida

ARV: anti-retroviral

BSL3: *biosafety level 3*/nível 3 de biossegurança

BSL4: *biosafety level 4*/nível 4 de biossegurança

CC-CKR2/CCR2: *cysteine-cysteine chemokine receptor 2*/receptor de quimiocina cisteína-cisteína tipo 2

CCR5: *cysteine-cysteine chemokine receptor 5*/receptor de quimiocina cisteína-cisteína tipo 5

CCR5: gene *CCR5*

CCR5Δ32: deleção de 32 pares de bases no gene *CCR5*

CD4: *cluster of differentation 4*/Grupamento de diferenciação 4

CD8: *cluster of differentation 8*/Grupamento de diferenciação 8

CDC: *Centers for Disease Control and Prevention*/Centros de Controle e Prevenção de Doenças

CHC: carcinoma hepatocelular

CRFs: *circulating recombinant forms*/formas recombinantes circulantes

CRISPR: *clustered regularly interspaced short palindromic repeats*/repetições palindrômicas curtas agrupadas e regularmente interespaçadas

CXCR4: *CXC chemokine receptor type 4*

DAAs: *direct-acting antiviral agents/antivirais de ação direta*

DNA: *deoxyribonucleic acid*/ácido desoxirribonucleico

EUA: Estados Unidos da América

FDA: *Food and Drug Administration*

HAART: *highly active antiretroviral therapy*/terapia antirretroviral altamente ativa

HBV: *hepatitis B virus*/vírus da hepatite B

HCV: *hepatitis C virus*/vírus da hepatite C

HCV+: HCV positivo

HCV-1: HCV genótipo 1

HCV-2: HCV genótipo 2

HCV-3: HCV genótipo 3

HCV-4: HCV genótipo 4

HCV-5: HCV genótipo 5

HCV-6: HCV genótipo 6

HCV-7: HCV genótipo 7

HIV: *human immunodeficiency virus*/vírus da imunodeficiência humana

HIV+: HIV positivo

HIV-1: HIV do tipo 1

HIV-2: HIV do tipo 2

HLA: *human leukocyte antigen*/antígeno leucocitário humano

INI: inibidor da integrase

IP/r: inibidor da protease com reforço de ritonavir

ITRN: inibidor da transcriptase reversa análogo de nucleosídeos

ITRNN: inibidor da transcriptase reversa não análogo de nucleosídeos

LACEN: Laboratório Central de Saúde Pública

MIP-1α/CCL3: *chemokine (C-C motif) ligand 3*/ligante de quimiocina 3

MIP-1β/CCL4: *chemokine (C-C motif) ligand 4*/ligante de quimiocina 4

NIH: *National Institutes of Health*

PEP: *post-exposure prophylaxis*/profilaxia pós-exposição

PCR: *polymerase chain reaction*/reação em cadeia da polimerase

PrEP: *pre-exposure prophylaxis*/profilaxia pré-exposição

RANTES/CCL5: *chemokine (C-C motif) ligand 5*/ligante de quimiocina 5

RNA: ribonucleic acid/ácido ribonucleico

ROCV: vírus Rocio

SABV: vírus Sabiá

SIV: *simian immunodeficiency virus*/vírus da imunodeficiência símia

SUS: Sistema Único de Saúde

TBEV: *tick-borne encephalitis virus*/vírus da encefalite transmitido por carrapatos

ZIKV: vírus Zika

Símbolos e unidades

Δ: delta

kb: kilobase

R_0: *basic reproductive number*

nm: nanômetro

LISTA DE FIGURAS E TABELAS

RESUMO

Esta tese apresenta uma série de trabalhos envolvendo diferentes fatores ambientais e imunogenéticos que contribuem para o estabelecimento das doenças emergentes, reemergentes e negligenciadas causadas por vírus no Brasil. A base para as discussões dos trabalhos é a perspectiva *One Health* (Saúde Única), na qual a emergência das doenças infecciosas é considerada como o resultado de interações entre fatores ambientais, humanos e de outros animais. Estão incluídos nesta tese dezesseis trabalhos científicos que abordam temas relacionados com diferentes aspectos das doenças infecciosas e das interações patógeno-hospedeiro. Tais trabalhos são artigos de revisão, comentários, uma hipótese e artigos de dados experimentais. Os seguintes temas foram abordados: I, importância do estudo da ecologia viral na prevenção e combate das doenças infecciosas emergentes e reemergentes; II, tecnologias e estratégias para a detecção de surtos e epidemias; III, salto de patógenos (*spillover*) e zoonoses no Brasil; IV, impacto do vento na dinâmica das doenças infecciosas; V, prevenção das doenças infecciosas emergentes, com enfoque nos melhores alvos para direcionamento de recursos e esforços; VI, aspectos históricos, biológicos, ecológicos e patogênicos dos vírus Sabiá e Rocio; VII, impactos dos exossomos sobre a infecção pelo HIV e terapia de células dendríticas contra o HIV; VIII, aspectos imunogenéticos da infecção pelo HCV; IX, efeitos de variantes genéticas sobre infecções virais, com foco em polimorfismos em genes de microRNAs; X, características do CCR5 (gene e proteína), com destaque para as potenciais implicações da ausência do CCR5 (através de técnicas de edição gênica ou em decorrência da variante genética CCR5Δ32) em diferentes contextos biológicos; XI; influências do CCR5 e CCR5Δ32 na infecção pelo *Tick-borne encephalitis virus* (vírus da encefalite transmitido por carrapatos); XII, resultados da investigação sobre os potenciais impactos do CCR5Δ32 na suscetibilidade à infecção pelo HCV, coinfecção HIV/HCV e doenças causadas pelo HCV (estudo avaliando 1.352 indivíduos); XIII, resultados da pesquisa sobre o papel do CCR5Δ32 na suscetibilidade à infecção pelo HBV e coinfecção HBV/HIV (estudo envolvendo 1.113 indivíduos); XIV, resultados da avaliação de níveis de quimiocinas/citocinas em sangue periférico de indivíduos HIV+ com diferentes tipos de progressão da infecção. O conjunto de trabalhos apresentados no corpo desta tese, junto

com as informações descritas nos capítulos de introdução e discussão, compõe um trabalho bastante amplo sobre os fatores genéticos, imunológicos e ecológicos das doenças infecciosas, com enfoque principal nas infecções que afetam a população brasileira. Por fim, esta tese salienta que os problemas causados pelas doenças infecciosas emergentes e reemergentes são extremamente complexos e que abordagens que levem em consideração a perspectiva *One Health* são importantes na prevenção e mitigação de tais problemas.

Palavras-chave: AIDS; CCR5; CCR5Δ32; coinfecção; doenças virais emergentes; doenças virais reemergentes; doenças infecciosas; doenças virais negligenciadas; ecologia; ecologia viral; epidemias; vírus da encefalite transmitido por carrapatos; exossomos; HBV; HCV; HIV; imunogenética; inflamação; interleucina-8; pandemias; salto de patógenos; Saúde Única; saúde global; saúde planetária; surtos; terapia de células dendríticas; vírus Rocio; vírus Sabiá.

ABSTRACT

This thesis presents a set of studies involving different environmental and immunogenetic factors that contribute to the establishment of emerging, reemerging, and neglected viral diseases in Brazil. Discussions are based on the One Health perspective, in which the emergence of infectious diseases is considered the result of interactions between environmental, human, and non-human animal factors. Sixteen scientific manuscripts addressing themes related to different aspects of infectious diseases and host-pathogen interactions are included in this thesis. Such studies include reviews, comments, one hypothesis, and original data articles. The following topics were addressed: I, the importance of studying viral ecology in the prevention and control of emerging and reemerging infectious diseases; II, technologies and strategies for detecting outbreaks and epidemics; III, pathogens jump (spillover) and zoonoses in Brazil; IV, the impact of wind on infectious diseases dynamics; V, prevention of emerging infectious diseases, with the focus on the best targets for allocation of resource and efforts; VI, historical, biological, ecological, and pathogenic aspects of Sabiá and Rocio viruses; VII, impacts of exosomes on HIV infection and dendritic cell-based immune therapy against HIV; VIII, immunogenetic aspects of HCV infection; IX, effects of genetic variants on viral infections, focusing on polymorphisms in microRNAs genes; X, characteristics of CCR5 (gene and protein), with emphasis on the potential implications of CCR5 absence (through gene editing techniques or due to the genetic variant CCR5Δ32) in different biological contexts; XI; influences of CCR5 and CCR5Δ32 on tick-borne encephalitis virus infection; XII, results of research on the potential impacts of CCR5Δ32 on susceptibility to HCV infection, HIV/HCV coinfection, and HCV-related diseases (study evaluating 1,352 individuals); XIII, data regarding the role of CCR5Δ32 in susceptibility to HBV infection and HBV/HIV coinfection (study involving 1,113 individuals); XIV, results of the evaluation of chemokine/cytokine levels in peripheral blood of HIV+ individuals with different profiles of infection progression. The set of articles presented in the body of this thesis, along with the information described in the introduction and discussion chapters, composes an extensive work on the genetic, immunological, and ecological factors of infectious diseases, with the main focus on infections that affect the Brazilian population.

Finally, this thesis highlights that the problems caused by emerging and reemerging infectious diseases are very complex and that approaches that take the One Health perspective into account are important in preventing and mitigating such problems.

Keywords: AIDS; CCR5; CCR5Δ32; coinfection; emerging viral diseases; reemerging viral diseases; infectious diseases; neglected viral diseases; ecology; viral ecology; epidemics; Tick-borne encephalitis virus; exosomes; HBV; HCV; HIV; immunogenetics; inflammation; interleukin-8; pandemics; pathogens jump; One Health; global health; planetary health; outbreaks; dendritic cell therapy; Rocio virus; Sabiá virus.

APRESENTAÇÃO E ESTRUTURAÇÃO DA TESE

Tendo como base a perspectiva *One Health* (ou Saúde Única), esta tese reúne um conjunto de trabalhos que exploram de forma teórica e experimental os fatores ambientais e imunogenéticos que contribuem para o estabelecimento das doenças emergentes, reemergentes e negligenciadas causadas por vírus no Brasil. O **Capítulo I** traz uma introdução aos temas que serão abordados nesta tese, além de listar os objetivos do trabalho.

No **Capítulo II** encontram-se cinco publicações que abordam diferentes temas relacionados com ecologia e doenças emergentes. No mesmo contexto, os **Capítulos III** e **IV** tratam de dois vírus selvagens pouco estudados pela comunidade científica: o vírus Sabiá e o vírus Rocio, respectivamente. Apesar destes trabalhos explorarem também o papel da genética humana no contexto das doenças infecciosas, os fatores ecológicos e virais são os temas centrais dos capítulos mencionados.

As interações entre patógeno e hospedeiro começam a ser tratadas de forma detalhada nos capítulos seguintes. A relação entre o HIV e os exossomos é explorada nos **Capítulos V** e **VI**, através de um artigo de revisão e um artigo de hipótese, respectivamente. Estes trabalhos estão focados nas discussões sobre os fatores imunogenéticos que influenciam o curso da infecção pelo HIV e a terapia de células dendríticas contra o HIV. O **Capítulo VII** apresenta o resultado da dosagem de citocinas séricas em indivíduos portadores do HIV com diferentes perfis clínicos, com enfoque nos níveis de IL-8. Ainda no contexto das relações patógeno-hospedeiro, o **Capítulo VIII** é formado por um artigo de revisão que discute as características imunogenéticas que impactam a infecção por outro vírus comum no Brasil, o HCV, cuja disseminação segue vias semelhantes à do HIV. Complementando essa discussão, o **Capítulo IX** descreve efeitos de variantes genéticas sobre infecções virais, com foco em polimorfismos em genes de microRNAs.

Em seguida, o papel do CCR5 (gene e proteína) em diferentes infecções virais é explorado. O **Capítulo X** apresenta uma publicação que aborda os prós e contras da ausência do CCR5 em humanos. O **Capítulo XI** traz uma discussão sobre o papel do CCR5 e da variante genética CCR5Δ32 sobre a infecção pelo *Tick-borne encephalitis virus*

através de um artigo de revisão. Este artigo também apresenta uma revisão da literatura sobre outros fatores genéticos que influenciam a infecção pelo patógeno. Posteriormente, o papel do CCR5Δ32 na infecção pelo HCV, coinfecção pelo HCV/HIV e doenças relacionadas ao HCV é apresentado no **Capítulo XII** em um artigo de dados. O **Capítulo XIII** apresenta outro artigo de dados que descreve os resultados de uma pesquisa sobre a influência do CCR5Δ32 na infecção pelo HBV e coinfecção HBV/HIV.

Por fim, o **Capítulo XIV** traz uma discussão geral sobre os temas tratados nesta tese, visando conectar as diversas abordagens usadas ao longo deste trabalho. No mesmo capítulo também estão apresentadas as conclusões e perspectivas deste trabalho. No **Anexo A** está descrita de forma resumida e selecionada a produção científica e acadêmica complementar que foi desenvolvida pelo autor deste trabalho ao longo de seu doutorado, mas que não foi diretamente incluída no texto precedente desta tese. Já o **Anexo B** apresenta as informações referentes às questões éticas envolvidas nos estudos apresentados.

CAPÍTULO I

Introdução e objetivos

INTRODUÇÃO

1. Saúde Planetária e Global

1.1. Saúde Planetária

Os problemas ambientais observados a nível local são mais facilmente identificados como fatores contribuintes de desequilíbrios da saúde humana e por isso recebem maior atenção da população, comunidade científica e órgãos governamentais, quando comparados a questões de um âmbito mais global. Por exemplo, a poluição do ar de uma metrópole pode ser facilmente reconhecida como causa de problemas respiratórios em seus moradores. Porém, mais recentemente, a ocorrência de diversos problemas de nível planetário contribuiu para que a "questão ambiental" fosse popularizada e sua importância reconhecida (Pignatti, 2004). Não há dúvidas de que a divulgação internacional de informações, relatórios, estudos e notícias através da internet teve papel crucial nesse processo.

Os principais problemas de ordem planetária enfrentados atualmente pela civilização humana envolvem mudanças climáticas, acidificação dos oceanos e fontes de água doce, degradação dos solos, escassez de água, superexploração das reservas pesqueiras, poluição atmosférica e a perda da biodiversidade (Pignatti, 2004; Whitmee et al., 2015; Hancock et al., 2017; Beaune et al., 2018; Weiss et al., 2018; Watts et al., 2017). Tais problemas tendem a ficar mais graves à medida que a população mundial cresce (Whitmee et al., 2015). Em razão de tal cenário, já é aceita a necessidade de um "movimento pela saúde planetária", envolvendo a comunidade global através de ações executadas em nível local, nacional e internacional, com foco na promoção da saúde do planeta Terra e das populações humanas (Horton et al., 2014).

Conceitualmente, Saúde Planetária pode ser entendida como o estado de saúde das civilizações humanas e dos sistemas naturais existentes na Terra. Um estado ideal de saúde planetária seria aquele no qual a população mundial usufrui de adequada saúde física, mental e social em um estado de harmonia com o meio ambiente, fazendo uso dos sistemas naturais de maneira parcimoniosa e renovável, de forma que as gerações atuais não

prejudiquem a existência das gerações futuras (Horton et al., 2014; Whitmee et al., 2015). Além de garantir o equilíbrio atmosférico e ambiental do planeta, a promoção da saúde planetária traz inúmeros benefícios para a saúde humana, que vão desde a redução do impacto das doenças crônicas causadas pela poluição (Guan et al., 2016) até a redução no número de casos de doenças infecciosas (Ostfeld, 2017).

Não há dúvidas de que a atividade humana está alterando de forma cada vez mais intensa os diferentes ecossistemas da Terra, indicando que os problemas de saúde pública também ficarão cada vez mais frequentes e mais graves (Myers et al., 2013). Por motivos como esses, ações focadas na preservação da saúde planetária devem ser entendidas, planejadas e postas em prática de forma urgente.

1.2. Saúde Global

O conceito de Saúde Global é muito similar ao conceito de Saúde Planetária, sendo que os objetivos das ações focadas na promoção de ambos são muito similares, principalmente no que se refere à promoção da saúde pública. Entretanto, os dois conceitos não são idênticos. A principal diferença entre eles é que a saúde planetária enfatiza a influência das questões planetárias (atmosféricas, climáticas e ambientais) sobre a saúde pública, colocando a "sustentabilidade" no centro das ações (Lerner e Berg, 2017). Apesar dessas questões também serem consideradas na saúde global, este termo geralmente é usado em discussões envolvendo agravos de saúde (por exemplo, doenças crônicas e infecciosas) que afetam a população mundial. De forma simplificada, as ações de saúde global estão focadas na promoção da saúde de todas as pessoas, independentemente das fronteiras políticas e geográficas (Koplan et al., 2009; Beaglehole e Bonita, 2010; Lerner e Berg, 2017), sem necessariamente enfatizar questões atmosféricas, climáticas ou ambientais.

As doenças infecciosas emergentes são consideradas ameaças à estabilidade global (Morens e Fauci, 2013). Dois exemplos de problemas de saúde global são as pandemias de influenza ocorridas no século XX (nos anos 1918, 1957 e 1968) (Kilbourne, 2006) e a pandemia de HIV/AIDS (Piot e Quinn, 2013). Ambas se sobrepõem às barreiras geográficas e são problemas compartilhados pela população mundial, exigindo respostas também de ordem global (Fineberg, 2014; Eisinger e Fauci, 2018). Após a popularização

do transporte aéreo internacional, o fluxo de pessoas por diferentes partes do mundo se tornou mais rápido e frequente. Dessa forma, é provável que problemas de saúde pública até então específicos de regiões e países particulares sejam cada vez mais tratados como problemas de saúde global.

2. *One Health* e as doenças infecciosas

2.1. Conceitos

Os problemas de saúde planetária e global deixam claro que a saúde das populações humanas está fortemente conectada com a saúde do planeta e seus ecossistemas. Por isso, relações desequilibradas das populações humanas com os ecossistemas terrestres podem afetar a saúde humana de diferentes maneiras. O modo como esses desequilíbrios se apresentam e suas consequências podem ser estudados e compreendidos através da abordagem *One Health*. Este termo pode ser traduzido para o português como Saúde Única. Nesta tese o autor optou por usar a expressão *One Health* com o objetivo de deixar as discussões em concordância com a literatura internacional. Deve-se ressaltar que é histórico o entendimento de que os humanos e outros animais vivem em um ambiente compartilhado e que, por isso, as questões de saúde ambiental e animal estão intimamente relacionadas. Porém, o conceito de *One Health* foi popularizado mais recentemente. O CDC apresenta *One Health* da seguinte forma:

> "*One Health recognizes that the health of people is connected to the health of animals and the environment. It is a collaborative, multisectoral, and trans-disciplinary approach - working at the local, regional, national, and global levels - with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment*" (CDC, 2018a).

No contexto das doenças infecciosas, *One Health* é uma perspectiva que considera o surgimento ou emergência de tais doenças como o resultado de desequilíbrios entre a "saúde humana", "saúde animal" (considerando os animais não humanos) e "saúde ambiental". Esses três fatores formam a tríade da *One Health*, sendo que o equilíbrio entre eles é essencial para que a saúde seja preservada e a emergência de doenças evitada, tanto

em nível local quanto em nível global (Bidaisee e Macpherson, 2014; Mwangi et al., 2016).

Além do conceito de *One Health*, alguns outros termos serão mencionados com frequência nesta tese. Com o objetivo de evitar possíveis confusões, os conceitos dos seguintes termos estão apresentados a seguir:

> - Endemia: "Ocorrência habitual de uma doença ou de um agente infeccioso em determinada área geográfica. Pode significar, também, a prevalência usual de determinada doença nessa área" (Brasil, 1985).
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> - Epidemia: "Aumento brusco, significativo e transitório da ocorrência de uma determinada doença numa população. Quando a área geográfica é restrita e o número de pessoas atingidas é pequeno, costuma-se usar o termo surto" (Brasil, 1985; grifo do autor).
>
> - Pandemia: "Epidemia de grandes proporções que atinge grande número de pessoas em uma vasta área geográfica (um ou mais continentes)" (Brasil, 1985).
>
> - Doenças infecciosas emergentes: "*Emerging infectious diseases are those whose incidence in humans has increased in the past 2 decades or threaten to increase in the near future*" (CDC, 2018b).
>
> - Doenças infecciosas reemergentes: "*Re-emerging infectious diseases are diseases that once were major health problems globally or in a particular country, and then declined dramatically, but are again becoming health problems for a significant proportion of the population (malaria and tuberculosis are examples)*" (NIH, 2007).
>
> - Doenças negligenciadas: "(...) um conjunto de doenças causadas por agentes infecciosos e parasitários (vírus, bactérias, protozoários e helmintos) que são endêmicas em populações de baixa renda vivendo, sobretudo em países em desenvolvimento na África, Ásia e nas Américas. O adjetivo "negligenciada" [...] tomou como base o fato de que por um lado elas não despertam o interesse das grandes empresas farmacêuticas multinacionais, que não veem nessas doenças compradores potenciais de novos medicamentos, e por outro o estudo dessas doenças vem sendo pouco financiado pelas agências de fomento" (De Souza, 2010).

Estes conceitos devem ser empregados junto com a definição da região geográfica que se aplicam, uma vez que um conceito aplicado a uma doença pode fazer sentido apenas em uma região em particular. Por exemplo, uma doença emergente em um país pode ser endêmica em outro (Boulus, 2001). Também é importante frisar que "doenças infecciosas reemergentes" podem ser consideradas como uma categoria entre as doenças emergentes

(NIH, 2007). Similarmente, as doenças negligenciadas também podem ser denominadas como emergentes e reemergentes (De Souza, 2010).

Apesar dos conceitos de doenças infecciosas emergentes e reemergentes serem usados com maior intensidade a partir dos anos de 1990, tais doenças fazem parte da história da humanidade. Deve-se lembrar das históricas epidemias de peste negra, gripe espanhola ou cólera, que na época em que ocorreram poderiam ser consideradas "doenças emergentes" na Europa (Waldman, 2001; Pedroso e Rocha, 2009). As doenças infecciosas também sempre fizeram parte do contexto histórico e social Brasileiro. Um exemplo são os variados problemas causados por tais doenças nos cortiços existentes da cidade do Rio de Janeiro no século XIX (Chalhoub, 2018).

2.2. Aplicações da One Health

A abordagem *One Health* é particularmente importante no estudo, diagnóstico e investigação das zoonoses, que são doenças infecciosas transmitidas de animais para humanos (Hubálek, 2003). Porém, humanos também podem transmitir doenças para animais (CDC, 2018c). Dessa forma, é possível classificar as zoonoses como doenças compartilhadas entre humanos e animais não humanos, independentemente da direção do fluxo de contágio. Foi estimado que 60-75% das doenças infecciosas emergentes são zoonoses (Taylor et al., 2001; Jones et al., 2008).

A raiva (doença causada pelo *Rabies virus*) é um exemplo clássico de zoonose. Porém, muitas doenças que atualmente são basicamente humanas já foram em algum momento doenças de animais não humanos e por isso também podem ser classificadas como zoonoses ou, mais adequadamente, classificadas como doenças de origem zoonótica (Hart et al., 1999; CDC, 2018c). Um exemplo clássico deste tipo de zoonose é a infecção pelo HIV, que apesar de ser uma doença humana, surgiu a partir de uma linhagem viral que infectava primatas não humanos (Hart et al., 1999). Outro exemplo é o sarampo, uma doença com origem em animais, mas atualmente praticamente restrita a humanos (Hart et al., 1999).

A *One Health* é muito efetiva para o estudo e combate das doenças infecciosas porque a emergência e a circulação de patógenos em populações humanas e animais é determinada por complexas interações entre essas diferentes populações e também por

fatores ambientais que interferem em tais relações. Por exemplo, essa abordagem já foi utilizada no estudo do vírus Ebola (Mwangi et al., 2016) e diferentes patógenos transmitidos por carrapatos (Vayssier-Taussat et al., 2015; Inci et al., 2016). Mais especificamente, a abordagem *One Health* ajuda na identificação dos fatores associados com a emergência das doenças infecciosas e até mesmo no controle de endemias, contribuindo para a mitigação das mesmas (Cunningham et al., 2017).

Apesar de não estarem diretamente evidenciados na tríade da *One Health* (animais não humanos, humanos e ambiente), os patógenos e suas características também são inevitavelmente levados em consideração quando as doenças infecciosas são abordadas dentro desta perspectiva. Levando isso em consideração, os patógenos deveriam ser representados com mais frequência e maior destaque nas figuras e esquemas de trabalhos científicos que apresentam a *One Health*, pois são cruciais nessa abordagem.

2.3. One Health, genômica e genética

É clássico o papel das técnicas de genética e biologia molecular no estudo das doenças infecciosas e na caracterização molecular dos agentes infecciosos. Por exemplo, as técnicas de PCR são mundialmente usadas na detecção de patógenos e no diagnóstico de infecções virais (DeBiasi e Tyler, 1999; Elnifro et al., 2000; Watzinger et al., 2006; Valones et al., 2009; Olofsson et al., 2011). A importância de técnicas básicas como a PCR é inquestionável. Elas continuarão sendo usadas na pesquisa e prática clínica das doenças infecciosas por muito tempo. Porém, as análises genômicas vêm ganhando cada vez mais espaço nesse cenário em razão de diversos motivos.

Atualmente é possível sequenciar de forma rápida, acurada e com custo relativamente baixo o material genético de patógenos. As plataformas de sequenciamento também estão se tornando cada vez mais fáceis de usar. Além disso, já existem diversas opções de sequenciadores portáteis, que facilitam o trabalho em campo. É possível dizer que as técnicas genômicas estão revolucionando o diagnóstico e vigilância das doenças infecciosas, além de ajudarem a entender a patogênese dessas doenças (Firth e Lipkin, 2013; Wohl et al., 2016; Yamagishi et al., 2017; Ashikawa et al., 2018).

A identificação dos diferentes patógenos encontrados em uma amostra ambiental ou clínica pode ser realizada através de técnicas de “metagenômica”. Ou seja, o que

antigamente só era possível de ser feito através de uma série de PCRs ou sequenciamento de diferentes regiões genômicas específicas com o objetivo de identificar microrganismos de forma individual, atualmente pode ser realizado simultaneamente através das análises de metagenômica. Tais técnicas ainda não fazem parte da rotina da maioria dos laboratórios responsáveis pela vigilância epidemiológica, principalmente em países em desenvolvimento. No entanto, na medida em que as ferramentas de metagenômica são popularizadas e tornam-se mais acessíveis (financeira e tecnicamente), podem resultar em enormes benefícios para a rápida identificação dos patógenos responsáveis por surtos e epidemias (Gardy e Loman, 2018).

A disponibilização dos dados genômicos de um patógeno responsável por uma emergência global através de plataformas digitais é de grande importância para que ações de estudo e mitigação sejam tomadas em nível internacional e de forma coordenada (Li et al., 2014; Gardy et al., 2015; Aarestrup e Koopmans, 2016; Gardy e Loman, 2018). Além de permitir que a comunidade global conheça quais cepas estão circulando nas regiões de ocorrência de endemias, surtos e epidemias, tais dados podem, por exemplo, facilitar o desenvolvimento de vacinas (Pizza et al., 2000).

Dados genômicos também permitem a reconstrução epidemiológica de um surto ou epidemia. Nesse sentido, análises filogenéticas podem trazer informações bastante precisas sobre a data provável de início de uma epidemia. Tal dado ajuda a entender as circunstâncias e prováveis eventos relacionados com a emergência de uma doença. Análises de transmissão de patógenos baseadas em dados genômicos permitem a identificação de indivíduos "*super spreaders*". Além disso, esses dados são usados para inferir as relações filogenéticas entre os patógenos, bem como permitem identificar regiões genômicas alvos de pressão seletiva (Wohl et al., 2016; Gardy e Loman, 2018). A evolução da virulência viral também pode ser avaliada através da filogenômica (Geoghegan e Holmes, 2018).

A aplicação das técnicas genômicas na vigilância e combate às doenças infecciosas emergentes está alinhada com a abordagem *One Health*. Porém, a aplicação de tais técnicas depende de uma série de fatores, como capacidade de coleta, preservação e processamento das amostras, capacidade de sequenciamento do material genético e disponibilidade de pessoal técnico capacitado para realizar e interpretar as análises (Gardy e Loman, 2018). Uma vez que essas dificuldades são ultrapassadas, os resultados são

fascinantes. Um bom exemplo foi o rápido entendimento das crises de saúde pública causadas pelo vírus Ebola entre os anos de 2013 e 2016 na África ocidental, que só foi possível em razão das modernas técnicas de sequencimento e análises genômicas (Gire et al., 2014; Park et al., 2015; Folarin et al., 2016; Wohl et al., 2016; Dudas et al., 2017). Similarmente, a elucidação da dinâmica da epidemia causada pelo vírus Zika (ZIKV) no Brasil e demais países da América também foi auxiliada por análises genômicas (Grubaugh et al., 2017; Metsky et al., 2017).

Novos avanços na área da genética ocorrem constantemente. Recentemente foram descritas metodologias de diagnóstico de doenças virais baseadas na tecnologia CRISPR/Cas, que provavelmente facilitarão ainda mais o trabalho de campo nas investigações de epidemias (Gootenberg et al., 2017; Chen et al., 2018; Chiu, 2018; Kocak e Gersbach, 2018; Myhrvold et al., 2018). Também recentemente foi descrito um modelo para a identificação dos prováveis reservatórios e vetores de um patógeno com base em análises genômicas (Babayan et al., 2018). Esse trabalho possui implicações bastante importantes, uma vez que tem o potencial de facilitar a identificação de componentes ecológicos de patógenos que emergem repentinamente, mas sobre os quais pouco se sabe (Woolhouse, 2018).

As discussões apresentadas neste tópico referem-se basicamente a análises genômicas baseadas no material genético dos patógenos. Entretanto, fatores genéticos humanos também impactam a suscetibilidade às infecções e progressão das doenças virais (Chapman e Hill, 2012; Hill, 2012). A influência dos fatores genéticos do hospedeiro sobre as doenças infecciosas será apresentada mais detalhadamente no tópico 4 deste capítulo.

Por fim, fica evidente que os profissionais da área da genética são cada vez mais importantes no estudo das doenças infecciosas emergentes. Atualmente, muitas etapas de investigação epidemiológica, desenvolvimento de ferramentas para estudo dos patógenos e diagnósticos das infecções são baseadas em dados genômicos. Para que tais ações sejam realizadas de forma correta, a participação de geneticistas e biólogos moleculares é fundamental.

2.4. Modos de transmissão das doenças infecciosas

A transmissão das doenças infecciosas emergentes pode acontecer de diferentes maneiras. Loh et al. (2015) dividiram os modos de transmissão da seguinte forma:

- Contato direto: contato pele-pele, arranhões, mordidas de animais, exposição a gotículas e contato com fluidos corporais, órgãos e tecidos.

- Transmissão aérea: através de partículas de poeira e pequenas gotículas suspensas no ar.

- Transmissão por vetores: por mordida/picada ou transferência mecânica por artrópodes.

- Transmissão oral: consumo de alimentos ou água contaminados.

- Ambiente contaminado ou fômite: contato indireto com o solo ou vegetação, contato com a água, transmissão indireta através de objetos inanimados contaminados.

Também deve ser mencionada a transmissão vertical, ou seja, aquela que ocorre da mãe para o filho. Por exemplo, a transmissão vertical teve um impacto importante no número de casos de crianças infectadas pelo HIV, principalmente no início/meados da pandemia (John e Kreiss, 1996). Mais recentemente, este modo de transmissão esteve envolvido nos casos de microcefalia e outros defeitos congênitos causados pelo ZIKV (Duarte et al., 2017; Nguyen et al., 2017).

De forma geral, os modos de transmissão variam de acordo com os fatores causais da emergência da doença infecciosa. Por exemplo, doenças que emergem em decorrência de modificações no uso da terra geralmente são transmitidas por vetores. Já as doenças resultantes do consumo de carne de animais selvagens estão associadas à transmissão através do contato direto com os animais (Loh et al., 2015).

2.5. Surgimento das doenças infecciosas

As doenças infecciosas podem ocorrer a partir do surgimento de “novos” agentes infecciosos na população humana ou em decorrência da introdução de patógenos já conhecidos em uma nova população suscetível, ou seja, até então não afetada pelo agente patogênico (Morse, 1995; De Carvalho et al., 2009; Luna e Da Silva, 2013). Levando em

consideração que os patógenos abordados no decorrer desta tese serão os vírus, este grupo taxonômico será enfatizado nos exemplos e discussões daqui em diante.

Apesar da dinâmica das infecções virais ser bastante complexa, as doenças infecciosas virais surgem nos humanos a partir de três formas básicas:

- Processos evolutivos criam novos vírus e novas variantes (muito comum em vírus de RNA) (Morse, 1993; Schatzmayr, 2001). A dinâmica das doenças infecciosas é altamente influenciada por diferentes processos evolutivos, como seleção natural, deriva, mutações, recombinações ou rearranjos gênicos. Ainda, coinfecções possibilitam a troca de material genético entre diferentes vírus, possibilitando a emergência de novas cepas virais (Metcalf et al., 2015). Por exemplo, a coinfecção de suínos por vírus *Influenza* de diferentes espécies, como aves ou humanos, possibilita a troca de informação genética entre os vírus e o surgimento de novas cepas virais humanas (Castrucci et al., 1993; Zhou et al., 1999; Brown, 2001; Ma et al., 2009).

- Um vírus tradicionalmente de animais não humanos cruza a barreira entre espécies e passa a infectar humanos (Morse, 1993; Schatzmayr, 2001). Este processo pode ser chamado de salto de patógenos ou *spillover*. Os hospedeiros originais do vírus da dengue são os primatas do Velho Mundo, mas após cruzar a barreira entre espécies, o vírus adquiriu a capacidade de causar epidemias em humanos (Parrish et al., 2008). A capacidade de um patógeno ultrapassar as barreiras entre espécies depende de uma variedade de fatores, incluindo as características biológicas dos hospedeiros, características virais, além de fatores ecológicos e populacionais (Plowright et al., 2017). Apesar do salto de patógenos ser geralmente entendido como um evento pontual, o movimento gradual de um patógeno animal em direção à população humana também pode ser considerado como *spillover* (Gardy e Loman, 2018). Vírus de primatas não-humanos apresentam maior capacidade de realizar *spillover* do que patógenos originalmente hospedados em aves, roedores e artrópodes, por exemplo (Walker et al., 2018). Além disso, vírus generalistas também apresentam maior capacidade de cruzar a barreira entre espécies do que vírus especialistas (Johnson et al., 2015).

- Um vírus até então restrito a um pequeno grupo populacional (animal ou humano) é disseminado entre um número grande de humanos (Morse, 1993; Schatzmayr, 2001). Um bom exemplo é o ZIKV, conhecido desde 1947. Sabia-se de sua circulação de forma limitada entre primatas não humanos e em grupos populacionais

específicos. Porém, após o vírus ser introduzido em novas populações suscetíveis, sua infecção se tornou epidêmica (Baud et al., 2017). Importantemente, dois pontos devem ser considerados neste exemplo específico: é possível que o ZIKV já circulasse em grupos populacionais maiores, mas sua circulação era desconhecida ou negligenciada. Ainda, a epidemia causada pelo ZIKV não foi devida à transmissão do tipo humano-humano, mas mediada por vetores (Baud et al., 2017). As condições que facilitam a disseminação das doenças infecciosas envolvem fatores humanos, dos animais não humanos, dos patógenos e do ambientes, e serão discutidos no tópico 2.5 desta tese.

É essencial levar em consideração que as duas primeiras formas mencionadas ajudam a explicar de forma bastante simplificada o surgimento de novas doenças infecciosas em humanos ou o surgimento de novas variantes/cepas. Já o terceiro processo é também uma explicação simplificada dos eventos envolvidos na emergência de uma nova doença, uma vez que parte do pressuposto da existência prévia do vírus em humanos, mesmo que de forma limitada a pequenos grupos populacionais ou indivíduos. Ainda, pode-se considerar a emergência de uma doença infecciosa a partir de dois processos básicos. O primeiro seria a introdução do patógeno em uma nova população suscetível (após o surgimento de uma nova cepa, *spillover* ou até mesmo em decorrência de um patógeno de origem ambiental). O segundo processo seria a disseminação do patógeno na população em que foi introduzido (Morse, 1995).

Especificamente em relação a doenças zoonóticas, entende-se a introdução de um novo patógeno animal na população humana como um processo gradual, envolvendo cinco etapas, no qual o patógeno sai do ciclo de circulação exclusiva entre animais (primeiro estágio) até adquirir a capacidade de realizar transmissão humano-humano, sem necessidade de hospedeiro intermediário, sendo este o último estágio da emergência de uma doença (Wolfe et al., 2007; Wolfe, 2009). Pode-se também considerar a emergência das doenças infecciosas com base na dinâmica da infecção e considerando um modelo que envolve três etapas básicas. Neste caso, há o estágio de pré-emergência (no qual estão surgem condições propícias para que ocorram eventos de *spillover*), seguido de um estágio de emergência (caracterizado por eventos de transmissão animais-humanos e alguns casos de transmissão humano-humano). Por último há o estágio de emergência pandêmica, no qual a transmissão humano-humano já está estabelecida e a doença pode adquirir um caráter epidêmico ou pandêmico (Morse et al., 2012).

Por fim, é importante ressaltar que o surgimento das doenças infecciosas representa um conjunto de processos entendidos, classificados e organizados de diferentes formas na literatura científica, conforme apresentado anteriormente. Muitas vezes o surgimento de uma nova doença em humanos não necessariamente representa um novo patógeno, pois este, muitas vezes, já circulava em outras espécies animais. O surgimento de um patógeno "novo" geralmente representa o surgimento de uma nova variante patogênica, com características que facilitam eventos de *spillover* ou que aumentam sua virulência. Além disso, existe uma confusão na literatura no que se refere a uma doença infecciosa "nova" e a "emergência" ou proliferação da doença na população humana. Levando essas discussões em consideração, o surgimento de uma nova doença infecciosa em humanos (considerando um patógeno proveniente de outros animais) pode ser simplificado da seguinte forma: um novo patógeno ou variante surge em decorrência de processos evolutivos em animais não humanos. Em seguida, dependendo da presença de características específicas do patógeno, das espécies hospedeiras e do ambiente, o patógeno/variante ultrapassa a barreira entre espécies. Caso o patógeno/variante tenha sucesso na infecção do hospedeiro humano, pode passar a infectar outros indivíduos. Com o passar do tempo e adaptações do patógeno ao novo hospedeiro, uma nova doença infecciosa humana pode surgir.

2.6. Fatores causais (ou "drivers") dos processos envolvidos na emergência das doenças infecciosas

Os processos envolvidos no surgimento e/ou emergência de uma nova doença infecciosa foram apresentados no tópico anterior. Mas quais seriam as "pressões" (causas ou *drivers*) para a ocorrência desses processos? A seguir serão mencionados os principais fatores causais dos processos envolvidos na emergência das doenças infecciosas, de acordo com os fatores da tríade *One Health*. Além disso, os fatores virais que impactam a emergência das doenças também serão apresentados.

2.6.1. Fatores humanos: biológicos e sociais

A aglomeração dos seres humanos em grupos e em locais como aldeias, cidades ou países favorece a circulação contínua de patógenos entre os indivíduos (Waldman, 2001),

sendo esta aglomeração um fenômeno que se intensificou após o período Neolítico (Bañuls et al., 2013). É possível que a expansão da população humana seja o fator que mais contribua para o surgimento das doenças infecciosas emergentes e reemergentes (Zanella, 2016). O número de pessoas vivendo em áreas urbanas é cada vez maior, sendo que a vida nas cidades representa uma contradição em relação às doenças infecciosas: ao mesmo tempo em que as áreas urbanas altamente povoadas criam as condições ideias para a emergência de surtos e epidemias, são nesses locais onde os serviços de saúde estão disponíveis de forma mais acessível à população (Vlahov et al., 2005; Segurado et al., 2016). Neste contexto, uma estrutura de saúde precária se torna um importante fator causal da emergência de doenças infecciosas, principalmente quando associado a um ineficaz sistema de vigilância epidemiológica (Morse, 1995; Luna, 2002).

A aglomeração dos seres humanos em cidades também está diretamente conectada com o fenômeno de urbanização. Além de estar associada a uma série de modificações da paisagem, como o desmatamento, sabe-se que a urbanização cria condições muito favoráveis para a emergência de doenças e manutenção de surtos e epidemias. Recentemente, Tian et al. (2018) mostraram que os surtos de hantaviroses na China estão associados com a urbanização e com os fatores associados a tal fenômeno (migrações, desenvolvimento econômico, entre outros). As larvas de mosquitos do gênero *Aedes* (vetores de diferentes vírus) são altamente favorecidas pelas condições ecológicas criadas em decorrência da urbanização (Zahouli et al., 2017). Este é um exemplo clássico, principalmente no Brasil, onde as campanhas de conscientização sobre a importância de se evitar a formação de criadouros do "mosquito da dengue" são amplamente divulgadas entre a população.

Por outro lado, um fenômeno conhecido como "desurbanização" (Eskew e Olival, 2018) talvez crie condições ainda mais favoráveis para as doenças infecciosas do que a urbanização. Por exemplo, sabe-se que o aumento do lixo em áreas urbanas fornece as condições ideais para o desenvolvimento de mosquitos transmissores de diversas doenças virais (Pignatti, 2004). A desurbanização caracteriza-se pelo abandono de áreas urbanas, resultando em uma queda nas condições socioeconômicas da região afetada, acompanhada pelo aumento do lixo e pela diminuição da infraestrutura de saneamento básico e controle de pragas. Tal cenário favorece a proliferação de animais portadores de zoonoses e de vetores de diferentes doenças (Eskew e Olival, 2018). Por isso pode-se considerar a

desurbanização como um fator tão importante quanto a urbanização quando se avalia a emergência das doenças infecciosas.

Além disso, as interações humanas estão cada vez mais facilitadas, tanto em nível local como internacional. A expressão “vetor cultural” é bastante adequada para ilustrar a disseminação das doenças infecciosas através das interações socioculturais (Paz e Bercini, 2009; Waldman, 2001). Neste contexto, o comércio internacional e o turismo são importantes estímulos da interação globalizada entre os seres humanos, tendo um papel importante na manutenção do vetor cultural das doenças infecciosas (Morse, 1995; Luna, 2002).

Os hábitos comportamentais humanos também podem facilitar o surgimento ou disseminação de doenças, principalmente tratando-se das doenças sexualmente transmissíveis, que podem ter sua transmissão aumentada ou diminuída de acordo com determinadas práticas sexuais. Sabe-se, por exemplo, que o uso de preservativos é uma escolha pessoal altamente eficaz para proteger contra a infecção pelo HIV. De forma similar, o uso de drogas injetáveis através de aparatos compartilhados também pode ser considerado como um “comportamento” que facilita a transmissão de doenças infecciosas (Morse, 1995).

O surgimento de grupos populacionais suscetíveis a uma doença específica contribui para o aumento do número de infecções. Por exemplo, a chegada de imigrantes não vacinados em uma área onde a circulação de um vírus é endêmica pode desencadear a elevação do número de casos de infecção pelo patógeno (Barata, 1997). Questões políticas e as guerras estimulam os fluxos migratórios e por isso também são fatores responsáveis pela emergência de surtos e epidemias (Morse, 1995; Luna, 2002).

A falta de controle de qualidade em bancos de sangue e hemoderivados também pode resultar na transmissão de doenças infecciosas para um grande número de pessoas, como aconteceu com a disseminação do HIV no início da pandemia (Luna, 2002). Devido ao atual rigoroso controle de qualidade aplicado nos bancos de sangue, esta não é mais uma realidade no Brasil e em países desenvolvidos, ao menos ao que se refere a patógenos como HIV, HCV e HBV. Porém, o risco dos bancos de sangue serem fontes de disseminação de patógenos desconhecidos ou que não fazem parte dos *screenings* tradicionais não deve ser negligenciado (Di Minno et al., 2016).

Os fatores mencionados acima desempenham grande influência sobre a emergência das doenças infecciosas, entretanto é a vulnerabilidade populacional que dita se uma nova doença emergirá ou não em determinada população (Pignatti, 2004; De Carvalho et al., 2009). A vacinação é um dos fatores que mais modificam a vulnerabilidade de uma população às doenças infecciosas de origem viral. Porém, só existem vacinas para um número reduzido de patógenos conhecidos. Além disso, as vacinas podem atuar na seleção de novas cepas patogênicas se a cobertura vacinal não é atingida de forma satisfatória (Read et al., 2015). Esse dado ressalta a importância das campanhas de vacinação atingirem a cobertura vacinal recomendada para cada vacina já existente, para que assim os benefícios da vacinação sejam obtidos de forma adequada e o maior número possível de pessoas seja beneficiado pelo uso das vacinas.

A vulnerabilidade populacional é afetada também pelas condições de saúde dos indivíduos. Além de fatores imunogenéticos do hospedeiro (discutidos no tópico 4 desta tese), o estado de saúde é influenciado por uma ampla gama de componentes sociais, biológicos e ambientais, tais como: tabagismo, estado nutricional, consumo de álcool, práticas sexuais e contato com poluentes. Sabe-se também que fatores como renda, etnia, gênero e cor de pele afetam os componentes mencionados, bem como o acesso aos serviços básicos de saúde (Pignatti, 2004; De Carvalho et al., 2009). O ato de vacinar os filhos também é influenciado por fatores como etnia e nível de escolaridades dos pais (Glatman-Freedman e Nichols, 2012; Wilson et al., 2015; Xeuatvongsa et al., 2017). Além disso, indivíduos com condições socioeconômicas não adequadas enfrentam dificuldade para acessar os serviços de vacinação (Wilson et al., 2016). Tal cenário demonstra que a vulnerabilidade (individual ou populacional) a uma nova doença é o resultado da interação de diversos aspectos. Neste contexto, quando o componente "humano" da tríade *One Health* é analisado, conclui-se que os fatores biológicos e sociais estão extremamente conectados. Isso deve ser levado em consideração quando se estuda o impacto dos fatores sociais ou biológicos sobre a emergência das doenças infecciosas.

2.6.2. Fatores associados aos animais não humanos

Animais não humanos hospedam e mantêm patógenos em determinados ecossistemas. Tais animais são chamados de "reservatórios" (Flores, 2007) e podem ser

considerados como “fontes” de doenças emergentes. Os morcegos são exemplos clássicos de animais reservatórios de uma variedade de patógenos (Calisher et al., 2006; Quan et al., 2013; Melaun et al., 2014; Schountz, 2014; Veikkolainen et al., 2014; Allocati et al., 2016; Hayman, 2016). Além disso, a amplificação dos vírus causadores de epidemias muitas vezes acontece em animais hospedeiros ou reservatórios selvagens, processo conhecido como “amplificação enzoótica”. Entretanto, há exceções. Muitos arbovírus, por exemplo, usam os humanos como hospedeiro amplificador, sustentando assim epidemias de grandes proporções (Donalisio et al., 2017). Também não se deve confundir o termo “reservatório de patógenos” com “reservatório de infecção” que, de forma simplificada, significa uma população ou ambiente no qual o patógeno circula permanentemente (Viana et al., 2014).

A relação entre humanos e animais não humanos é histórica e muito diversificada. A carne dos animais é usada pelos humanos para a alimentação, os animais auxiliam os humanos em trabalhos agrícolas, bem como fornecem segurança e companhia aos humanos. Em troca, os animais recebem alimentação, abrigo e cuidados básicos. Apesar dessa relação trazer inúmeros benefícios para ambos, ela facilita a emergência de doenças zoonóticas.

O transporte de animais com finalidade esportiva e expositiva (em circos, feiras agropecuárias ou parques zoológicos), assim como o tráfico de animais silvestres, são atividades que contribuem para a disseminação de patógenos (Zanella, 2016). Os animais reservatórios podem transportar inúmeros microrganismos. Além disso, os patógenos podem infectar um animal antes da viagem e serem transmitidos para espécies locais ao final da viagem, criando a possibilidade de estabelecimento de novos e diferentes reservatórios de patógenos no local de destino para onde o animal foi transportado. A exposição de animais em locais com grande visitação de humanos, como feiras e zoológicos, pode criar condições favoráveis para a exposição de humanos aos patógenos hospedados em animais, seja entre o público visitante ou entre as equipes de manejo dos animais, como tratadores e veterinários.

A criação de animais para fornecimento de alimentos para os humanos desempenha importante papel na emergência das doenças infecciosas. A criação e manejo desses animais geralmente acontecem em ambientes de confinamento, onde um grande número de indivíduos é mantido em condições controladas. Tais condições facilitam a circulação de patógenos entre os animais, possibilitando o surgimento de novas cepas ou de

microrganismos mais virulentos. A emergência de surtos/epidemias do vírus *Influenza* e vírus do gênero *Henipavirus* (como *o Nipah virus*) já teve a participação de animais de criação, por exemplo (Bayry, 2003) . Além disso, a criação de animais é uma atividade que facilita eventos de *spillover* em razão do contato que os criadores têm com um grande número de animais. Importantemente, as condições nas quais os animais são criados, assim como as estratégias de vacinação, facilitam ou não a emergência de doenças infecciosas humanas a partir de suínos, aves e outras espécies de criação (Tomley e Shirley, 2009; IFAH, 2013).

O consumo e o comércio de carne de caça, principalmente de espécies exóticas, é outra atividade associada ao surgimento de infecções (Zanella, 2016; Nava et al., 2017). Tradicionalmente, a carne de animais exóticos vendidos em feiras ou comércio local é chamada de "*bushmeat*". Já "*wild meat*" refere-se também a carne de animais exóticos, mas neste caso a carne é obtida através de caça e então destinada ao consumo próprio do caçador ou de sua família, sem a finalidade de venda. Apesar do consumo e comércio de carne de animais selvagens não sustentar epidemias (apenas surtos), é possível que essas atividades facilitem eventos de *spillover*, que não aconteceriam necessariamente através do consumo da carne, mas em decorrência do contato do sangue do animal com o caçador, facilitado por cortes na pele ou através das mucosas do caçador (Wolfe et al., 2005; Nava et al., 2017). Apesar deste exemplo estar inserido no sub-tópico "fatores animais" desta tese, ele também poderia estar mencionado entre os "fatores humanos" discutidos no sub-tópico anterior, pois a caça e o comércio são essencialmente fatores culturais humanos. Ainda, o consumo de carne de animais selvagens também causa importantes perdas para a biodiversidade, contribuindo para o "fator ambiental" na emergência das doenças infecciosas (Wolfe et al., 2005; Roger et al., 2016; Benítez-López et al., 2017; Brashares e Gaynor, 2017). Da mesma forma, a agricultura e a pecuária são atividades diretamente relacionadas com o desmatamento e, também por esse motivo, facilitam o surgimento de surtos e epidemias (McMichael et al., 2007; Gottdenker et al., 2014; Machovina et al., 2015; Busch e Ferretti-Gallon, 2017).

Em razão da grande interação com os humanos, os animais de companhia (*pets*) são fontes adicionais de zoonoses e podem contribuir para a disseminação de patógenos entre a população humana. Este risco é aumentado quando os animais de companhia são animais exóticos, uma vez que a chance dessas espécies hospedarem microrganismos patogênicos

e/ou desconhecidos pode ser maior do que aquela verificada em animais como cães e gatos (Vasconcellos, 2001; Zanella, 2016).

Outros animais que também têm grande contato com os humanos são as “pragas urbanas”, como os ratos e os pombos. Diversas doenças infecciosas estão relacionadas com a presença desses animais. Conhecidos tradicionalmente como portadores de fungos causadores da criptococose, um estudo realizado no Brasil demonstrou que os pombos urbanos podem adicionalmente ser importantes reservatórios de arbovírus (Ramos et al., 2017). Ratos podem hospedar diferentes tipos de patógenos humanos e representam um risco para populações que vivem em áreas infestadas (Himsworth et al., 2013; CDC, 2017).

Aves migratórias podem transportar patógenos por diferentes e distantes regiões geográficas. Sabe-se que a emergência de cepas do vírus *Influenza* é constantemente afetada pela circulação das aves entre diferentes regiões, uma vez que tais animais são reservatórios do vírus (Kawamoto et al., 2005; Causey e Edwards, 2008).

Por fim, é importante mencionar que a emergência de uma ampla variedade de doenças virais é dependente da presença de vetores, sendo estas chamadas de *vector-borne diseases*. No Brasil, os mosquitos são responsáveis pela disseminação de infecções como febre chikungunya, febre por ZIKV, febre de Mayaro, dengue, febre amarela, entre outros (Figueiredo, 2007; Figueiredo, 2016; Mota et al., 2016). Da mesma forma, carrapatos podem transmitir bactérias, parasitas e vírus patogênicos (Rodríguez et al., 2018). Os vetores devem ser considerados como importantes focos de estratégias de controle das doenças infecciosas, principalmente em áreas de endemia das doenças transmitidas por esses animais.

2.6.3. Fatores ambientais

Além de abrigarem uma grande variedade de espécies animais e vegetais, países com uma rica biodiversidade abrigam um número grande de patógenos, sendo este o cenário encontrado no Brasil (Boulus, 2001; Keesing et al., 2010). Além disso, em razão de sua vasta biodiversidade e da ocorrência de desequilíbrios sociais e ambientais, o Brasil é considerado um *hot spot* para a emergência de doenças infecciosas (Allen et al., 2017; Nava et al., 2017).

A degradação do ambiente e a perda da biodiversidade são importantes fatores responsáveis pelo surgimento de novas doenças infecciosas, particularmente as zoonoses. A preservação da biodiversidade contribui para a manutenção das doenças "na selva", longe das áreas urbanas (Piggnati, 2004; Ostfeld, 2009). A prevalência de mosquitos e carrapatos hospedando patógenos é menor em áreas com maior biodiversidade do que em áreas degradadas (Ostfeld, 2009). Vetores generalistas, quando presentes em áreas biodiversas, podem buscar alimento em uma maior variedade de espécies que geralmente hospedam um número menor de patógenos. Em áreas degradadas, vetores generalistas buscam alimento em uma menor variedade de espécies, que geralmente concentrando um número maior de patógenos (Ostfeld, 2009). Nesse contexto, a perda da biodiversidade contribui para a emergência e para a o aumento da transmissão de doenças infecciosas (Keesing et al., 2010), o que está relacionado ao fato de que, em locais com natureza degradada, geralmente há maior receptividade aos patógenos, facilitando a proliferação dessas doenças (De Carvalho et al., 2009).

Apesar da biodiversidade ser geralmente associada com densas florestas tropicais que abrigam variadas espécies animais e vegetais, os solos também contêm uma rica diversidade de espécies, incluindo inúmeros patógenos (*Bacillus anthracis*, por exemplo). Por isso, o uso do solo pode afetar essa biodiversidade e, consequentemente, impactar a saúde humana (Wall et al., 2015). Na maioria dos casos, o uso do solo pelos humanos através da agricultura, irrigação, desmatamento, fragmentação de habitat, urbanização e desurbanização está associado a um aumento na transmissão de patógenos. Porém, deve-se ponderar que algumas evidências sugerem que essas modificações podem gerar alterações variadas na dinâmica das doenças infecciosas, incluindo a redução da transmissão de patógenos em algumas situações (Gottdenker et al., 2014). Em torno de 10% dos estudos que avaliaram os impactos do uso da terra sobre a emergência das doenças infecciosas observaram uma redução na transmissão de patógenos em decorrência dessas modificações antropogênicas. Entretanto, os fatores envolvidos nessa redução ainda são pouco entendidos (Gottdenker et al., 2014).

O maior contato da população com patógenos é facilitado principalmente pelo desmatamento, causado geralmente por fatores como construção de moradias, atividade agropecuária e expansão dos meios de transporte (Boulus, 2001). Entretanto, o surgimento de uma doença infecciosa em decorrência de distúrbios no meio ambiente geralmente afeta

as populações de forma temporalmente distinta. A degradação ambiental impacta primeiramente a população mais próxima à área degradada. Posteriormente, as populações alocadas em regiões mais distantes do foco da degradação também poderão ser afetadas (Pignatti, 2004; De Carvalho et al., 2009), seja porque a doença tomou proporções epidêmicas através da transmissão homem-homem ou porque o patógeno está sendo disseminado através de vetores.

Para que um patógeno se estabeleça em uma nova população e tenha a capacidade de causar um surto ou até mesmo uma epidemia, não basta que ele simplesmente seja introduzido na nova população. As condições populacionais e ambientais precisam ser adequadas a esta introdução. Conforme já mencionado, regiões ambientalmente degradadas são mais propícias ao surgimento de novas doenças. Por exemplo, vetores animais encontram maior facilidade para ocupar nichos ecológicos vagos nessas regiões, em razão da baixa competição ou ausência de predadores. Por consequência, os patógenos transmitidos por tais vetores também se farão presentes e o estabelecimento de uma nova doença pode acontecer (Pignatti, 2004). Vetores que se alimentam de sangue de um número maior de espécies (vetores generalistas) também parecem carrear menos patógenos do que aqueles que se alimentam de uma baixa diversidade de espécies (vetores especialistas), o que geralmente é verificado em áreas degradadas (Ostfeld, 2009).

Além da perda da biodiversidade, eventos climáticos extremos já são aceitos como importantes *drivers* das doenças infecciosas emergentes (Nava et al., 2017; Watts et al., 2017). Eventos naturais como secas ou longos períodos chuvosos modificam o ciclo de vida de muitos vetores e, por isso, impactam o número de casos de doenças infecciosas (Luna, 2002). Projetos de engenharia que modificam de forma importante a natureza, como a construção de represas e rodovias, desempenham efeitos similares aos causados por eventos climáticos naturais, sendo também responsáveis por modificações no número de casos de infecções (Luna, 2002).

É consenso que o equilíbrio ambiental é essencial para que a emergência de novas doenças seja evitada (Boulus, 2001; Pietrzak et al., 2018). Neste contexto, a proteção da biodiversidade planetária deve ser implementada como uma importante estratégia de garantia da saúde das populações humanas (Whitmee et al., 2015). Para que isso aconteça, é fundamental que a compreensão da associação entre os desequilíbrios ambientais e a

emergência das doenças infecciosas seja disseminada entre a população (Pietrzak et al., 2018).

Por fim, é importante mencionar que fatores ambientais não relacionados com desequilíbrios ambientais também influenciam a dinâmica das doenças infecciosas. Um bom exemplo é o vento, que tem um papel tanto na disseminação dos patógenos através de partículas suspensas no ar (Aliabadi et al., 2011; Ssematimba et al., 2012), quanto como modificador do comportamento de vetores animais como os mosquitos do gênero *Anopheles*, transmissores da malária. Por exemplo, o vento modifica a direção da dispersão de CO_2 do ar, molécula usada pelos mosquitos para localizar os humanos. Dessa forma, dependendo da direção do vento, os mosquitos terão maior ou menor chance de localizar e picar os humanos (Endo e Eltahir, 2018a; Endo e Eltahir, 2018b).

2.6.4. Fatores associados aos patógenos

Além de fatores ambientais, humanos e de outros animais, as características virais também ditarão se um vírus será capaz de causar apenas casos isolados de infecção, um surto, uma epidemia ou até mesmo uma pandemia. Vírus transmitidos de animais para humanos e que possuem a capacidade de infectar diferentes hospedeiros apresentam maior capacidade de gerar epidemias (Johnson et al., 2015). Vírus de primatas não-humanos têm maiores chances de estabelecer transmissão humano-humano do que vírus provenientes de aves, roedores ou artrópodes (Geoghegan et al., 2016; Walker et al., 2018). A transmissão do tipo humano-humano também é mais provável de acontecer com vírus generalistas, que podem infectar células do fígado, sistema nervoso central ou trato respiratório (Walker et al., 2018). É importante destacar que a transmissão do tipo humano-humano é considerada como um fator básico para que um vírus cause uma epidemia (Plowright et al., 2017; Morse et al., 2012). Porém, a transmissão humano-humano pode não ser essencial em alguns casos. A transmissão mediada por vetores é eficaz para sustentar epidemias e surtos, como no caso das arboviroses, por exemplo (Whitehead et al., 2007; Lessler et al., 2016).

Outras características virais aumentam a chance de um vírus estabelecer um padrão de transmissão humano-humano e emergir sob a forma de epidemia, sendo elas: capacidade de causar infecção com baixa mortalidade do hospedeiro e/ou de forma crônica, ausência de envelope viral, ausência de vetor para mediar a transmissão, partículas

virais de pequeno tamanho (<75nm em diâmetro) e genoma com até dois segmentos (Geoghegan et al., 2016; Walker et al., 2018). Vírus com genoma de RNA apresentam maiores chances de sofrer mutações e rearranjos gênicos, o que aumenta as chances de adaptação a novos hospedeiros (Nichol et al., 2000).

Quando se estuda a dinâmica das doenças infecciosas e a capacidade de um determinado patógeno causar uma epidemia, é muito comum se deparar com o símbolo "R_0". O significado de tal símbolo é "*basic reproductive number*". No campo da ecologia, R_0 representa o número esperado de descendentes que um indivíduo típico terá ao longo da vida (Reluga et al., 2009). Já no estudo da epidemiologia das doenças infecciosas, o R_0 representa o número médio (*average*) de casos secundários de uma doença infecciosa produzido por um único evento de infecção, em uma população completamente suscetível (Dietz, 1993; Woolhouse et al., 2005; Gardy e Loman, 2018). Um padrão $R_0=0$ significa que o patógeno não tem capacidade de transmissão humano-humano ou não está sendo transmitido entre humanos. Um padrão de transmissão $R_0<1$ configura a falta de capacidade de causar uma epidemia. Um surto pode ser possível, mas dependerá de um grande número de infecções a partir da fonte original do patógeno. O padrão $R_0>1$ representa um padrão de transmissão passível de causar uma epidemia (Woolhouse et al., 2005; Lloyd-Smith et al., 2009). $R_0=1$ é compatível com a transmissão endêmica (Garnett, 2002) ou representa transição entre $R_0<1$ e $R_0>1$ (Woolhouse et al., 2005). Um vírus pode ultrapassar a barreira entre espécies (*spillover*), mas se não apresentar $R_0>1$, não causará uma epidemia (May et al., 2001). A forma de calcular o R_0 pode ser obtida em diferentes fontes (Dietz, 1993; Breban et al., 2007).

2.7. Estratégias de combate e vigilância das doenças infecciosas

De acordo com Segurado et al. (2016), vigilância epidemiológica pode ser definida da seguinte forma:

> "A vigilância epidemiológica consiste na coleta regular e sistemática de dados de ocorrência de problemas de saúde considerados prioritários, com o propósito de nortear as ações de prevenção e controle, bem como avaliá-las, e trabalha com a lógica da notificação compulsória de doenças, ou seja, a comunicação obrigatória à autoridade sanitária da ocorrência de cada caso das doenças sob vigilância" (p. 30).

Para que a vigilância das doenças emergentes e reemergentes aconteça de forma efetiva, inúmeros profissionais precisam estar envolvidos e diferentes ações devem ser promovidas conjuntamente. Além das equipes de saúde responsáveis pelo tratamento dos doentes, os profissionais envolvidos na investigação epidemiológica dos surtos e epidemias "em campo" têm um papel essencial no controle dessas urgências de saúde (Barata, 1997; Paz e Bercini, 2009). De forma complementar, o subdiagnóstico e a subnotificação das doenças infecciosas devem ser combatidos através do treinamento dos profissionais responsáveis por tais ações. Muitas vezes isso requer o envolvimento de uma equipe multidisciplinar, incluindo profissionais das ciências sociais, visto que muitas doenças infecciosas estão intimamente relacionadas com hábitos socioculturais (Grisotti, 2010).

Pesquisadores da área da virologia ambiental investigam principalmente vírus encontrados na água e efluentes. Levando em consideração que o Brasil tem um grave problema com o tratamento de esgoto e com a implantação de infraestrutura de saneamento básico de forma geral, o monitoramento de vírus transmitidos pela água (exemplos: vírus da hepatite A e E, poliovírus e rotavírus) deve ser intensificado (Prado e Miagostovich, 2014). Além disso, deve-se levar em consideração que muitas das doenças infecciosas humanas são ou já foram essencialmente zoonoses. Dessa forma, os profissionais da área veterinária desempenham um papel fundamental no controle e na execução de medidas de vigilância e prevenção das doenças emergentes (Paz e Bercini, 2009; Zanella, 2016).

Conhecer a geografia médica das regiões avaliadas ajuda na identificação das possíveis doenças infecciosas com potencial para emergir na região de análise, além de auxiliar na predição da dinâmica dessas doenças. Por exemplo, ambientes próximos a florestas geralmente estão associados a surtos, endemias e epidemias com padrões de transmissão diferentes daqueles observados em metrópoles (Boulus, 2001); Os casos de dengue são comuns nas cidades, onde as condições urbanas são propícias à proliferação de mosquitos. Já as habitações próximas de áreas florestais podem facilitar o contato dos humanos com carrapatos ou artrópodes como os triatomíneos, possibilitando a transmissão de diferentes patógenos hospedados nestes vetores (Vayssier-Taussat et al., 2015; Inci et al., 2016; Vieira et al., 2018). Além disso, a distribuição geográfica das doenças infecciosas apresenta uma série de padrões que podem ser identificados através da biogeografia (Murray et al., 2015). Técnicas de geoprocessamento podem ser úteis para a

representação visual dos dados epidemiológicos no contexto da geografia médica (Barata, 1997). O uso de tais ferramentas deve ser estimulado entre os profissionais de saúde e pesquisadores envolvidos na investigação de surtos e epidemias.

Um sistema de informação que permita o compartilhamento de dados entre diferentes setores é essencial para que a vigilância epidemiológica funcione na prática, permitindo a rápida detecção e notificação das doenças (Barata, 1997; Zanella, 2016). Essa ação deve envolver órgãos responsáveis pela saúde humana, veterinária e ambiental, com troca de informações entre diferentes estados e países quando existe a ameaça da ocorrência de epidemias ou pandemias (Zanella, 2016). Existem inúmeras plataformas *on-line* com o objetivo de monitorar os casos de doenças infecciosas ao redor do mundo (Christaki, 2015; O'Shea, 2017). Uma delas é o *HealthMap* (https://www.healthmap.org/pt/), que utiliza diferentes tipos de informações disponíveis na internet para monitorar a ocorrência de doenças infecciosas ao redor no mundo (Freifeld et al., 2008).

Além dos problemas causados aos indivíduos diretamente afetados, as epidemias causam importantes impactos sobre os serviços de saúde pública. Tais impactos são amplificados caso não existam vacinas ou tratamentos conhecidos contra o agente patogênico (Donalisio et al., 2017). Por isso, o abastecimento e manutenção dos estoques de vacinas e medicamentos antivirais é essencial (Zanella, 2016). Além disso, a busca de novas terapias para as doenças infecciosas emergentes e reemergentes deve ser incentivada.

Apesar dos atuais sistemas de controle de qualidade dos bancos de sangue brasileiros serem efetivos para evitar a transmissão de patógenos como o HIV, HCV e HBV em transfusões sanguíneas, os *screenings* (triagens) não detectam muitos vírus reemergentes ou negligenciados. Por isso a implementação de *screenings* mais amplos parece ser essencial para minimizar a chance de transmissão de patógenos emergentes através de transfusão sanguínea e uso de hemoderivados, apesar dessa ação muitas vezes ser limitada por questões de custo (Marks et al., 2016). Alternativamente à aplicação de *sceenings* de amplo espectro, pode-se investir em estratégias de inativação de patógenos que apresentem baixo custo e sejam eficientes e de fácil aplicação (Schmidt et al., 2014).

A existência de laboratórios com capacidade de realizar o diagnóstico das doenças emergentes de forma adequada e ágil é um fator essencial para a obtenção de uma estrutura

nacional eficaz de vigilância epidemiológica (Waldman, 2001; Paz e Bercini, 2009). Isso demanda a existência de infraestrutura disponível para tais práticas diagnósticas além de pessoal altamente qualificado (Barata, 1997; Waldman, 2001).

Os laboratórios onde são realizados o diagnóstico e procedimentos de pesquisa de microrganismos patogênicos são divididos em quatro níveis de biossegurança, classificados de acordo com a letalidade e outras características dos patógenos manipulados. A estrutura física de cada laboratório, bem como o preparo dos profissionais que neles trabalham, é planejada de forma a proteger os profissionais e a comunidade externa. Especificamente, os nívels de biossegurança desses laboratórios são divididos da seguinte forma: *biosafety level 1* (BSL1), *biosafety level 2* (BSL2), *biosafety level 3* (BSL3) e *biosafety level 4* (BSL4, o mais elevado). Em laboratórios BSL4 são onde as pesquisas com vírus como Ebola, Marburg e Lassa são realizadas (Bayot e King, 2019). Apesar de já existirem no Brasil laboratórios BSL3 (Simonetti, 2014) e um laboratório BSL4 (Lyra, 2014), é urgente a expansão do número de laboratórios com elevado nível de biossegurança no Brasil, onde patógenos com alto potencial de letalidade possam ser estudados de forma segura (Cardoso e Navarro, 2007). Essa carência em termos de laboratórios com alto nível de biossegurança aumenta a dependência do Brasil em relação a outros países para a identificação, isolamento e estudo das amostras de alto risco coletadas no território nacional (Schatzmayr, 2001).

O manejo de agentes patogênicos, animais ou amostras biológicas em ambiente laboratorial também deve garantir a segurança da comunidade. O armazenamento e pesquisa de patógenos de alta letalidade e com o potencial de serem utilizados como arma biológica deve ser conduzido em locais e instituições munidas de sistemas de segurança robustos e eficientes (Barata, 1997; Cardoso e Navarro, 2007). A varíola é considerada erradicada no mundo desde 1980 e apenas poucos estoques do vírus são mantidos legalmente no CDC (EUA) e no *State Research Center of Virology and Biotechnology VECTOR* (localizado na Rússia), apesar de *vials* contendo o vírus e armazenados acidentalmente terem sido encontrados em 2014 nas dependências do NIH (EUA) (Reardon, 2014). Caso a varíola fosse usada como arma biológica atualmente, as consequências seriam graves, uma vez que uma parcela muito pequena da população mundial possui imunidade vacinal para varíola e os estoques de vacina são escassos (Waldman, 2001). Esse exemplo sugere que a capacidade nacional para isolar, armazenar e

estudar vírus altamente patogênicos é extremamente importante e depende de uma estrutura laboratorial robusta, que deve ser administrada por profissionais altamente capacitados. Conforme mencionado anteriormente, no Brasil ainda há a necessidade de construção de novos laboratórios BSL3 e BSL4, os únicos onde patógenos de alta letalidade podem ser manipulados de forma segura. Conjuntamente, também há a necessidade de investir na formação de profissionais capacitados para trabalhar em tais locais.

As ações voltadas ao controle das doenças emergentes não podem envolver apenas os serviços de saúde. O setor de pesquisa deve estar intensamente envolvido na investigação dessas doenças, podendo auxiliar com a elucidação dos aspectos básicos dos patógenos, no desenvolvimento tecnológico voltado à detecção de surtos, no diagnóstico laboratorial das infecções, e no desenvolvimento de vacinas e terapias inovadoras (Paz e Bercini, 2009; Donalisio et al., 2017). A rede brasileira de laboratórios de saúde pública (LACENs) e os laboratórios federais devem atuar em conjunto com o *background* oferecido pelos laboratórios universitários (Luna, 2002). Ainda, as instituições privadas também podem estar envolvidas nesse processo (Luna, 2002; Paz e Bercini, 2009).

Modelos estatísticos podem ajudar na identificação de áreas com alto risco para emergência das doenças infecciosas, apontando regiões onde as estratégias de prevenção devem ser adotadas de forma mais intensa (Jones et al., 2008; Allen et al., 2017; Wilkinson et al., 2018). Apesar das novas tecnologias serem extremamente benéficas e eficazes para o estudo das doenças infecciosas, é importante mencionar que o padrão de aglomeração e distribuição geográfica de casos, os modos de transmissão, as taxas de infecção, bem como os grupos mais vulneráveis ainda podem ser identificados através de abordagens clássicas da epidemiologia descritiva (Barata, 1997). Complementariamente, as medidas de vigilância epidemiológica devem ser fortalecidas no Brasil em ações que levem em consideração os patógenos, seus vetores, reservatórios, hospedeiros e a população vulnerável de forma conjunta, a partir da perspectiva *One Health*. Além disso, apesar da intensificação dessas ações em situações de surtos e epidemias ser essencial, o alto risco da emergência de novas doenças no Brasil faz com que a vigilância epidemiológica sobre tais ameaças à saúde pública deva ser constante em nosso país (Paz e Bercini, 2009; Lima-Camara, 2016; Donalisio et al., 2017).

Por fim, a população deve ser conscientizada sobre como as infecções acontecem. Esse é um passo essencial para que as medidas protetivas contra as doenças infecciosas sejam aplicadas na vida cotidiana de cada indivíduo (Boulus, 2001). Levando em conta os custos humanos e monetários das doenças infecciosas, estratégias voltadas para a prevenção são muito vantajosas (Heymann e Dar, 2014). Os custos para mitigar uma epidemia podem ser muitas vezes mais elevados do que os investimentos em prevenção (Castillo-Chavez et al., 2015).

3. Doenças virais negligenciadas, emergentes e reemergentes no Brasil

O Brasil está entre os países com os maiores números de doenças tropicais emergentes e reemergentes (Mackey et al., 2014). Conforme discutido em detalhe nos tópicos anteriores, as características naturais, sociais e políticas brasileiras são propícias para a emergência de diferentes doenças infecciosas, uma vez que o Brasil abriga inúmeros patógenos humanos em potencial e, ao mesmo tempo, apresenta uma série de condições favoráveis à disseminação das doenças infecciosas (Luna, 2002).

As mortes causadas por algumas doenças infecciosas e parasitárias diminuíram consideravelmente no Brasil ao longo do século XX. Essa redução deve-se a melhorias no desenvolvimento do país, que facilitaram o acesso da população aos serviços de saúde e vacinação e reduziram problemas relacionados ao saneamento básico, apesar da persistência e proliferação de algumas doenças como a dengue e a febre amarela (Luna e Da Silva, 2013).

No Brasil, as seguintes doenças entram no grupo daquelas consideradas como emergentes e reemergentes: dengue, HIV/AIDS, cólera, leishmanioses, febre amarela, hepatite C, doenças transmitidas por alimentos, hantaviroses, leptospirose, febre maculosa (riquetiose), influenza pandêmica e as infecções hospitalares (Luna e Da Silva, 2013).

De acordo com uma equipe de trabalho formada pela Academia Brasileira de Ciências, as doenças negligenciadas mais importantes no Brasil são: doença de Chagas, leishmanioses, malária, filarioses, micobacterioses (hanseníase e tuberculose), clamidioses e riquetioses, raiva, hantavírus, hepatites virais, gastroenterites virais, paracoccidiodomicose e outras micoses profundas, envenenamento por toxinas

(principalmente de animais peçonhentos), dengue, febre amarela e outras arboviroses (De Souza, 2010).

Considerando que muitas doenças negligenciadas podem ser classificadas como emergentes ou reemergentes, é possível que esses dois últimos termos sejam suficientes para denominar as doenças negligenciadas no Brasil (De Souza, 2010). O conceito de doenças negligenciadas é muitas vezes pouco preciso e contraditório, pois algumas doenças ditas "negligenciadas" recebem bastante atenção por parte da indústria farmacêutica e do governo, como é o caso da dengue (Luna e Da Silva, 2013). É importante levar em consideração que muitas doenças ditas emergentes ou "novas" já circulavam na população há bastante tempo, só não eram identificadas (Grisotti, 2010). Além disso, o que significa uma doença "nova" ou "emergente" vai depender de questões geográficas, históricas, sociais, epidemiológicas, da capacidade científica de reconhecê-la como tal e até mesmo de interpretações filosóficas e semânticas (Grisotti, 2010).

Há um grupo de doenças cujos índices de morbimortalidade apresentam queda ou estabilidade, mas que continuam representando um problema de saúde pública no Brasil, tais como: hanseníase, tuberculose, tracoma, malária, doença meningocócica, geohelmintíases e protozooses intestinais, cisticercose, toxoplasmose, febre tifoide, sífilis e outras doenças sexualmente transmissíveis, infecção por *Yersinia pestis*, varicela, micoses sistêmicas e hidatidose (Luna e Da Silva, 2013). Recentemente, toxoplasmose e sífilis voltaram a ser importantes agravos de saúde pública no Brasil (Cooper et al., 2016; Reinehr et al., 2017; CEVS, 2018). Ainda, existe o grupo de doenças que apresentam tendência de declínio no Brasil, com possibilidade de serem controladas ou até mesmo eliminadas, sendo elas: doenças imunopreveníveis, pneumonias e infecções por *Influenza*, doenças diarreicas, hepatites A e B, esquistossomose, doença de Chagas, raiva, filariose linfática e oncocercose (Luna e Da Silva, 2013). Das doenças mencionadas, nesta tese serão abordadas mais detalhadamente aquelas causadas pelo HIV, HCV e HBV.

3.1. HIV/AIDS

O HIV pertence à família *Retroviridae* e ao gênero *Lentivirus*. É um vírus esférico (~100 nm), apresenta membrana lipídica e envelope viral (GACB, 2016). As glicoproteínas do envelope gp41 e gp120 são as principais responsáveis pela interação do HIV com a

superfície da célula hospedeira (Gorry et al., 2004; Korsman et al., 2014). O genoma do HIV é composto por duas fitas simples de RNA, encontra-se no interior de um capsídeo cônico e o genoma do HIV compreende os seguintes genes: *gag*, *pol*, *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, *vpx* e *tev* (GACB, 2016).

Existem duas espécies de HIV: o HIV do tipo 1 (HIV-1) e o HIV do tipo 2 (HIV-2). O HIV-1 é a espécie de maior circulação no mundo. O HIV-2 circula principalmente nos países africanos, apesar de também ser encontrado em outras regiões geográficas. Sabe-se que o HIV-2 é menos patogênico do que o HIV-1 (Campbell-Yesufu e Gandhi, 2011; GACB, 2016). O HIV-2 é dividido nos seguintes grupos: A, B, C, D, F, G e H. Já o HIV-1 divide-se nos seguintes grupos: M, N, O e P. O grupo M (o "M" é derivado da palavra "*main*") é dividido nos seguintes subtipos: A (A1, A2 e A3), B, C, D, F (F1 e F2), G, H, J e K. Também existem as formas recombinantes desses subtipos, conhecidas como CRFs (*circulating recombinant forms*, ou formas recombinantes circulantes). O grupo M e, em particular, os subtipos A1, B, C e CRF02_AG (forma recombinante) estão entre os responsáveis pelo maior número de casos de infecção no mundo (Tebit e Arts, 2011; Korsman et al., 2014; Librelloto et al., 2015; GACB, 2016). Os subtipos B, C e a forma recombinante BC são cepas circulantes no Rio Grande do Sul (Librelloto et al., 2015).

Evidências filogenéticas indicam que o HIV-1 é derivado do vírus da imunodeficiência símia (*simian immunodeficiency virus*, SIV), um vírus de primatas não humanos (Sharp e Hahn, 2011; Tebit e Arts, 2011). Acredita-se que as cepas que deram origem ao HIV passaram a infectar humanos através de eventos de *spillover* devido à exposição de pessoas ao sangue de primatas não humanos. Atividades de caça e manipulação de carne de tais primatas são os prováveis *drivers* dos eventos de *spillover* que facilitaram a emergência do HIV (Peeters et al., 2002; Sharp e Hahn, 2011). Estima-se que a introdução do HIV em humanos aconteceu entre os anos de 1920 e 1940 (Faria et al., 2014; GACB, 2016). É provável que a pandemia de HIV tenha iniciado na República Democrática do Congo (antigo Zaire) na década de 1960, mais especificamente na cidade de Kinshasa. O sistema de transporte fluvial e rodoviário do Congo, junto à prostituição e outros fatores desconhecidos, facilitaram a saída do HIV do interior da África para o resto do mundo (Faria et al., 2014).

Quando não tratada, a infecção pelo HIV causa a síndrome da imunodeficiência adquirida (AIDS), uma doença caracterizada principalmente pela depleção das células do

sistema imunológico (Maartens et al., 2014). Os primeiros casos de AIDS foram relatados pelo CDC no início da década de 1980 nos EUA, quando um grupo de homossexuais masculinos foi diagnosticado com um tipo de infecção pulmonar fúngica característica de indivíduos imunodeprimidos (CDC, 1981). Porém, sabe-se que, após a infecção, o HIV permanece latente no indivíduo infectado por vários anos antes de causar AIDS (Bacchetti e Moss, 1989; Siliciano e Siliciano, 2004; Maartens et al., 2014), indicando que, provavelmente, a circulação do HIV nos EUA já acontecia antes da década de 1980. Além disso, posteriormente aos primeiros relatos na literatura médica dos casos de AIDS, o HIV foi identificado em amostras de material biológico coletadas entre as décadas de 1950 e 1960 (Nahmias et al., 1986; Zhu et al., 1998; Worobey et al., 2008). Este mesmo tipo de evidência também indicou a circulação do HIV nos EUA na década de 1970 (Cohen, 2016; Worobey et al., 2016). Em conjunto, essas informações confirmam que o HIV já circulava por diferentes locais do mundo muitos anos antes da detecção do início da pandemia.

Historicamente sabe-se que a busca pela identificação do agente causador da AIDS foi marcada pela disputa entre dois grupos de pesquisa: um americano, coordenado por Robert Gallo, e um grupo francês do Instituto Pasteur de Paris, liderado por Luc Montagnier. Após um intenso período de disputas (Gallo, 1994), o mérito pela descoberta do HIV como causador da AIDS foi compartilhado entre os dois grupos de pesquisa (Gallo e Montagnier, 2003).

A transmissão do HIV ocorre através das seguintes formas: parenteral (transfusão de sangue, compartilhamento de agulhas contaminadas ou acidentes com materiais perfuro-cortantes contaminados); via sexual (sexo anal, vaginal e oral); transmissão vertical (da mãe para o filho, durante a gravidez, no momento do parto ou através do aleitamento) (Patel et al., 2014). Porém, é importante destacar que o risco de infecção varia conforme o tipo de exposição ao HIV. Por exemplo, o risco de infecção através do sexo anal é alto, já o sexo oral configura um risco baixo (Patel et al., 2014). Além disso, apesar da transmissão do HIV através da transfusão de sangue poder ser uma realidade em alguns países (Moore et al., 2001), sabe-se que a testagem e triagem das bolsas de sangue em hemocentros representam estratégias efetivas para evitar a transmissão do HIV através dessa rota.

Estima-se que 36,9 [31,1 - 43,9] milhões de pessoas estavam infectadas pelo HIV no mundo todo no ano de 2017 (UNAIDS, 2018). No Brasil, foram notificados 194.217

casos de infecção pelo HIV entre o período de 2007 e 2017 (Rio Grande do Sul, 2018). O Rio Grande do Sul apresenta o pior cenário epidemiológico de todo o País, contabilizando o maior número de casos de detecção de gestantes com HIV (~10/1.000 nascidos vivos, conforme dados do ano de 2015). Entre as cidades brasileiras, Porto Alegre apresentou a maior taxa de detecção de HIV em 2015: 22,9 casos/1.000 nascidos vivos (Brasil, 2017a). Dados mais recentes apontam uma redução nos números referentes ao cenário estadual, mas o Rio Grande do Sul ainda ocupa a primeira posição na taxa de detecção de gestantes com HIV no País (Rio Grande do Sul, 2018).

As principais células-alvo do HIV são os leucócitos T $CD4^+$ que expressam os co-receptores CCR5 ou CXCR4. Após a interação do HIV com o receptor CD4 e um dos co-receptores (geralmente o CCR5), ocorre a fusão vírus-célula e a penetração do genoma e de proteínas virais no citoplasma. Uma vez que o HIV é um retrovírus, a transcriptase reversa viral transforma o material genético do tipo RNA em DNA, que então é integrado ao genoma da célula hospedeira. Quando integrado ao genoma do hospedeiro, o HIV assume a característica de pró-virus e pode permanecer latente por muitos anos. Quando sai da latência, as proteínas codificadas na sequência do pró-virus são transcritas pela maquinaria de transcrição do hospedeiro. Após, as proteínas virais recém transcritas e traduzidas podem formar novas partículas virais viáveis e infectar novas células (Maartens et al., 2014).

A infecção pelo HIV caracteriza-se por um período agudo, no qual o indivíduo pode ou não apresentar sintomas. Quando presentes, os sintomas caracterizam-se por febre, dores do corpo, suores noturnos, entre outros (Cohen et al., 2010; Maartens et al., 2014). Esses sintomas são inespecíficos e a infecção aguda pelo HIV pode não ser identificada porque é muito similar a outras doenças comuns, como um resfriado, por exemplo. Após a infecção aguda, ocorre um período de latência, no qual o HIV replica-se no hospedeiro sem causar sinais ou sintomas. Este período assintomático dura em torno de sete anos (Maartens et al., 2014). Porém, quando contínua em decorrência da ausência de tratamento, a replicação viral ocasiona morte das células do sistema imunológico, acarretando um quadro progressivo de imunodeficiência. Quando este quadro compromete as funções imunológicas básicas, como a defesa do organismo contra patógenos oportunistas, a AIDS é diagnosticada (Maartens et al., 2014).

Uma pequena parcela (<1%) dos indivíduos infectados pelo HIV mantém a carga viral em níveis indetectáveis, mesmo sem tratamento farmacológico. Esses indivíduos são conhecidos como “controladores de elite”. Acredita-se que fatores imunogenéticos do hospedeiro estejam envolvidos nessa capacidade de controle da infecção, compreendendo variantes de genes *HLA* (*human leukocyte antigens*), sub-populações de células T $CD8^+$ e variantes dos genes *KIR*. Porém, os exatos fatores que fazem de um indivíduo um controlador de elite não são completamente entendidos (Deeks et al., 2015). Indivíduos denominados “não progressores de longo termo” (do inglês, *long-term non-progressors*) mantêm contagens altas de células T $CD4^+$ e $CD8^+$ sem o uso de terapia farmacológica, apresentando carga viral detectável e por tempo superior a dez anos (Kumar, 2013; Valverde-Villegas et al., 2015). Existem também indivíduos HIV+ que apresentam um curso de infecção mais rápido do que o observado na maioria dos pacientes. São os chamados “progressores rápidos” (de Medeiros et al., 2016). Além de fatores virais, características genéticas e imunológicas do hospedeiro são também responsáveis por esses perfis diferenciados de progressão da infecção pelo HIV (Kumar, 2013; Valverde-Villegas et al., 2015; Jacobs et al., 2017).

A detecção da infecção pelo HIV pode ser realizada através de testes sorológicos, moleculares e virológicos (Parekh et al., 2019). Os testes rápidos representam uma ferramenta bastante útil para a testagem de um grande número de amostras, podendo ser aplicados fora do ambiente laboratorial. Caso um teste rápido resulte em um resultado “positivo”, testes confirmatórios serão realizados. O Ministério da Saúde disponibiliza de forma detalhada as recomendações e os fluxogramas que devem ser aplicados para a testagem de HIV no Brasil (Brasil, 2013). Os programas de testagem da população para a infecção pelo HIV reduziram o número de pacientes diagnosticados com quadros de AIDS. Atualmente, é comum uma pessoa descobrir que é portadora do HIV antes de manifestar os primeiros sintomas da AIDS (Brasil, 2018a). Entretanto, estima-se que em torno de 500 mil pessoas de países da América Latina e Caribe vivam com o HIV sem saber (OPAS, 2018).

O tratamento da infecção pelo HIV é feito através do uso de uma combinação de fármacos artirretrovirais (ARVs). Quando o tratamento é feito de forma correta, a carga viral se torna indetectável pelos métodos tradicionais de testagem e o indivíduo passa a usufruir de uma vida muito similar a de um indivíduo não portador do vírus. Considerando

o cenário atual de tratamento, a infecção pelo HIV já pode ser considerada uma doença crônica, apesar dos indivíduos HIV+ sofrerem de problemas inerentes ao tratamento e aos estresses fisiológicos causados pela infecção crônica às células do sistema imune e a diferentes órgãos, sendo a inflamação crônica um dos principais problemas (Deeks et al., 2013a; Deeks et al., 2013b). Um longo caminho foi percorrido antes de um tratamento efetivo contra o HIV estar disponível. Até o ano de 1986 não se conhecia tratamento efetivo. A monoterapia foi introduzida em 1978 e usada até 1991. Entre 1992 e 1995 foi utilizada a terapia dupla, e entre 1996 e 2002 a terapia antirretroviral altamente ativa (HAART, *highly active antiretroviral therapy*) foi introduzida. As terapias de resgate surgiram entre os anos 2003 e 2007 (Scheffer, 2012).

Existe uma gama muito grande de ARVs que atuam sobre diferentes estágios de replicação do HIV. Os tipos mais comuns de ARV são: inibidores de fusão, inibidores dos co-receptores (CCR5 ou CXCR4), inibidores de ligação vírus-célula, inibidores de protease (IP), inibidores de maturação, inibidores da transcriptase reversa não análogos de nucleosídeos (ITRNN), inibidores da transcriptase reversa análogos de nucleosídeos (ITRN) e inibidores da integrase (INI) (Deeks et al., 2015). Entre 1987 e 2012, mais de trinta ARVs foram aprovados pela FDA (Scheffer, 2012). O esquema terapêutico preferencial recomendado pelo Ministério da Saúde é composto por dois ITRN associados à outra classe de antirretrovirais [ITRNN, IP/r (inibidor de protease com reforço de ritonavir) ou INI] (Brasil, 2018b).

A partir de 2008 surgiram evidências de que os ARVs poderiam ser usados em estratégias de prevenção contra o HIV (Scheffer, 2012), o que hoje é uma realidade (Riddell et al., 2018). No Brasil, o Sistema Único de Saúde (SUS) já disponibiliza a PrEP (*pre-exposure prophylaxis* ou profilaxia pré-exposição). A PrEP consiste na tomada diária de um ARV por um indivíduo HIV negativo, mas que apresenta comportamento de risco para a infecção. A presença do ARV no organismo do indivíduo protege de forma efetiva contra a infecção pelo HIV em caso de exposição, por isso é considerada uma ação preventiva (Brasil, 2018c; Riddell et al., 2018). A PrEP é um dos componentes da estratégia brasileira de prevenção combinada contra o HIV, da qual também fazem parte: testagem para o HIV; uso regular de preservativos; diagnóstico oportuno e tratamento adequado de infecções sexualmente transmissíveis; redução de danos; gerenciamento de

vulnerabilidades; supressão da replicação viral pelo tratamento antirretroviral; imunizações (Brasil, 2018c).

Outro esquema também disponível pelo SUS é a PEP (*post-exposure prophylaxis* ou profilaxia pós-exposição), que consiste no uso de ARVs por indivíduos expostos ou potencialmente expostos ao HIV, seja em acidentes de trabalho ou através do sexo sem o uso de preservativos. Quanto mais cedo iniciada após o evento de potencial exposição ao vírus, mais efetiva é a PEP contra a infecção pelo HIV (Brasil, 2016).

Apesar dos avanços no tratamento e prevenção do HIV serem inquestionáveis, muitos desafios ainda existem. Mesmo quando a replicação do HIV é suprimida a níveis indetectáveis, o vírus permanece latente em algumas células ou locais microanatômicos muito limitados. Essas células/locais são conhecidas como "reservatórios virais" e representam um desafio para o efetivo tratamento da infecção pelo HIV. Atualmente não há qualquer terapia que elimine por completo o HIV dos reservatórios virais. Por esse motivo, caso a ART seja cessada, o HIV volta a se replicar nas células do indivíduo infectado (Blankson et al., 2002).

Além dos amplamente debatidos problemas e estigmas sociais enfrentados pelos portadores do HIV (Mahajan et al., 2008; Kose et al., 2012; Rueda et al., 2016), existe uma série de problemas fisiológicos relacionados à infecção crônica, incluindo a manutenção do vírus nos reservatórios virais, a inflamação crônica e a ativação imune, mesmo quando os indivíduos estão sob ART. Sabe-se que esse quadro acarreta problemas em diferentes órgãos, fazendo com que indivíduos infectados pelo HIV sofram precocemente de problemas de saúde tipicamente enfrentados apenas em idades mais avançadas (Deeks, 2011; Deeks et al., 2013a; Deeks et al., 2013b).

Iniciar o tratamento o mais cedo possível após a detecção da infecção pelo HIV limita o número e o tamanho dos reservatórios virais, diminui o risco de novas infecções e reduz o quadro de inflamação crônica (Deeks et al., 2015). Em decorrência desses efeitos, desde 2013 o Ministério da Saúde recomenda o início da ART imediatamente após o diagnóstico da infecção pelo HIV, independentemente da contagem de células T $CD4^+$ do paciente (Rio Grande do Sul, 2018).

3.2. Hepatites virais: HCV e HBV

3.2.1. HCV

O HCV pertencente ao gênero *Hepacivirus* e à família *Flaviviridae*. É um vírus envelopado e apresenta material genético do tipo RNA com aproximadamente 9.600 nucleotídeos. A partícula viral é formada por três proteínas estruturais (Core, E1 e E2) e sete proteínas envolvidas na replicação viral (p7, NS2, NS3, NS4A, NS4B, NS5A e NS5B). Existem sete genótipos de HCV, sendo o HCV-1, HCV-2, HCV-3, HCV-4, HCV-5 e HCV-6 os que apresentam maior importância epidemiológica. O HCV-7 circula apenas em regiões específicas do continente africano (Simmonds et al., 2005; Murphy et al., 2015; Kim et al., 2016).

Populações do mundo inteiro enfrentam problemas de saúde causados pelo HCV. Dessa forma, a infecção por este vírus é considerada um problema de saúde global (Global Burden of Hepatitis C Working Group, 2004). O número de pessoas infectadas cronicamente pelo HCV no mundo está entre 64 e 103 milhões (Manns et al., 2017). O número de casos de infecção pelo HCV no território nacional também é muito alto. A prevalência de soropositivos para HCV no Brasil, considerando adultos e adolescentes, é de 1,38% (Pereira et al., 2013). Apesar do número de casos de infecções no Brasil estar diminuindo, os casos de doenças hepáticas em decorrência do HCV estão aumentando (Ferreira et al., 2015). Uma possível explicação para este fenômeno é o aumento da expectativa de vida nos pacientes infectados: morre-se menos em decorrência da infecção, porém o número de doenças causadas pelo patógeno aumenta ao longo da vida do paciente. Da mesma forma que o Rio Grande do Sul enfrenta um sério problema em relação à infecção pelo HIV, a situação epidemiológica da infecção pelo HCV no Estado também é alarmante. Dados de 2016 indicaram que a maior taxa de incidência de infecção pelo HCV no Brasil é a registrada no Rio Grande do Sul (Brasil, 2017b).

As principais vias pelas quais um indivíduo pode se infectar pelo HCV são a sexual, percutânea e perinatal. Ou seja, as formas de infecção pelo HCV são bastante similares àquelas relacionadas à infecção pelo HIV (Shepard et al., 2005). As maneiras pelas quais a infecção pelo HCV acontece, assim como os fatores de risco para a infecção, podem variar conforme as condições sociais, políticas e econômicas de cada país. Por

exemplo, apesar do Brasil contar com um sistema de testagem nos bancos de sangue que previne a infecção pelo HCV através de transfusão sanguínea, este problema ainda é uma realidade em alguns países em desenvolvimento. Em países desenvolvidos, o uso de drogas injetáveis é considerado um importante facilitador da infecção (Shepard et al., 2005). Acredita-se que o HCV seja em torno de 10 vezes mais infeccioso do que o HIV em situações de exposição percutânea a sangue contaminado (Gerberding, 1994; Coutinho, 1998; Budd e Robertson, 2005). Essa informação ajuda a explicar por que o compartilhamento de aparatos para uso de drogas injetáveis é um fator de risco tão importante para a infecção pelo HCV.

O HCV possui alto tropismo pelas células hepáticas, por isso a maior parte dos problemas relacionados à infecção ocorre no fígado (Ding et al., 2014). Porém, o curso clínico da infecção é variado e os problemas hepáticos também variam de pessoa para pessoa. Quando a infecção pelo HCV ocorre, o organismo do indivíduo infectado pode eliminar naturalmente o vírus. Em torno de 15 a 45% dos indivíduos infectados eliminam o HCV em até seis meses após a infecção ter acontecido (Lingala e Ghany, 2015). Porém, aqueles que não o eliminam podem desenvolver hepatite C crônica e sofrer com os problemas causados por essa condição (Lingala e Ghany, 2015; Ahmad, 2017). Tais problemas são variados, sendo os mais comuns a fibrose hepática, cirrose hepática e carcinoma hepatocelular (CHC). Entre os pacientes com hepatite C crônica, 20 a 30% desenvolvem cirrose e 1 a 4% desenvolvem CHC (Lingala e Ghany, 2015). É importante destacar que nem sempre a infecção crônica progride para o CHC (Mitchell et al., 2015).

A coinfecção HCV/HIV é um problema comum (Maier e Wu, 2002; Sethi e Sterling, 2006), que ocorre em um terço dos indivíduos infectados pelo HIV (Hernandez e Sherman, 2011). Entre usuários de drogas injetáveis, a situação é ainda mais problemática, sendo observada em 90-95% desses indivíduos (Maier e Wu, 2002). A coinfecção é uma condição bastante preocupante, pois a dupla infecção intensifica os problemas de saúde causados por cada um dos vírus. Por exemplo, a infecção pelo HIV faz com que a progressão da infecção pelo HCV seja mais rápida e o risco de morte seja mais elevado do que aquele observado em pacientes monoinfectados (Maier e Wu, 2002; Operskalski e Kovacs, 2011). Outro aspecto da coinfecção refere-se aos danos causados pelo tratamento, uma vez que a terapia antirretroviral apresenta hepatotoxicidade, agravando o quadro do

paciente coinfectado. Porém, os benefícios do tratamento geralmente superam os riscos da hepatotoxicidade (Rockstroh, 2005; Rockstroh et al., 2005).

O diagnóstico da infecção pelo HCV é feito através de testes sorológicos ou detecção do RNA viral, dependendo do estágio da infecção (Irshad et al., 2013; Ahmad, 2017). Além da detecção direta ou indireta do patógeno, exames que avaliam a função hepática também são geralmente realizados (Ahmad, 2017). Estes exames são importantes para determinar o estado clínico do paciente e o estágio da infecção.

O objetivo do tratamento da infecção pelo HCV é eliminar o vírus do organismo. Apesar dos tratamentos baseados em interferon terem sido muito comuns, atualmente o uso dos medicamentos antivirais fazem parte dos tratamentos mais recomendados. Os antivirais usados para tratar a infecção pelo HCV são conhecidos como antivirais de ação direta (DAAs, do inglês *direct-acting antiviral agents*) (Ahmad, 2017; Manns et al., 2017). Tais medicamentos interferem em diferentes etapas do ciclo viral e, quando usados em combinação (dois ou três), podem promover a cura da infecção em mais de 90% dos pacientes tratados (Manns et al., 2017). Além do uso de antivirais, o tratamento da infecção também visa a prevenção ou interrupção dos problemas hepáticos causados pelo HCV (Ahmad, 2017).

Características virais, fatores do hospedeiro e componentes do ambiente são os aspectos que mais impactam a infecção pelo HCV, considerando a suscetibilidade e progressão da doença (Lingala e Ghany, 2015). As altas taxas de mutação do HCV afetam principalmente o tratamento, devido ao surgimento de mutações de resistência (Kliemann et al., 2016a; Kliemann et al., 2016b). Genes do sistema imune e suas variantes estão entre os principais componentes do hospedeiro atuantes na modulação de suscetibilidade à infecção pelo HCV, coinfecção HCV/HIV e progressão das doenças hepáticas (Yee, 2004; Chapman e Hill, 2012; da Silva et al., 2014; Valverde-Villegas et al., 2017a).

Os agravos causados pelo HCV vão além dos problemas de saúde. Quando um indivíduo é portador do vírus, uma série de problemas sociais o acompanha. O status de "HCV+" impacta de diferentes formas a dinâmica de relacionamentos pessoais. Além disso, os problemas de saúde em decorrência da infecção geram gastos com políticas de saúde pública destinadas à prevenção de novas infecções e tratamento das doenças hepáticas. Dependendo do estado de saúde, os indivíduos infectados também deixam de trabalhar e produzir adequadamente. Este cenário gera graves problemas econômicos

(Leigh et al., 2001; Brown e Gaglio, 2003). Dessa forma, assim como a maioria das outras doenças infecciosas, a infecção pelo HCV é também um problema econômico e, principalmente, social. Por isso, novamente a abordagem *One Health* se torna essencial para o combate e estudo desta infecção.

3.2.2. HBV

O HBV que infecta humanos pertence ao gênero *Orthohepadnavirus* e à família *Hepadnaviridae* (Schaefer, 2007), é um vírus envelopado, com genoma de DNA e apresenta dez genótipos (nomeados de A até J). Seu material genético é circular e parcialmente de dupla fita, com aproximadamente 3,2 kb (Yuen et al., 2018). Sete proteínas são codificadas pelo genoma do HBV: HBx, core, polimerase, L-HBsAg, M-HBsAg, S-HBsAg e precore/HBeAg (Lamontagne et al., 2016). Assim como o HCV, o HBV tem tropismo principalmente por hepatócitos e causa infecção aguda e crônica em humanos (Yuen et al., 2018). O *sodium taurocholate cotransporting polypeptide* (NTCP) é o receptor utilizado pelo vírus para penetrar nas células do hospedeiro (Yan et al., 2012; Li, 2015).

Quando um indivíduo é infectado pelo HBV, três desfechos são possíveis: I, infecção aguda (tipicamente assintomática, sendo que geralmente o organismo elimina o vírus em até seis meses); II, infecção oculta (o organismo não elimina o vírus, que permanece no organismo em baixos níveis, sendo difícil de ser detectado através de testes sorológicos); III, infecção crônica (o organismo não consegue eliminar o vírus, permanecendo cronicamente infectado) (Lamontagne et al., 2016). O desfecho da infecção depende de fatores virais e da capacidade do sistema imune de cada organismo manejar a infecção (Seeger e Mason, 2015; Yuen et al., 2018). Entre os fatores do hospedeiro, os polimorfismos genéticos em genes do sistema imune têm destacada influência no curso da infecção pelo HBV (Moudi et al., 2016).

Apenas uma pequena parcela (5-10%) dos indivíduos infectados desenvolve infecção crônica (Liang, 2009; Liaw e Chu, 2009; Moudi et al., 2016). Porém, este tipo de infecção é uma situação preocupante, uma vez que pode causar inflamação, fibrose, cirrose e CHC (Yuen et al., 2018). Entre os portadores crônicos do vírus, 15-40% sofrem com cirrose hepática (Tang et al., 2018) e 25-40% desenvolvem CHC (Yuen et al., 2018). Os

mecanismos pelos quais o HBV provoca CHC ainda não são completamente compreendidos (Seeger e Mason, 2015).

Em torno de 290 milhões de pessoas estão infectadas pelo HBV no mundo (Polaris Observatory Collaborators, 2018). A América Latina está entre as regiões globais com as menores taxas de infecção crônica (Yuen et al., 2018). O Brasil contabilizou 218.257 casos confirmados de hepatite B entre os anos de 1999 e 2017, sendo que o maior número de casos é encontrado nas regiões Sul e Sudeste (Brasil, 2018d).

A principal forma de diagnóstico da infecção pelo HBV é o teste sorológico, que visa a detecção de HBsAg no soro. Além dos exames sorológicos, testes moleculares também podem ser empregados (Yuen et al., 2018). Destaca-se que dependendo dos tipos de anticorpos detectados no soro, bem como da presença ou ausência do DNA viral, é possível verificar se um indivíduo possui infecção ativa, foi infectado no passado e eliminou o vírus ou, ainda, se está imunizado contra o vírus (Tang et al., 2018). Exames com o objetivo de indicar a saúde hepática também são geralmente realizados nos pacientes infectados (Yuen et al., 2018).

O tratamento da infecção pelo HBV é feito com o uso de antivirais (lamivudina, adefovir, entecavir, entre outros). Além dos antivirais, o uso de imunomoduladores como o interferon pode ser necessário (Tang et al., 2018; Yuen et al., 2018). O tratamento não promove a completa eliminação do vírus do organismo (cura da infecção), porém a cura funcional, caracterizada pela eliminação do HBsAg, pode ser atingida em uma parcela dos pacientes tratados (Yuen et al., 2018).

Em torno de 10% dos indivíduos HIV+ são portadores do HBV (Yuen et al., 2018). O HIV pode acelerar a patogênese do HBV, sendo que os indivíduos coinfectados apresentam um maior risco de morte do que indivíduos que portam apenas um dos vírus (Kourtis et al., 2012; Singh et al., 2017). Ou seja, da mesma forma que a coinfecção HIV/HCV, a coinfecção HIV/HBV pode ser considerada uma situação preocupante.

A transmissão do HBV acontece através da exposição a sangue ou fluidos contaminados, como sêmen e fluido vaginal (MacLachlan e Cowie, 2015). Essas diferentes vias de transmissão facilitam a disseminação do HBV entre a população humana. Além disso, outros fatores virais são favoráveis à disseminação do HBV, tais como: capacidade de provocar infecção persistente e assintomática, alta resistência fora do organismo humano e longo período de incubação. Esses fatores conferem vantagens adaptativas ao

HBV, facilitando sua disseminação entre as populações humanas de diferentes partes do mundo (Araujo et al., 2011). A principal forma de prevenção da infecção pelo HBV é a vacinação, que é eficaz contra os dez genótipos virais (Yuen et al., 2018). A vacina contra o vírus é fornecida no Brasil gratuitamente pelo SUS (Brasil, 2018e). O HBV é menos variado em termos genótipos e subtipos do que o HCV e HIV, por exemplo. Por esse motivo, o desenvolvimento da vacina contra o HBV foi possível enquanto que ainda não há vacinas para o HCV e HIV (Steckelberg, 2017).

3.3. Vírus selvagens negligenciados: Sabiá e Rocio

Além dos patógenos amplamente conhecidos pela comunidade científica, o Brasil abriga um grupo de vírus que, apesar de já descritos na literatura, são pouco estudados e, por isso, o conhecimento sobre seus aspectos biológicos e potencial patogênico é escasso. Esses patógenos são os vírus exóticos, podendo também ser chamados de selvagens ou negligenciados.

Apesar do termo "vírus exótico" ser tradicionalmente empregado para denominar patógenos importados ou que recentemente emergiram de outros países (Dowdle, 1980), esse termo também pode ser usado para se referir aos patógenos encontrados no território nacional, mas sobre os quais pouco se conhece. Isso se justifica porque o termo "exótico" também pode significar "excêntrico" ou "estranho". Além disso, é importante mencionar que os vírus causadores de febres hemorrágicas também podem ser denominados "exóticos" (Geisbert e Jahrling, 2004). Alternativamente, tais vírus podem ser chamados de "selvagens" ou "de campo", pois, quando estudadas, as cepas sob análise são aquelas encontradas circulando na natureza (Moraes e Jaramillo, 2007). Por fim, esses patógenos podem ainda entrar na categoria dos vírus "negligenciados", pois despertam pouca atenção da comunidade científica e médica. Conforme mencionado anteriormente, as doenças negligenciadas também estão englobadas na categoria de emergentes e reemergentes (De Souza, 2010).

Dois exemplos de vírus brasileiros selvagens e negligenciados são o Sabiá (SABV) e o Rocio (ROCV). O SABV é um vírus envelopado, com genoma de RNA e pertencente à família *Arenaviridae* (Coimbra et al., 1994; Gonzalez et al., 1996; Buchmeier et al., 2007). Quatro casos de infecção pelo SABV foram registrados até hoje, sendo dois casos fatais

registrados no estado de São Paulo (devido à exposição natural ao vírus) e dois casos não fatais adquiridos em ambiente laboratorial, um no Brasil e outro nos EUA (Vasconcelos et al., 1993; CDC, 1994; Coimbra et al., 1994; Coimbra et al., 2001). O SABV pode causar um quadro de febre hemorrágica severa em humanos, com alto potencial de letalidade (Cardoso e Navarro, 2007). A doença causada pelo SABV é denominada de *Brazilian hemorrhagic fever* (febre hemorrágica brasileira) (CDC, 2013). Apesar do modo de transmissão do SABV ser desconhecido, acredita-se que seja através de aerossóis contendo partículas virais (Barry et al., 1995). Os estudos envolvendo o SABV devem ser realizados em laboratórios BSL4 (Chosewood e Wilson, 2009), sendo que o CDC coloca o SABV entre os 67 agentes da *Select Agents and Toxins List*. Sobre a lista, o CDC declara: "*The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant product*" (CDC, 2018d).

Já o ROCV é um *Flavivirus* (Figueiredo, 2000) transmitido por mosquitos (Lopes et al., 1981; Mitchell e Forattini, 1984; Mitchell et al., 1986; Laporta et al., 2012). Esse vírus foi responsável por um surto de encefalite ocorrido na região do Vale do Ribeira e da Baixada Santista, no sudeste Brasileiro, entre os anos de 1975 e 1980 (Iversson et al., 1989; Figueiredo, 2000). O surto causou ~100 mortes e deixou mais de 200 pessoas com sequelas (Figueiredo, 2000). Trabalhos avaliando amostras de cavalos do território brasileiro não encontraram anticorpos anti-ROCV nesses animais (Pauvolid-Corrêa et al., 2011; Silva et al., 2013). Porém, outros trabalhos indicam a circulação do ROCV em animais selvagens (Lopes et al., 1978; Mitchell e Forattini, 1984; Figueiredo, 2007; de Barros et al., 2011, Pauvolid-Corrêa et al., 2014; Silva et al., 2014), fazendo com que a ocorrência de novos surtos de encefalite por ROCV na população Brasileira seja possível. Mesmo frente à constante ameaça à saúde pública configurada pela potencial reemergência do ROCV, são escassos os estudos com foco nesse vírus.

Apesar do último caso conhecido de infecção pelo SABV ter ocorrido em 1999 (Coimbra et al., 2001) e o surto causado pelo ROCV ter acabado em 1980 (Figueiredo, 2000), os estudos sobre esses patógenos devem ser estimulados. Especificamente no caso do SABV, deve-se confirmar seus modos de transmissão e localizar as espécies que atuam como hospedeiros/reservatórios desse patógeno. Já em relação ao ROCV, a vigilância em relação à circulação desse vírus em espécies selvagens e vetores é importante para detectar

precocemente um potencial novo surto de encefalite por ROCV. De forma geral, reunir as informações disponíveis sobre o SABV e o ROCV e estudar os aspectos biológicos e patogênicos dos mesmos é essencial para combater de forma rápida e efetiva esses vírus caso eles voltem a infectar humanos.

4. Imunogenética e doenças infecciosas

4.1. Fatores imunológicos: foco nos exossomos

A patogênese e o curso clínico das doenças infecciosas clássicas estão descritos nos livros-texto de medicina, veterinária e biologia. Entretanto, é muito comum que diferentes indivíduos mostrem padrões diferentes de suscetibilidade a determinadas infecções, respondam de forma variada aos tratamentos disponíveis, bem como apresentem particularidades na progressão das doenças. Além dos fatores relacionados aos patógenos e ao ambiente, já discutidos anteriormente, fatores imunológicos do hospedeiro apresentam grande influência na suscetibilidade e progressão das doenças infecciosas. Tais fatores estão englobados no componente humano da *One Health*.

Vários trabalhos evidenciaram o papel de citocinas, quimiocinas e sub-populações celulares na progressão diferenciada da infecção pelo HIV, por exemplo (Ferre et al., 2009; Owen et al., 2010; Yan et al., 2013; Valverde-Villegas et al., 2015; de Medeiros et al., 2016; Platten et al., 2016; Jacobs et al., 2017; Gutiérrez-Rivas et al., 2018). Porém, recentemente, o papel das microvesículas celulares na infecção pelo HIV e outras doenças infecciosas tem sido descrito de forma crescente. Existe uma ampla variedade de microvesículas celulares (Raposo e Stoorvogel, 2013). Entre elas, os exossomos estão entre as mais estudadas (Lawson et al., 2016) e são considerados importantes estruturas moduladoras das interações do tipo patógeno-hospedeiro (Schorey et al., 2015).

Exossomos são vesículas de aproximadamente 30-100 nm liberadas por diferentes células no meio extracelular (Mincheva-Nilsson e Baranov, 2010). Morfologicamente, apresentam estrutura esférica, embora em fotomicrografias feitas com microscópio eletrônico apareçam com formato similar ao de hemácias humanas (Mincheva-Nilsson e Baranov, 2010; Gennebäck et al., 2013). Os exossomos são formados em corpos

multivesiculares no citoplasma das células e liberados a partir de evaginações da membrana plasmática (Mincheva-Nilsson e Baranov, 2010; Raposo e Stoorvogel, 2013).

Diferentes moléculas podem ser encontradas nos exossomos: proteínas, lipídeos, DNA, RNA, microRNAs, entre outras (Madison e Okeoma, 2015; Jia et al., 2017). A principal função biológica atribuída aos exossomos é o transporte de tais moléculas de forma estável e por longas distâncias, permitindo a comunicação entre células e tecidos de diferentes locais do organismo (Robbins e Morelli, 2014; de la Torre Gomez et al., 2018).

A relação entre exossomos e as doenças infecciosas emergiu de forma mais intensa quando a teoria do exossomo troiano foi publicada em 2003 (Gould et al., 2003). Tal hipótese defende que processos evolutivos forneceram ao HIV e a outros retrovírus a capacidade de usurpar a maquinaria de brotamento e transporte de exossomos para infectar novas células "escondidos" do sistema imune. Ou seja, os exossomos poderiam ser usados pelos vírus como uma ferramenta de evasão do sistema imune (Gould et al., 2003). Posteriormente, o papel dos exossomos em diferentes infecções começou a ser evidenciado de forma mais robusta. Há indícios de que os exossomos influenciem o curso das infecções por diferentes vírus, incluindo citomegalovirus, vírus Epstein-Barr, vírus da hepatite A, vírus do papiloma humano, vírus linfotrópico da célula T humana, herpesvirus, vírus da família *Bunyaviridae* (Anderson et al., 2016; Raab-Traub e Dittmer, 2017), vírus da dengue (Vora et al., 2018), vírus da encefalite transmitido por carrapatos (TBEV, *Tick-borne encephalitis virus*) (Zhou et al., 2018) e vírus Ebola (Pleet et al., 2016). Basicamente, o papel dos exossomos nas interações patógeno-hospedeiro pode acontecer das seguintes formas: através do transporte de moléculas derivadas de patógenos; transporte dos patógenos no interior dos exossomos; ou através da modulação do sistema imune (Zhang et al., 2018).

Por fim, é interessante mencionar que o uso dos exossomos como ferramentas para o transporte altamente regulado de biomoléculas e fármacos já começou a ser explorado (Ha et al., 2016; Jiang e Gao, 2017; Kaminski et al., 2017; Luan et al., 2017; Bunggulawa et al., 2018). É possível que, em breve, exossomos sejam usados para carrear fármacos ou moléculas imunoreguladoras (como os microRNAs) em direção a células-alvo de forma direcionada e regulada. Também há evidências indicando que os exossomos podem ser utilizados como biomarcadores clínicos de infecções virais (Zhang et al., 2018).

A era das pesquisas envolvendo exossomos e outras microvesículas celulares está apenas iniciando, mas é promissora. Entender os diferentes papeis dos exossomos nas infecções virais pode indicar novos alvos terapêuticos, potencialmente resultando em avanços no tratamento dessas doenças.

4.2. Fatores genéticos

Da mesma forma que fatores imunológicos *per se* impactam diferentes aspectos das doenças infecciosas, componentes genéticos individuais do hospedeiro são importantes moduladores da suscetibilidade às infecções e progressão diferenciada dessas doenças. A Tabela 1 apresenta alguns exemplos de efeitos de polimorfismos genéticos humanos sobre diferentes infecções.

Tabela 1. Exemplos de polimorfismos genéticos que influenciam diferentes aspectos de doenças infecciosas.

Patógeno ou doença	**Fenótipo ou efeito**	**População**	**Polimorfismo**	**Gene**
HIV/AIDS	Carga viral	Europeia	rs9264942	*HLA-C*
		Europeia	rs2395029	*HLA-B*, *HCP5*
		Africano-Americana	rs2523608	*HLA-B*
	Controle do HIV-1	Europeia	rs9264942	*HLA-C*
		Europeia	rs4418214	*MICA*
		Europeia	rs2395029	*HLA-B*, *HCP5*
		Europeia	rs3131018	*PSORS1C3*
		Africano-Americana	rs2523608	*HLA-B*
		Africano-Americana	rs2255221	Intergênico
		Africano-Americana	rs2523590	*HLA-B*
		Africano-Americana	rs9262632	Intergênico
	Progressão da infecção	Europeia	rs9261174	*ZNRD1*, *RNF39*
		Europeu-Americana	rs11884476	*PARD3B*
		Europeia	rs2395029	*HLA-B*, *HCP5*
		Europeia	rs2234358	*CXCR6*
HCV/hepatite C	Eliminação do vírus	Europeia	rs8099917	*IL28B*
HBV/hepatite B	Infecção crônica	Asiática	rs3077	*HLA-DPA1*
		Asiática	rs9277535	*HLA-DPB1*
Dengue	Síndrome de choque	Asiática	rs3132468	*MICB*
		Asiática	rs3765524	*PLCE1*
Malária severa	Suscetibilidade	Africana	rs11036238	*HBB*
Tuberculose	Suscetibilidade	Africana	rs4334126	18q11.2 (*GATA6*, *CTAGE1*, *RBBP8*, *CABLES1*)
Hanseníase	Suscetibilidade	Asiática	rs3764147	*LACC1*
		Asiática	rs9302752	*NOD2*
		Asiática	rs3088362	*CCDC122*
		Asiática	rs602875	*HLA-DR-DQ*
		Asiática	rs6478108	*TNFSF15*
		Asiática	rs42490	*RIPK2*
Doença meningocócica	Proteção	Europeia	rs1065489	*CFH*
		Europeia	rs426736	*CFHR3*
Doença de Creutzfeldt-Jakob	Suscetibilidade	Europeia, Papua Nova Guiné	rs1799990	*PRNP*

Fonte: Adaptada de Chapman e Hill (2012).

Porém, não apenas os polimorfismos que modificam de forma direta a expressão de proteínas através de alteração da sequência gênica têm efeitos relevantes sobre as doenças causadas por patógenos. Estão cada vez mais evidentes os efeitos de variantes genéticas em processos envolvendo moléculas reguladoras da expressão gênica, como os microRNAs, sobre as infecções pelo HIV, HBV, HCV e HPV (Kulkarni et al., 2011; Bae et al., 2012; Blais et al., 2012; Cheong et al., 2013; Liu et al., 2013; Song et al., 2013; Peckham-Gregory et al., 2016; Al-Qahtani et al., 2017; Sajjad et al., 2017; Tian et al., 2017).

Os patógenos sempre foram uma ameaça à saúde humana. Como consequência, diversas forças evolutivas moldaram o genoma humano com características protetivas contra as infecções. Pode-se dizer que o genoma da espécie humana foi moldado pelos patógenos que interagiram com as diferentes populações (Barreiro e Quintana-Murci, 2010; Bañlus et al., 2013; Karlsson et al., 2014). Tais interações resultaram na seleção de genes especializados na resposta imune contra infecções. Um exemplo clássico são os genes *TLRs*, que codificam receptores de membrana e intracelulares específicos para o reconhecimento de patógenos (Barreiro e Quintana-Murci, 2010).

A arquitetura genética da suscetibilidade às doenças infecciosas pode ser explicada de três formas: I, Variantes comuns: polimorfismos de alta frequência, identificáveis por estudos do tipo *genome-wide*. II, Variantes monogênicas raras: são polimorfismos de alta penetrância ou variantes evolutivamente jovens. III, Múltiplas/diversas variantes raras: representam múltiplas variantes de baixa penetrância que, em conjunto, explicariam a suscetibilidade às infecções (Hill, 2012). Porém, apesar de competirem em muitos aspectos, essas três formas de explicar a suscetibilidade genética às doenças infecciosas não são mutuamente excludentes. Da mesma forma que existem variantes monogênicas de alta penetrância que sozinhas modificam a suscetibilidade a determinado patógeno (Hill, 2012), a maior parte da suscetibilidade às infecções é ditada por múltiplos genes e variantes genéticas (Tibayrenc, 2007). Um exemplo de variante genética de alta penetrância é o CCR5Δ32 que, quando em homozigose, confere alta proteção contra a infecção pelo HIV (Liu et al., 1996; Dean et al., 1996). Este exemplo será discutido detalhadamente no tópico 4.3 desta tese.

É importante lembrar que as interações do tipo patógeno-hospedeiro estão em constante mudança à medida que os patógenos mutam e a população humana troca informação gênica. Dessa forma, as interações do tipo patógeno-hospedeiro devem ser

consideradas como evolutivamente dinâmicas e constantes (Barreiro e Quintana-Murci, 2010; Bañlus et al., 2013). Isso explica por que variantes genéticas que já foram vantajosas em determinado contexto histórico ou ecológico podem se tornar fatores de risco para doenças em outros momentos. Da mesma forma, a fixação de uma característica genética no genoma humano em decorrência de uma doença infecciosa que afligiu a humanidade no passado pode ser atualmente vantajosa em outros contextos (Karlsson et al., 2014). Novamente o CCR5Δ32 serve como um bom exemplo. Acredita-se que epidemias passadas, como a de Peste Negra na Europa, tenham ajudado a fixar esta variante no genoma humano (Galvani e Novembre, 2005), a qual atualmente é vantajosa em termos de proteção contra a infecção pelo HIV.

Além de facilitar a identificação de populações humanas que apresentam maior ou menor suscetibilidade às doenças infecciosas, o estudo de polimorfismos genéticos pode trazer importantes avanços para o tratamento de tais doenças. Conhecer o efeito de um polimorfismo sobre uma doença específica pode trazer *insights* para o desenvolvimento de novos medicamentos, vacinas e formas de prevenção. Avanços na terapia contra o HIV e formulação de uma vacina contra a malária foram impulsionados por estudos envolvendo polimorfismos genéticos (Hill, 2012).

Os estudos do tipo caso-controle são poderosas estratégias para a identificação de variantes genéticas que modificam a suscetibilidade às infecções (Hill, 2012). Por exemplo, utilizando estudos caso-controle, o grupo do Laboratório de Imunobiologia e Imunogenética da UFRGS já descreveu diversos efeitos estatisticamente significativos de variantes genéticas sobre distintos aspectos das infecções pelo HCV e HIV na população brasileira (da Silva et al., 2011; da Silva et al., 2014; Valverde-Villegas et al., 2017a; Valverde-Villegas et al., 2017b).

Além dos estudos tipo caso-controle, pesquisas envolvendo animais geneticamente modificados (por exemplo, camundongos nocauteados para algum gene) podem ajudar a elucidar o impacto de determinado gene/proteína sobre uma doença específica. Por fim, é importante ressaltar que os estudos que avaliam grandes números de variantes genéticas (*genome-wide studies*) são importantes ferramentas de exploração da influência de genes e polimorfismos sobre as doenças infecciosas (Frodsham e Hill, 2004).

Alguns genes específicos destacam-se no estudo das doenças infecciosas, sendo o *CCR5* um dos mais conhecidos em razão da sua relação com a infecção pelo HIV (Scurci

et al., 2018). Porém, a influência do *CCR5* vai além da infecção por este vírus. A seguir serão discutidos aspectos básicos deste gene, da proteína CCR5 e da variante CCR5Δ32 em diferentes contextos biológicos.

4.3. Quimiocinas, CCR5 e CCR5Δ32

As quimiocinas são citocinas quimiotáticas que medeiam a ativação e migração celular através da interação com receptores acoplados à proteína G, ou "receptores de quimiocinas" (Luster, 1998; Allen et al., 2007). É importante ressaltar que também existem receptores de quimiocinas atípicos, que atuam independentemente das proteínas G (Griffith et al., 2014). Existem quatro tipos básicos de receptores de quimiocinas: receptor de quimiocinas C, receptores de quimiocinas CC, receptores de quimiocinas CXC e receptores de quimiocinas CX3C (Allen et al., 2007; Turner et al., 2014). O "C" refer-se a uma cisteína e o "X" refere-se a um aminoácido não-cisteína (Allen et al., 2007). Basicamente, a migração celular mediada por quimiocinas ocorre através da quimiotaxia, que é a locomoção das células em resposta a um gradiente quimioatrativo (Chung et al., 2001).

As interações entre quimiocinas e receptores têm um papel crucial na manutenção de processos fisiológicos e patológicos envolvendo a migração celular, incluindo o direcionamento de leucócitos para os locais de inflamação (Luster, 1998; Kufareva et al., 2015; Allen et al., 2007). Diferentes células expressam diferentes receptores de quimiocinas e essa expressão pode ser constitutiva ou induzível (Luster, 1998). Além disso, diferentes quimiocinas, ou "ligantes", estimulam ou inibem funções celulares específicas. Para que os processos fisiológicos associados às quimiocinas não se tornem eventos patológicos, é essencial que as interações entre receptores e ligantes sejam altamente reguladas (Luster, 1998; Chen et al., 2017). A perda do equilíbrio das interações quimiocinas-receptores e a consequente ação desregulada das células do sistema imune podem desencadear diferentes doenças humanas (Bernardini et al., 2016; Chen et al., 2017).

O conceito de ampla redundância em relação às interações entre quimiocinas e receptores, no qual é aceito que mais de um ligante pode interagir com diferentes receptores (Mantovani, 1999), é atualmente questionável em alguns aspectos. Acredita-se

que o sistema de reconhecimento de quimiocinas é mais refinado e específico do que descrito anteriormente (Allen et al., 2007). Por exemplo, o CCR5 desempenha uma ação não redundante no recrutamento de células T de memória para os pulmões em resposta a infecções virais (Kohlmeier et al., 2008). Nesse sentido, a expressão diferenciada ou defeitos na ação de algum receptor de quimiocina específico pode explicar padrões diferenciados de migração celular e função imune. No entanto, para entender os processos anormais das interações entre quimiocinas e receptores, é essencial conhecer os aspectos biológicos de cada receptor. Neste contexto, o CCR5 é o foco desta tese.

O CCR5 tornou-se popular depois do seu papel como co-receptor do HIV ser descoberto na metade da década de 1990 (Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Drajic et al., 1996). Porém, o envolvimento do CCR5 em diferentes doenças é cada vez melhor entendido e discutido por diferentes autores (Lu et al., 2017; Butrym et al., 2018; Gao et al., 2018; Jiao et al., 2018; Singh et al., 2018; Wang et al., 2018). Da mesma forma, os bloqueadores do CCR5 são drogas promissoras para o tratamento de diferentes doenças de fundo inflamatório (Halama et al., 2016; Moy et al., 2017; Puengel et al., 2017; Coppola et al., 2018; Shah e Savjani, 2018; Vangelista e Vento, 2018).

A caracterização funcional do CCR5 ocorreu em 1996. Samson et al. (1996a) clonaram o gene *Chem13*, atualmente denominado *CCR5*, que codifica a molécula CCR5. Naquele ano, os mesmos autores nomearam o receptor alvo de "CKR-5" e mostraram que ele tinha alta homologia (75%) com CC-CKR2, agora conhecido como CCR2 (Samson et al., 1996a). No mesmo ano, Raport et al. (1996) e Combadiere et al. (1996) também clonaram o *CCR5* e encontraram uma semelhança com o *CCR2* de aproximadamente 70%. Naquela época já se sabia que o CCR5 era estimulado pelas quimiocinas MIP-1α/CCL3, MIP-1β/CCL4 e RANTES/CCL5 (Combadiere et al., 1996; Raport et al., 1996) e expresso principalmente em leucócitos, mas também em diferentes tecidos, incluindo timo e baço (Raport et al., 1996).

Ainda no mesmo ano, Samson et al. (1996b) e Liu et al. (1996) demonstraram que uma variante genética do *CCR5*, conhecida como CCR5Δ32 (devido a uma deleção de 32 pares de bases na região codificante do gene), era um forte fator de resistência contra a infecção pelo HIV-1. Complementando esses estudos, Dean et al. (1996) demonstraram, através de dados de diferentes coortes, que o CCR5Δ32 protegia contra a infecção pelo

HIV em indivíduos homozigotos para o alelo variante (Δ32) e promovia progressão lenta para AIDS em indivíduos heterozigotos para o alelo Δ32.

Em uma época em que a epidemia de AIDS era particularmente alarmante, os resultados mencionados tiveram um grande impacto na comunidade científica que procurava urgentemente fatores de resistência contra o HIV. Além disso, estudos envolvendo o CCR5Δ32 também se destacaram fora da comunidade de pesquisa biomédica. Nesse contexto, trabalhos de divulgação científica como o artigo publicado por O'Brien e Dean (1997) na *Scientific American* foram importantes para a popularização da função do CCR5 e da variante CCR5Δ32 na infecção pelo HIV. Mais de vinte anos depois, em 2009, a relação entre o CCR5 e a infecção pelo HIV mais uma vez chamou a atenção da comunidade global após uma publicação de Hütter et al. (2009) no *New England Journal of Medicine*. Nesse estudo, os autores descreveram o primeiro caso de controle de longo prazo da infecção pelo HIV obtido após um paciente infectado pelo HIV que sofria de leucemia receber um transplante de medula óssea com células de um doador homozigoto para o CCR5Δ32 (Hütter et al., 2009). Este paciente é atualmente conhecido como "Paciente de Berlim", encontra-se com boa saúde e tem papel ativo em campanhas e ações de combate ao HIV (Brown, 2015). Recentemente, foi publicado o relato do segundo caso de remissão sustentada da infecção pelo HIV (Gupta et al., 2019) após procedimento similar ao descrito por Hütter et al. (2009). Esses dois casos demonstram que a cura da infecção pelo HIV é possível, além de ajudarem no entendimento dos reservatórios virais e sobre as potenciais formas de eliminá-los. De importância, esses relatos também dão esperança aos pacientes infectados no que se refere à perspectiva de melhores tratamentos e servem de estímulo à comunidade científica comprometida com o fim da pandemia de HIV/AIDS.

Detalhes interessantes sobre a história das pesquisas iniciais envolvendo o CCR5 podem ser encontrados no artigo de Parmentier (2015). Deve-se ressaltar que, além do papel do CCR5 na infecção pelo HIV ser extensivamente estudado desde 1996, apenas recentemente o envolvimento do CCR5 na fisiopatologia de outras doenças, como o câncer (de Oliveira et al., 2014; Weitzenfeld e Ben-Baruch, 2014) e doenças inflamatórias (Balistreri et al., 2007; Martin-Blondel et al., 2016; Troncoso et al., 2018; Kaminski et al., 2019), passou a ser entendido em maior detalhe.

De forma geral, muitos avanços científicos e médicos já foram alcançados através do estudo do CCR5. O maior exemplo é o Maraviroque, um bloqueador do CCR5 eficaz no tratamento da infecção pelo HIV (Dorr et al., 2005; Fätkenheuer et al., 2005; Fätkenheuer et al., 2008; Gulick et al., 2008). Os bloqueadores do CCR5 também demonstraram ter potencial para serem usados no tratamento de outras doenças, especialmente o câncer (Velasco-Velázquez et al., 2012; Mencarelli et al., 2013; Sicoli et al., 2014).

Embora muito conhecimento tenha sido acumulado ao longo de mais de 20 anos de pesquisa sobre diferentes aspectos do CCR5, ainda há poucos estudos que abordam as funções do CCR5 fora do contexto da infecção pelo HIV. Dessa forma, esta tese traz uma atualização sobre a biologia do CCR5 e sua importância na regulação da resposta imune, além de apresentar resultados sobre os impactos da variante CCR5Δ32 na infecção pelo HCV, coinfecção HIV/HCV, infecção pelo HBV e coinfecção HIV/HBV. Os potenciais impactos do CCR5 e do CCR5Δ32 na infecção pelo TBEV também serão abordados. Tais informações são essenciais para guiar as terapias gênicas e farmacológicas baseadas na modulação da expressão do CCR5.

4.4. TBEV e o CCR5: uma interação emergente e ainda pouco explorada

No Brasil, as doenças transmitidas por carrapatos podem ser consideradas negligenciadas. Apesar dessas doenças receberem pouca atenção da comunidade científica e médica, elas são um problema em diferentes regiões do País (Szabó et al., 2013). Olhando para o período de 2004 e 2017, Reck et al. (2018) relataram 70 casos de parasitismo humano por carrapatos apenas no estado do Rio Grande do Sul. Os casos envolveram carrapatos das espécies *Amblyomma parkeri, A. aureolatum, A. ovale, A. dubitatum, A. fuscum, A. incisum, A. longirostre, Haemaphysalis juxtakochi, Rhipicephalus sanguineus, R. microplus* e *Ornithodoros brasiliensis.*

O TBEV é um vírus com genoma do tipo RNA, pertencente à família *Flaviviridae* e ao gênero *Flavivirus.* O TBEV é transmitido principalmente por carrapatos das espécies *Ixodes ricinus* e *I. persulcatus.* A infecção por esse vírus pode causar um grave quadro de encefalite, sendo um importante problema de saúde pública principalmente em países da Europa (Gritsun et al., 2003; Süss, 2011; Zavadska et al., 2013; Kazimírová et al., 2017).

Até onde se sabe, não há registro da circulação do TBEV no Brasil. Também não há registros de *I. ricinus* e *I. persulcatus* no Estado do Rio Grande do Sul (Evans et al., 2000; Reck et al., 2018) ou em outras regiões brasileiras (Dantas-Torres et al., 2009), embora outras espécies do gênero *Ixodes* (Evans et al., 2000; Dantas-Torres et al., 2009; Reck et al., 2018) e do complexo *Ixodes ricinus* (Michel et al., 2017) sejam encontradas no Brasil. Levando em consideração os relatos da presença de carrapatos do gênero *Ixodes* no território nacional, a possibilidade de entrada do TBEV no Brasil não pode ser ignorada.

Diferentes estudos indicaram que o CCR5 e o CCR5Δ32 apresentam destacado papel na suscetibilidade à infecção pelo TBEV e patogênese da doença (Kindberg et al., 2008; Mickienė et al., 2014; Grygorczuk et al., 2016; Michlmayr et al., 2016; Ignatieva et al., 2017; Thangamani et al., 2017). Considerando que o Maraviroque (bloqueador do CCR5) já foi introduzido no Brasil para o tratamento da infecção pelo HIV (CONITEC, 2012; Brites et al., 2015), uma atenção sobre a influência do bloqueio do CCR5 na suscetibilidade à infecção pelo TBEV e talvez outros vírus transmitidos por carrapatos pode ser estendida à população brasileira. Além disso, embora ainda não haja evidências da circulação do TBEV no Brasil, os papeis do CCR5 e do CCR5Δ32 na infecção pelo TBEV representam interessantes questões em aberto a serem exploradas.

OBJETIVOS

Objetivo geral

Abordar de forma integrada os principais fatores imunogenéticos e ambientais que contribuem para o estabelecimento de doenças virais emergentes, reemergentes e negligenciadas no Brasil, com enfoque na perspectiva *One Health*.

Objetivos específicos

- Revisar os aspectos epidemiológicos, ecológicos e genéticos que norteiam o estudo, prevenção e mitigação das doenças infecciosas.

- Caracterizar de forma crítica o cenário atual das doenças emergentes, reemergentes e negligenciadas causadas por vírus no Brasil, usando como modelos de estudo os vírus Sabiá, Rocio, HIV e HCV.

- Descrever os fatores imunológicos e genéticos que influenciam as infecções pelo HIV e HCV, com enfoque na genética do hospedeiro e no papel dos exossomos.

- Determinar os níveis de citocinas/quimiocinas relacionadas à inflamação em indivíduos HIV+ com os diferentes perfis de progressão à AIDS.

- Discutir efeitos de polimorfismos genéticos sobre diferentes infecções virais, com foco em variantes de genes de microRNAs.

- Revisar os aspectos básicos do CCR5 e abordar seus impactos nas doenças infecciosas, usando como modelo de estudo o *Tick-borne encephalitis virus*.

- Avaliar o impacto do polimorfismo CCR5Δ32 na suscetibilidade à infecção pelo HCV, coinfecção HIV/HCV e doenças causadas pelo HCV.

- Investigar os efeitos do CCR5Δ32 na suscetibilidade à infecção pelo HBV e coinfecção HIV/HBV.

CAPÍTULO II

Ecologia e doenças emergentes

Este capítulo apresenta três trabalhos (*Letter to the editor)* publicados no periódico *Brazilian Journal of Infectious Diseases*, um trabalho (*Correspondence*) publicado no *The Lancet Planetary Health* e um quinto trabalho (*Mini-Review*) aceito para publicação na revista *Journal of Infection and Public Health*. Os cinco trabalhos abordam diferentes temas relacionados com ecologia e doenças emergentes. Por ordem de apresentação, as publicações são:

Ellwanger JH e Chies JAB (2016) Emergent diseases in emergent countries: we must study viral ecology to prevent new epidemics. Braz J Infect Dis 20: 403-404. doi: 10.1016/j.bjid.2016.02.003

Ellwanger JH, Kaminski VL e Chies JAB (2017) How to detect new viral outbreaks or epidemics? We need to survey the circulation of viruses in humans and other animals using fast, sensible, cheap, and broad-spectrum methodologies. Braz J Infect Dis 21: 211-212. doi: 10.1016/j.bjid.2016.12.001

Ellwanger JH e Chies JAB (2018) Zoonotic spillover and emerging viral diseases - Time to intensify zoonoses surveillance in Brazil. Braz J Infect Dis 22: 76-78. doi: 10.1016/j.bjid.2017.11.003

Ellwanger JH e Chies JAB (2018) Wind: a neglected factor in the spread of infectious diseases. Lancet Planet Health 2: e475. doi: 10.1016/S2542-5196(18)30238-9

Ellwanger JH, Kaminski VL e Chies JAB (2019) Emerging infectious disease prevention: where should we invest our resources and efforts? J Infect Public Health, *No prelo*.

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Letter to the Editor

Emergent diseases in emergent countries: we must study viral ecology to prevent new epidemics

Recently Africa has seen the resurgence of Ebola virus in the largest outbreak hitherto recorded in the current history. This outbreak scared people worldwide and required the intervention of the World Health Organization, governments of several countries, medical and research institutions and non-governmental organizations such as Doctors Without Borders. In Brazil, the number of dengue cases in 2015 was the largest ever recorded (1,649,008 probable cases[1]) and the increasing number of people infected with Chikungunya and Zika viruses shows that the country is not prepared to deal with the proliferation of the mosquito *Aedes aegypti*, the main vector of Dengue, Chikungunya, and Zika viruses. Additionally, big events like the Olympic Games (that will be held in Brazil in 2016) can facilitate the international spread of these viral diseases. This alert was recently reinforced by Bogoch and colleagues when discussing the probable pattern of the Zika virus spread from Brazil to other countries.[2] The examples of diseases caused by different viruses demonstrate one crucial fact: neglected viruses emerge and proliferate, when favorable ecological conditions are in place. What does that mean? Environmental disturbances and their influence on these pathogens are and will increasingly be a global problem. This becomes an even greater concern when scientific communities and government do not give the necessary attention to this issue.

Environmental disturbances are also largely related to socioeconomic factors. Thus, it should be considered that epidemics are a problem with social and environmental aspects. For example, economic and social factors (such as wildlife hunting) associated with environmental factors (such as deforestation) are quoted as facilitators for the emergence of zoonotic diseases. These factors facilitate the transmission of viruses from wild animal to humans (an excellent review on these issues was conducted by Pike and collegues[3]).

Returning to the example of the most recent Ebola virus outbreak. It is believed that disordered human occupation of forest areas has contributed to the passage of the virus from their natural hosts to the human population. In addition, poor living conditions and the precarious health institutions of affected countries must have contributed to pathogen dissemination. Furthermore, Dengue, Chikungunya, and Zika viruses infect people through mosquitoes bite, and the proliferation of these vectors is facilitated by increasing urban waste, allowing water accumulation, and thus creating an environment conducive to the proliferation of mosquito larvae. Again, these examples highlight how environmental disturbances interfere in the viral ecology and contribute to the increase of cases of viral diseases.

Hemorrhagic fevers caused by Ebola virus and even the Dengue virus are classic examples of how severe can be the infection by these pathogens. Similarly, cases of microcephaly in children born from mothers infected with Zika virus represent another example of immediate problems caused by viral epidemics in population terms. Here, it is important to note that, despite being very probable, the relationship between the virus and this brain malformation has yet to be confirmed. There are also long-term problems caused by the emergence of viral pathogens. HIV, for example, was discovered few decades ago. However, when ecological and social factors beneficial to HIV proliferation emerged, the world witnessed the rise of a pandemic hitherto unimagined, which last over 30 years and yet seems to be far from being controlled.

The Brazilian biodiversity is extremely wide, so it should still hide countless unknown pathogens. Not less important, we should emphasize the Brazilian (always mentioned as a continental country) diversity in terms of climate, geographical differences, and even uses and habits of the population. As previously stated, pathogens will emerge when ecological conditions are favorable. Therefore, a pertinent question would be: Is Brazil a "nursery room" for the emerging of brand new viral diseases? (or even more troubling: How many different potential human pathogens are already present in our environment just waiting for the right conditions to show up?) Pathogens already known, but neglected, may also resurge and come back to frighten the population and those responsible for public health agencies. The only way to get around this is by trying to identify early on in the wild nature viruses with potential to cause future outbreaks in the human population and study the ecology and the genetics of these pathogens. This would allow us to identify what changes in environmental and social factors may contribute to the emergence of an epidemic. Similarly, it would allow us to fight against future epidemics when it occurs. At the international level, there are already actions that would help recognizing viruses that can

cause epidemics. It could be highlighted the Global Viral initiative (www.globalviral.org), that has the mission of "(...) to promote understanding, exploration and stewardship of the microbial world". Similarly, the Brazilian scientific community should turn its attention to these problems and take the opportunity to explore this research field yet so little studied in our country. This will have a very positive impact not only on the generation of knowledge but also on the health of the population, which now suffers the effects of diseases that so far have not received the deserved attention from the scientific and governmental communities.

Conflict of interest

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Letter to the Editor

How to detect new viral outbreaks or epidemics? We need to survey the circulation of viruses in humans and other animals using fast, sensible, cheap, and broad-spectrum methodologies

Dear Editor,

"One Health" is the concept that unifies (I) human, (II) animal, and (III) environmental health.[1,2] According to this concept, any condition that affects one of these three actors will affect the health of all of them. Emerging and reemerging diseases can be recognized as disorders in the One Health. Such diseases can arise in response to environmental disturbances caused by human action. For example, interferences on wildlife habitats can predispose wild animals to approach or even to live in urban areas, creating ideal conditions for pathogens to jump from these animals to humans. In other words, the emergence of a disease can be the result of lack of synergism between various socioecological factors.

When a new zoonotic disease arises in a human population, it is mandatory to quickly detect and identify such a new pathological agent. This early detection is an important way to prevent outbreaks and avoid epidemics. For example, if the circulation of HIV had been identified in Africa right after the virus transition from wild primates to humans, perhaps the HIV pandemic could have been avoided. Similarly, to prevent the reemergence of a viral disease in a human population, early identification, monitoring, and survey of the viruses circulating among humans is essential. This monitoring should be primarily done in sentinel populations (I) living in places near to the habitats of animals considered to be important viral reservoirs (for example rodents, bats, pigs, and monkeys); (II) living or working near animal breeding regions or regions of slaughtering of livestock animals, since these sites provide ideal conditions for the emergence of human viral diseases due to cohabitation of humans and non-human animals; and (III) living in areas infested by viral vectors (especially mosquitoes). Still, this monitoring should be carried out among patients that seek health services showing signs of viral infections. However, a practical problem is the lack of tools and methodologies to perform this control.

Recently, a Brazilian research group developed a DNA microarray methodology (SMAvirusChip) that allows the screening of more than 400 viruses transmitted by arthropods and small mammals, using only one biological sample.[3] It is important to highlight that the SMAvirusChip can identify viruses that pose a constant concern for public health authorities (Chikungunya, Dengue, Zika, among others) or intrigue the scientific community, as is the case of Sabiá and Rocio viruses (both viruses identified in human patients but for which still lacks genetic/evolutionary or even ecological information). Based on oligonucleotide probes, this tool allows the precise identification of viruses that are often difficult to diagnose by conventional immunoassays due to cross-reactivity. For example, the results of immunoassays aiming at detecting the Zika virus can be inconclusive due to cross-reactivity of antibodies induced by other flaviviruses, such as Dengue or Yellow Fever. For us, the detection of a broad spectrum of viruses is a unique feature of the SMAvirusChip. On the other hand, the feasibility of large-scale use of methodologies such as SMAvirusChip still needs to be evaluated. Of course, the cost for the application of this microarray methodology may be an important obstacle, especially in developing countries. However, this is an example of a tool that could be used for monitoring the circulation of viruses among humans and other animal species.

The development of tools to identify a broad spectrum of viral pathogens in a fast, sensible, and cheap way is a global need. Such tools would primarily be applied to healthcare services of developing countries located in tropical regions. Knowing the pathogens that circulate in a given population will allow the detection of even small variations in circulation patterns. Several fronts could be envisaged in such survey: first, methodologies such as these would facilitate the diagnosis of viral diseases of which the causative agents often remains unidentified. In these cases, even if the identification of the pathogens does not have clinical implications or therapeutic consequences, the data is of epidemiological importance. Second, it would allow the survey of different viruses hosted in human populations. Third, these tools could be used in health services for the screening of blood bags, for

example. Fourth, in viral ecology studies, these tools would facilitate the work of researchers that target viral diversity amongst non-human animals. In these different contexts, the spectrum of pathogens detected in research activities would also be expanded.

Permanent monitoring of the circulation of viruses in risk areas can be considered a strategy within the scope of "constant interventions". Matua et al.[4] defined this term as strategies "undertaken at individual, community, and institutional levels following an epidemic and to be continued in the aftermath, that is in-between the outbreaks". The "constant interventions" were recommended by the authors in the context of the last outbreak of Ebola in Africa; we consider it useful to be applied in other situations, previous to the establishment of a significant number of infectious cases, in the monitoring of endemic, emerging, and reemerging diseases. The same authors state that "In essence, 'constant interventions' are intended to keep populations and institutions in 'high-risk' areas ready, fully prepared, and constantly aware of the risk of Ebola outbreak recurrence". In our opinion, this definition fits properly not only for Ebola but for many other viral diseases.

In conclusion, viruses with a great potential to trigger new epidemics are already "out there". Monitoring of the circulation of viruses in biological samples (human and non-human) is essential for early identification of the next outbreaks or epidemics. The need for such a surveillance and monitoring behavior is even greater in developing tropical countries where so often humans live disharmoniously with many domestic and wild animals. The proposed surveillance would be even more effective if performed with samples from (I) vectors, (II) resident viral reservoirs, and (III) migratory animals such as birds, and (IV) humans. Together, data gathered from humans and other animals (wild, livestock, and domesticated) would show where the viral hotspots are, enabling measures to control the spread of viral pathogens. Such strategy could not only reduce health problems but also the economic losses caused by the infection and even death of animals of economic interest (for the food industry, for example). Importantly, only with the development of methodologies that allow the identification of a broad spectrum of viruses this monitoring can be achieved effectively. In developing and tropical countries like Brazil, this type of initiative should be encouraged not only as a form of support for technological development, but mainly as a public health strategy and a way to preserve the One Health.

Conflicts of interest

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Letter to the Editor

Zoonotic spillover and emerging viral diseases – time to intensify zoonoses surveillance in Brazil

Dear Editor:

Are we prepared to face newly emerging viral diseases? This question is as recurrent as the emergence of new viruses and the reemergence of neglected infectious diseases. Of note, Central and South America are considered world hotspots for the emergence of new mammalian viral zoonoses.[1] Due to the size of its territory, Brazil lies at the center of these hotspots.

An assessment of viral diversity across mammalian orders has identified bats as the main reservoir of new viruses in Brazil.[1] As one of the world's most biodiverse countries, Brazil harbors a large diversity of bat species, as well as a number of species that might act as hosts of as yet unknown pathogens. In this context, the arrival of exogenous viruses and their adaptation to our diverse environmental and socio-ecological conditions should also be taken into consideration.

The yellow fever outbreak this year and the recent spread of Zika virus (ZIKV) from Brazil to other American countries[2] are poignant examples of the failure of our country's strategies for monitoring and controlling outbreaks and epidemics. They clearly reflect a lack of political interest in basic public health measures and epidemiological surveillance. At the same time, they result from failure to implement new technologies for population-level monitoring of virus circulation between humans and zoonotic animals.

To avoid potentially serious mistakes, such as the late detection of ZIKV in our country, a different attitude is required. In addition to improve vector control of known diseases, such as arthropod-borne infections, surveillance of the circulation of viruses among bats and other mammalian species in the Brazilian territory must be reinforced. In addition, of obvious interest to public health, the rapid detection of new viruses among humans is essential.

We believe that a rapid detection of zoonotic spillovers will only be possible if our knowledge about the viruses circulating in non-human animals of high zoonotic potential is channeled into a powerful and effective surveillance system. According to Plowright et al.,[3] a "zoonotic spillover" is defined as the "transmission of a pathogen from a vertebrate animal to a human". We would like to highlight the importance of detecting when a pathogen crosses the boundary from its natural reservoir to start circulating among humans, a complex event involving environmental, pathogen, and host factors.[3] Following a zoonotic spillover, human-to-human transmission is essential to sustain an epidemic or pandemic.[3,4] As a matter of fact, most zoonotic viruses will never cause infections that spread extensively among the human population.[4] Nevertheless, viruses hitherto attracting little medical interest can unexpectedly become a threat to public health. For example, until the recent discovery of the link between ZIKV and microcephaly and other congenital problems, ZIKV infection was not considered clinically or epidemiologically important.

Efforts toward the surveillance of virus circulation in humans can build on samples collected for other purposes.[2] For example, the screening of blood bank samples for the presence of zoonotic viruses might represent a promising strategy requiring a relatively minor investment. As previously discussed,[5] we encourage the use of broad spectrum methods for the detection of emerging epidemics and pandemics. Monitoring approaches based on the simultaneous detection of genomic sequences of different viruses appear to be both cost-effective and fast, while covering a broad spectrum of pathogens.[2,5] However, we understand the importance of focusing monitoring efforts on those human groups most susceptible to coming into contact with new viruses. Moreover, considering the vast number of animal pathogens,[3] monitoring strategies should target (I) viruses showing a particularly high potential for human-to-human transmission, and (II) viruses already circulating in other human populations. Indiscriminate surveys may not be effective in predicting pandemics.[4]

Here, we suggest a number of initiatives for the prevention, detection, monitoring, and interruption of outbreaks and epidemics in Brazil (Fig. 1). Additional actions may be suggested and applied to complement these initiatives. Importantly, our initial suggestions must be viewed in the context of a best case scenario in terms of political, economic, and public health conditions in Brazil. Under current circumstances, the implementation of various of these initiatives will be difficult or remain incomplete. Despite the potential difficulties, the measures suggested here are important to improve the Brazilian system for epidemiological control of viral pathogens. The resources needed to stop or mitigate an epidemic would

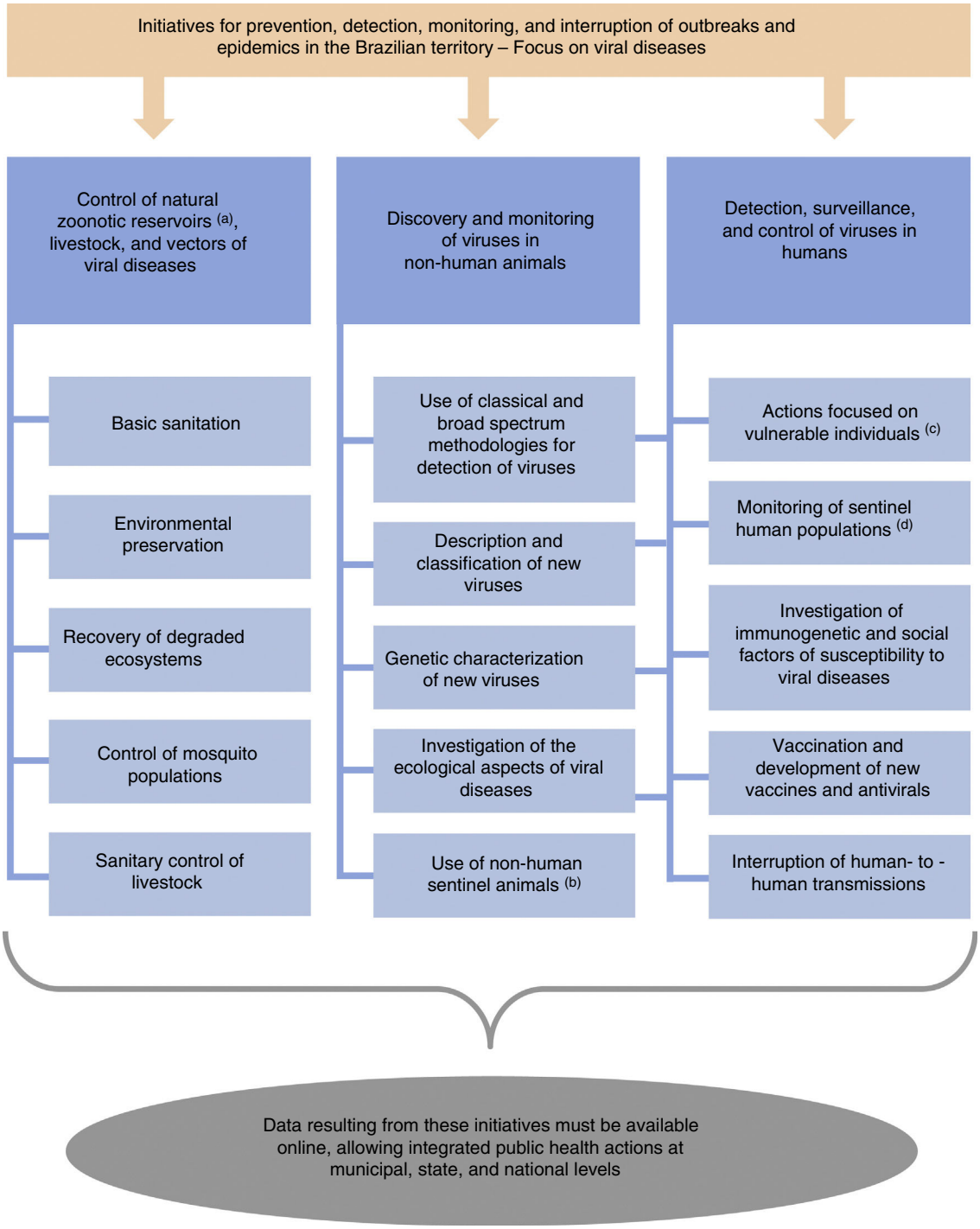

Fig. 1 – Initiatives for prevention, detection, and interruption of outbreaks and epidemics in the Brazilian territory, with focus on viral diseases. [a]Bats, dogs, cats, rodents, pigeons, among others. [b]Non-human animals experimentally or naturally exposed to risk areas. [c]Mainly injecting drug users and sex workers (these populations are more susceptible to viral transmission among humans). [d]Hunters, individuals living in rural areas, and those in close contact with livestock (these groups of people are more susceptible to zoonotic spillover). In addition, blood donors or individuals who consent to donate biological samples for research activities can be used as human sentinels in strategies for the surveillance of zoonotic diseases.

certainly greatly exceed those necessary to finance prevention strategies. Finally, these measures suggested by us are essential to prevent Brazil from becoming, yet again, a nursery for new zoonoses.

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Wind: a neglected factor in the spread of infectious diseases

Emerging and reemerging infectious diseases can result from unbalanced human, animal, and environmental factors. This concept belongs to the One Health perspective and has a growing role in discussions on global health. In addition to affecting the emergence of new or neglected infectious diseases or even the maintenance of a given condition at a population level, different factors of the One Health triad (humans, non-human animals, and environment) also have an effect on human-to-human transmission of pathogens. For example, host genetic traits might increase or decrease individual susceptibility to viral diseases,[1] zoonoses can be transmitted from wild animals to humans due to the sharing of habitats or deforestation and hunting,[2] and unregulated urbanisation and bad sanitation could increase the occurrence of mosquito breeding sites, favouring the transmission of arthropod-borne diseases.[3]

The aforementioned examples are classic and even considered commonplace when analysing the factors involved in the dynamics of the spread of infectious diseases, although often they are unable to provide us with a complete understanding of the complex behaviour of certain outbreaks. In this sense, the identification of neglected factors within the One Health components is urgently needed. In our view, the study by Noriko Endo and Elfatih Eltahir[4] published in *The Lancet Planetary Health* (Sep 1, p e406–13) is an important contribution, because it shows that the wind speed and direction affect malaria transmission. The simplicity and the beauty of the proposed model, relies on the fact that such factors naturally affect the behavior of *Anopheles* mosquitoes. The main points of this study that we would like to highlight is that no extreme environmental alterations, including degradation of the natural environment, were needed to identify and explain potential differences in the dynamics of mosquito populations. Instead, the authors considered naturally occurring components. Although the study's conclusions were obtained from the analysis of a single scenario and pathogen, they have broader implications. They indicate that wind should be taken into account as a crucial factor in the transmission of various diseases, not just those that are air-borne, because it could modulate the dynamics of different vectors and pathogens. In other words, despite being relatively neglected and mainly considered in terms of its direct effects on pathogen dispersion, wind's capacity to carry and disperse signal molecules (eg, CO_2), or other as yet unidentified features, make it a more important factor in the environmental component of the One Health concept.

The consequences of our associations and contact with other species, and of the human activity on natural or modified environments, are issues that are continuously raised and discussed. Practical recommendations on preservation of biodiversity and prevention of disease emergence from the close contact between humans and the natural world have recently been published in Brazil.[5] In line with this initiative, Endo and Eltahir[4] suggest actions focused on counteracting some of the unwanted effects of wind on malaria transmission, including that "villages should be located downwind of reservoirs to reduce the incidence of malaria". Turning the results of scientific studies into functionally applicable recommendations is not always possible, but such attempts should be encouraged. Taken together, the findings discussed here point to the urgent need for inclusion of the effects of wind in the One Health approach focused on understanding and control of arthropod-borne diseases. The potential effects of wind on the behaviour of other vectors must also be investigated.

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Emerging infectious disease prevention: where should we invest our resources and efforts?

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Abstract

Strategies focused on the prevention of emerging infectious disease outbreaks are currently in the spotlight of discussions among researchers committed to infectious disease control. In this mini-review, we provided a brief update on this discussion and characterized the three main targets for investments in emerging infectious disease prevention: animals, human sentinels for spillover events, and the general human population. Furthermore, the pros and cons of each target are highlighted. Despite the particularities of the proposed targets, each of them can fill different gaps in the surveillance of infectious diseases. When all three targets are focused on together, they create a powerful strategy of emerging infectious disease prevention.

Keywords: infectious diseases, outbreaks, epidemics, zoonosis, pathogens, spillover, global health.

Introduction

Although it seems paradoxical considering the general advances in health sciences, emerging infectious diseases (EIDs) have become more frequent in recent decades [1]. Environmental changes due to human activity, increased international mobility, poor public health systems, and microbial adaptations are some of the main drivers of this problem [2, 3]. To efficiently combat EIDs, scientific and governmental communities use different approaches focused on the prediction, rapid detection, and surveillance of pathogens with the potential to cause outbreaks, epidemics, and even pandemics. However, the high pathogen diversity in nature makes the prediction of which pathogens have a real potential of causing diseases in humans a large challenge. Considering this fact, researchers committed to EID prevention frequently need to make complex decisions about the best aspect they should focus their actions and financial resources on, which are often highly limited, especially in low-income countries.

Targets for investments in emerging infectious disease prevention

The discovery of new potential human pathogens is a useful strategy for EID prevention [4]. However, given the high cost and uncertainty about its effectiveness, this strategy has been highly criticized [5]. Considering that it is expensive to test animal populations for previously unrecognized pathogens, testing for pathogens that have already crossed the barrier between animals and humans (spillover) [6] could be a more cost-efficient use of limited resources. Additionally, the early detection of EIDs based on effective surveillance of pathogens circulating in human populations would be easier than the attempt to predict "when and where" EID events will occur [5, 7]. In our opinion, efforts expended in each of the strategies mentioned above promote different impacts on EID prevention. To explain and discuss such strategies, it is necessary to help researchers organize and optimize their actions to obtain the best return from the scarce resources available for this purpose. Most EIDs are zoonoses [1], and considering the basic steps of zoonotic disease emergence [8], we summarized here the major investment targets for EID prevention (Figure 1). EID drivers and pathogen characteristics that facilitate spillover are not included in our discussion. In brief, a simple question should be asked: who should be tested/evaluated to provide a better understanding of the actual prevalence of EIDs?

- *Target 1: animals.* The investigation of already known or even unknown microorganisms hosted in animals would help in the identification and tracking of pathogens that, at some point, may cause human diseases. However, most of the pathogens circulating in wild animals will never

cause human diseases or pandemics [6, 9]. In addition, this strategy can be very costly and has little immediate practical applicability [5]. However, this approach is valuable in outbreak situations. For example, the development of a vaccine or therapeutic strategies for new human diseases can be accelerated if the genome sequences or other biological aspects of the pathogen are already available before the outbreak event [3, 4]. Of note, focusing investigations on key animals (e.g., companion animals, livestock, and select wild animals, such as bats) may be more advantageous since they are in close contact with humans and can act as spillover intermediates [8, 10]. In the context of EID prevention, targeting broad-spectrum pathogen detection in animals is an exploratory-type strategy.

- *Target 2: human sentinels for spillover events.* Zoonotic pathogens found in human biological samples represent a small number of microorganisms that have successfully moved from animals to humans [6]. Individuals in close and frequent contact with wild animals and livestock (e.g., hunters, farmers, and veterinarians) can act as human sentinels of recent spillover events [7]. Scientists who work with human sentinels direct their efforts to detect pathogens that already infect humans but have little epidemiological importance. Once a new human pathogen is detected, response actions such as the elucidation of its medical importance and surveillance intensification can be taken. However, if the spillover is not rapidly identified or if adequate control measures are not taken, the pathogen will have the opportunity to spread among the human population.

- *Target 3: general human population.* Screenings performed in blood donor samples or samples from other specific groups may be useful to detect the circulation of emerging pathogens at a population level. This action requires an adequate laboratory and technical structure. In this context, it is important to emphasize that low-income countries will require substantial efforts concerning investments to build laboratories and train staff to address the diagnosis of infectious diseases [11]. The strategies mentioned in the previous topics can help to determine which pathogens should be included in such screenings. A fundamental feature of pathogens that cause epidemics or pandemics is the ability to move between humans through direct human-to-human transmission [6, 9]. Once observed, this specific transmission pattern should not be neglected. However, various microorganisms circulating in humans are neither pathogenic nor epidemiologically important. Therefore, microbial screening in the general population can be very useful for EID prevention but can sometimes trigger false alarms.

The prevention strategies summarized in targets 2 and 3 (targeting human sentinels or the general human population) present a surveillance-type characteristic. In addition to the three targets mentioned here, vectors cannot be overlooked in EID prevention since they play important roles in pathogen transmission from animals to humans as well as in sustaining outbreaks. For example,

mosquitoes sustain the endemic, epidemic, and sylvatic cycles of Zika virus [12] and Dengue virus [13]. Additionally, ticks are responsible for the transmission of various pathogens between animals and humans [14]. For these reasons, the surveillance of pathogens in vectors must also be encouraged and expanded.

Pivotal considerations and perspectives: strengthen the basics and invest in new technologies

The transmission of pathogens between humans is divided into direct and indirect modes. The direct transmission modes are vertical (e.g., transplacental or during vaginal birth), sexual (e.g., genital-genital or oral-genital), nonsexual direct contact (e.g., kissing or touching), and airborne (e.g., respiratory tract-respiratory tract). The indirect transmission modes are environmental (e.g., infected water-oral or contaminated food-oral), fomites (e.g., needle-blood or doorknob-hand), and vector-borne (e.g., cutaneous penetration or vector fecal deposition) [15]. Direct human-to-human transmission should receive special attention in EID prevention strategies since, as mentioned before, this is the mode of transmission that sustains epidemics [6, 9]. However, indirect modes of transmission are crucial in some cases. For example, the role of mosquitoes in epidemics caused by arboviruses is extremely relevant and cannot be neglected [12, 13].

Many advances have already been made in developing pipelines and strategies to prevent and mitigate outbreaks. For example, once the etiologic agent of an infectious disease is detected, conduits to stop the transmission chain can be easily established based on the knowledge of the transmission modes responsible for the pathogen transmission. However, these actions often run counter to factors such as the lack of basic sanitation and low education levels of the population affected by a disease outbreak, factors that hamper the adequate establishment of strategies to mitigate infectious diseases. Thus, EID prevention is dependent on the strengthening of basic social and environmental factors, such as population access to education and health services, as well as environmental preservation. In other words, it is necessary to prioritize the One Health approach, in which human, animal, and environmental factors are considered together in EID prevention and mitigation strategies [16-18].

In addition to focusing on basic strategies, investing in new diagnostic technologies and tools of pathogen detection is crucial. Classically, epidemiological surveillance in human populations requires the selection of which pathogens will be monitored. For this task, it is necessary to know which pathogens circulate in a given population and which pathogens have relevance to be monitored at a population level and to have cost-effective diagnostic tools to perform the surveillance quickly and effectively. New diagnostic technologies are changing this

scenario and reducing these problems, such as DNA microarray platforms capable of detecting several pathogen species in a single biological sample [19-21]. Additionally, portable sequencers [22, 23] and CRISPR/Cas-based methods [24-26] are important new tools for pathogen diagnostics in the field. However, methods for pathogen screening or rapid and precise microbial detection are generally expensive, and there is still a need to develop cost-effective tools to allow their implementation even in low-income countries. Although the tools mentioned above are not yet part of most laboratories and are still not available for all teams responsible for epidemiological surveillance, they exemplify how investing in the development of new diagnostic technologies can positively impact surveillance strategies.

In the case of strategies focused on pathogen exploration, metagenomic strategies allow the identification of which groups of pathogens are present in environmental, animal, or clinical samples, in addition to knowing the abundance of pathogen groups found in the sample. In addition, the obtained DNA sequences can be used for phylogenetic and phylogeographic analyses, allowing for the understanding of evolutionary aspects of the pathogens, the reconstruction of transmission chains, and even the estimation of the probable starting date of an outbreak [27-29]. Metagenomics tools are constantly being refined [30], and their use in strategies for pathogen exploration is increasing. In addition to discovering new pathogens circulating in humans, when metagenomic studies are performed using samples from vectors or wildlife [31, 32], they allow the discovery of new pathogens with the potential of experiencing spillover. However, it is important to remember that spillover is a complex process [6], and after a new virus is identified in wildlife, it is necessary to evaluate very carefully whether it represents a threat to human health [33].

Concluding remarks

In conclusion, human and monetary investments focused on animals, humans, and vectors meet different demands and support the elucidation of different questions in the EID context. Taken together, they are profoundly complementary and give rise to powerful strategies for EID prevention. In essence, all the strategies discussed here are based on surveillance and early detection of human health threats. Researchers and governmental agencies should choose the appropriate targets for their future investments based on the pros and cons of each of them and take into account the available resources and the most urgent needs of their communities and countries or the global scenario. Answering the question mentioned before, we suggest the following approaches: animals and human sentinels for spillover events could be targeted in research initiatives, in which a large number of resources are available. Additionally, the general human population should be evaluated

as part of public health programs. In the best possible scenario, the three groups should be targeted together.

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No competing interests to declare.

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Figure legend

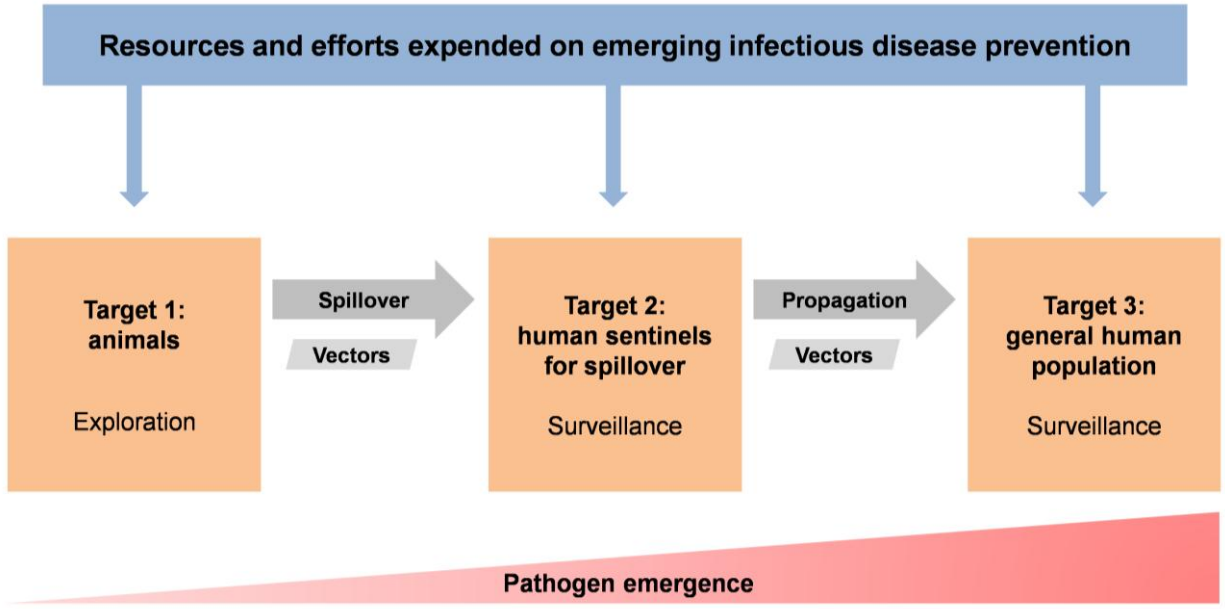

Figure 1. Targets for investments in EID prevention.

CAPÍTULO III

Keeping track of hidden dangers - The short history of the Sabiá virus

Este capítulo apresenta o seguinte artigo de revisão publicado no periódico *Revista da Sociedade Brasileira de Medicina Tropical*:

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Review Article

Keeping track of hidden dangers - The short history of the Sabiá virus

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Abstract

Emerging infectious diseases are a global threat. In countries like Brazil, where biodiversity is high and public health conditions in terms of infrastructure and medical care are often precarious, emerging diseases are particularly worrisome. The lack of monitoring strategies to identify pathogens with the potential to cause outbreaks or epidemics is another problem in Brazil and other developing countries. In this article, we present the history of the Sabiá virus (SABV), a pathogen that was described in the 1990s in Brazil. Several aspects of the biology and ecology of the SABV remain unknown. The SABV has the potential to cause hemorrhagic fever in humans. To date, four cases of human infections have been reported worldwide; two were naturally acquired (both in Brazil), whereas the other two were linked to occupational exposure in the laboratory environment (one in Brazil and one in the USA). In this review, we summarize the basic biological and ecological characteristics of the SABV. This is the first work to gather all available data on the historical aspects involving the cases of SABV infection along with an update on its characteristic features.

Keywords: Arenaviridae. Emerging viral disease. Sabiá virus. Viral ecology.

INTRODUCTION

Brazil has abundant biodiversity. Brazilian ecosystems likely hide many pathogens that can cause infectious diseases in humans. This is relevant for public health as these pathogens could cause diseases with unknown characteristics. When pathogens are newly introduced into the human population, this poses significant challenges and dangers as the population is not immunized against these organisms. Potential vaccines have yet to be developed, which typically requires a time-consuming process. Moreover, the lack of knowledge on the biology and ecology of these infectious agents represents an obstacle to prevention and containment strategies of potential epidemics.

In this review, we are highlighting the Sabiá virus (SABV), which, although described in the 1990s, has not been well studied. Briefly, it is known that this arenavirus circulates in Brazil and can cause hemorrhagic fever in humans. However, little is known about the virus' biology and ecology.

Considering the paucity of information on this pathogen, the aims of this article were to gather available information on the SABV and to describe the four reported cases of SABV infection within a historical perspective. Some aspects of SABV ecology and biology will also briefly be discussed.

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METHODOLOGY

This study is a narrative review. We consulted the following regional and international databases: *Biblioteca Virtual em Saúde* (BVS; http://bvsalud.org/), MEDLINE (via PubMed; https://www.ncbi.nlm.nih.gov/pubmed), and The Scientific Electronic Library Online (SciELO; http://www.scielo.org/). Keywords used to search for articles on the SABV included *sabia virus* and *SABV*. Using the search term *sabia virus*, we found 622, 788, and 9 articles in the BVS, MEDLINE, and SciELO, respectively. The search term *SABV* uncovered 3 articles in the BVS, 10 in MEDLINE, and none in SciELO. After we analyzed the articles through their abstracts or full texts regarding its relevance, 24 of them were selected to be included in our review. When necessary, reference lists of previously published studies were consulted to select and locate articles. Eight works discussing topics such as *arenavirus*, *emerging virus*, and *viral ecology* were also included in this review aiming to complement our discussion on these topics.

Basic aspects of the Sabiá virus

Classification and geographic distribution of the Sabiá virus and other arenaviruses - The SABV belongs to the family *Arenaviridae*, and within these arenaviruses to the Tacaribe or New World complex. Arenaviruses are enveloped negative-stranded RNA viruses[1-3]. In addition to the SABV, the New World complex includes the following 17 viruses: Allpahuayo (Peru), Amapari (Brazil), Bear Canyon (California, USA)

Chapare (Bolivia), Cupixi (Brazil), Flexal (Brazil), Guanarito (Venezuela), Junin (Argentina), Latino (Bolivia), Machupo (Bolivia), Oliveros (Argentina), Parana (Paraguay), Pichinde (Colombia), Pirital (Venezuela), Tacaribe (Trinidad), Tamiami (Florida, USA), and Whitewater Arroyo (Southwestern USA.). It seems that Brazil is the natural place of occurrence of the SABV (reviewed in Radoshitzky et al.[4]).

In 1996, phylogenetic analyses (of a portion of the nucleocapsid gene) resulted in the classification of the SABV as a unique member of the clade B of the New World arenaviruses[2]. Previous studies that had evaluated the complete glycoprotein precursor, complete nucleoprotein, and polymerase genes as well as the phylogenetic relationships among New World arenaviruses had resulted in the same classification[5-7]. According to Radoshitzky et al.[4], eight arenaviruses belong to the clade B: Amapari, Chapare, Cupixi, Guanarito, Junin, Machupo, Sabiá, and Tacaribe. Non-genetic factors such as geographical regions of occurrence, hosts, or the potential to cause epidemics are not good indicators to correlate different groups of arenaviruses[5].

Viral host and route of infection - Commonly, naturally acquired human infections by arenaviruses are caused by viruses with rodent hosts[8]. Rodents are likely also reservoirs for the SABV. Recently, Bisordi et al.[9] identified arenavirus sequences in 5 of 55 vesper mice (*Calomys tener*) that were caught in the area where the second naturally acquired infection with the SABV was identified (Espírito Santo do Pinhal, São Paulo State, Brazil); however, all sequences were different from those that had been described for the SABV. Notably, their study resulted in the identification of a new arenavirus, the Pinhal virus[9].

Generally, arenaviruses are transmitted through contact with contaminated rodent urine[10]. Thus, inhalation of dust mixed with rodent urine is the probable route of infection by the SABV in nature. Future studies should aim at identifying the presence of the virus in samples of excreta and biological fluids of rodents.

Clinical implications and pathogenesis of Sabiá virus infections - Arenaviruses found in the Americas that are pathogenic in humans include Chapare, Flexal, Junin, Pichinde, Guanarito, Machupo, Tacaribe, and the SABV. The potential of the Allpahuayo, Amapari, Cupixi, Parana, Pirital, Latino, and Oliveros viruses to cause diseases in humans is unknown (reviewed in Koma et al.[11]).

In general, symptoms caused by the SABV are abdominal and epigastric pain, bleeding gums, conjunctival petechiae, conjunctivitis, cough, diarrhea, difficulty walking, fever, headache, hematemesis, hemorrhage, leukopenia, malaise, myalgia, nausea, somnolence, sore throat, tonic-clonic seizures, tremors, vomiting, weakness, and shock[1,12].

An *in vitro* study performed by Radoshitzky et al.[13] indicated that the SABV infects host cells through the transferrin receptor 1 (TfR1) as virus replication was halted by administering an anti-TfR1 antibody. Based on these data, it is plausible that the hemorrhagic fever caused by the SABV is related to the virus' capacity to bind to human TfR1. Other study *in vitro* also shown that the SABV Z protein interacts with the retinoic acid-inducible gene I product (RIG-I) and downregulates the cellular beta interferon response[14].

The SABV is considered a dangerous pathogen as it can induce a severe hemorrhagic fever that can rapidly progress to the death of the infected individual[15].There is no specific treatment for the disease or immunoprophylaxis for the virus. One might ask, *Why, almost 30 years after the first report of human SABV infection, is knowledge on SABV biology and pathogenesis still so limited?* Similarly, considering that the last case of naturally acquired human infection was reported in 1999, *How can the absence of new cases within the last 17 years be explained?* Some potential answers might be: I) New human infections are occurring but are not being reported due to the similarity of the clinical symptoms with those of other viral infections; II) The habitat of the reservoir species is not conducive to human interaction, which could hinder the infection of humans by the virus; and III) The transmission capacity of the virus from natural reservoir species to humans is low. However, it is important to identify the reservoir species of the SABV. Once the reservoir species is known, experimental studies aimed at elucidating the pathophysiological aspects of SABV infection should be conducted.

Viral detection and potential anti-viral strategies - Detection of the SABV in humans remains a clinical and methodological challenge. Machado et al.[16] report the need for developing techniques to detect the SABV without having to manipulate infectious viral samples. Currently, there is a well-described reverse transcription polymerase chain reaction (RT-PCR) assay for the rapid detection of the SABV[17].

Figueiredo[18] suggested that the vaccine against the Junin virus (that is already being used in Argentina) could also induce immunity to the SABV. However, his hypothesis needs to be confirmed. Recently, Golden et al.[19] described a DNA vaccine that could be used as a pan-arenavirus immunotherapeutic.

The compounds ST-193 and ST-294 have been shown to possess anti-viral activity for arenaviruses[20-22]. The target of ST-193 is likely a segment of about 30 amino acids within the envelope of the glycoprotein 2 subunit[21]. ST-294 targets the interaction of the transmembrane fusion subunit with the stable signal peptide, and then interferes with envelope glycoprotein-mediated membrane fusion[22]. However, these results were primarily derived from experiments focused on the Lassa and Junin viruses[21,22]. Although there is no sufficient evidence to confirm that ST-193 and ST-294 can be used for the SABV, it is possible that these compounds also have anti-SABV activity. The similarities in envelope composition between the SABV, Lassa, and Junin viruses need to be assessed to be able to extrapolate the results[21,22] mentioned above.

Clinical and experimental evidence has shown that the antiviral drug ribavirin can be used to treat SABV infection[23,24]. As Radoshitzky et al.[25] have suggested, antiviral drugs focused on viral transcription and replication can work through different routes. Because of that, structural studies on arenaviral RNA-dependent RNA polymerase and nucleoprotein could facilitate the development of antiviral drugs that are effective for the treatment of New World arenavirus infections.

Four documented cases of Sabiá virus infection

Only four cases of SABV infection have been reported to date (**Table 1**). Herein, infections that occurred outside the

TABLE 1

Summary of the four cases of Sabiá virus infection.

Case	Year	Location	Situation	Outcome	Reference
First	1990	São Paulo State, Brazil	Naturally acquired infection	Fatal	Coimbra et al.[1]
Second	1992	Pará State, Brazil	Occupational exposure (laboratory environment)	Non-fatal	Vasconcelos et al.[27]
Third	1994	Yale University, Connecticut (USA)	Occupational exposure (laboratory environment)	Non-fatal	CDC[28]
Fourth	1999	São Paulo State, Brazil	Naturally acquired infection	Fatal	Coimbra et al.[12]

USA: United States of America; **CDC:** Centers for Disease Control and Prevention.

laboratory environment will be termed *naturally acquired infections*. Detailed information on the relevant aspects of each case are presented below.

First (index) case - The SABV was first isolated in 1990 from a fatal case of hemorrhagic fever in São Paulo State (Southeastern Brazil). This index case was a 25-year-old female agricultural engineer who experienced fever, myalgia, headache, nausea, vomiting, and weakness for 12 days before seeking medical attention. She eventually developed conjunctival petechiae, hematemesis, vaginal bleeding, increased sleepiness, tremors, difficulty walking, and generalized tonic-clonic seizures; she died of the infection on the fourth day of hospital admission. The necropsy indicated necrosis of and hemorrhage in various organs[1].

After a series of tests performed on a blood sample of the patient at the *Instituto Adolfo Lutz* (Brazil), *Instituto Evandro Chagas* (Brazil), Yale Arbovirus Research Unit (USA), and U.S. Army Medical Research Institute of Infectious Diseases (USA), the causative agent of the disease was identified as a new virus of the family *Arenaviridae*. The pathogen was named after the district where the patient lived, Sabiá. Until then, only four arenaviruses causing hemorrhagic fevers were known: Lassa, Junin, Machupo, and Guaranito[1]. SABV causes the disease now known as *Brazilian hemorrhagic fever* or *São Paulo hemorrhagic fever*[26]

Second case - While working on the characterization of the SABV in a reference laboratory in Pará State (Brazil), a 39-year-old male laboratory technician was infected with the virus (probably through aerosol) in 1992. He developed a prolonged influenza-like illness. However, this case was not fatal, possibly because the patient had sought medical attention and received treatment (basically fluid control) soon after the first symptoms appeared[1,27].

Third case - Similar to the second case, the third case of infection with clinical disease caused by the SABV happened in 1994. It was an accidental laboratory exposure, likely linked to aerosol transmission. During routine work in a biosafety level 3 laboratory, a 46-year-old male research scientist at Yale University (USA) was infected with the virus after an accident involving a bucket centrifuge. After centrifugation of approximately 240mL supernatant from a tissue culture infected with SABV, the researcher realized that the tube used for the centrifugation was broken, and that a large portion of container contents (approximately 100mL) had leaked into the rotor of the centrifuge. The infection possibly happened by a leaking rotor or during the cleaning/opening procedure of the centrifuge, although the researcher was not directly exposed to the leaked content and used personal protective equipment[8,23,28,29]. Human error was recognized as the principal factor causing this incident[30].

This patient fell ill but survived after being hospitalized and receiving the antiviral drug ribavirin. Based on this case, it could be suggested that ribavirin could be used to treat future cases of SABV infection[23]. Supporting this, evidence exists that ribavirin can be used to treat Argentine hemorrhagic fever, which is caused by the Junin virus[24].

Considering that two SABV infections occurred during work routines in reference laboratories with biosafety equipment, it is likely that the SABV is transmitted through aerosol. Thus, additional biosafety precautions should be undertaken when handling the virus[23]. Currently, it is recommended to handle the SABV in biosafety level 4 laboratories[31].

Fourth case - The fourth case of SABV infection was reported in 1999 and was naturally acquired. A 32-year-old male coffee-grain machine operator, resident of a rural area (Espírito Santo do Pinhal) of the São Paulo State, presented with a febrile illness. After hospitalization for seven days, the patient died. It was found that the SABV was responsible for the pathological condition of the patient[12]. Similar to the second case, little information is available in the literature on this fourth case of SABV infection.

Eco-epidemiological aspects of naturally acquired Sabiá virus infections

Due to the scarcity of studies on the identification of the SABV host species and its circulation among different animals, little is known about the eco-epidemiological aspects of naturally acquired SABV infections. Similarly, to the best of our knowledge, no serological study has been conducted in populations living in locations where the two cases of natural acquired SABV infections were identified. Serological and epidemiological studies are particularly necessary to assess

the epidemiology, routes of transmission, and prevalence of infection of the virus among humans. Importantly, subclinical SABV infections might occur without being identified.

The difference in locations (urban area *vs.* rural region of São Paulo State) of the two naturally acquired infections is worthy of further investigation. Due to the typical characteristics (activities in open areas, proximity to forest environments, among others) of the rural environment that propitiate the closest coexistence of humans and wild rodents, infections by arenaviruses are quite plausible. Farming activities undertaken by the infected man are thought to have facilitated his exposure to rodent excreta[12].

The SABV circulation in non-human animals of urban areas was little investigated. Recently, Bisordi et al.[9] performed a serological investigation among rodents that were captured in the city (Espirito Santo do Pinhal) where this case was reported to identify the host rodent species of the SABV. A total of 412 rodents of 7 different species (*Necromys lasiurus*, n = 164; *Akodon* spp., n = 116; *Calomys tener*, n = 68; *Mus musculus*, n = 55; *Oligoryzomys nigripes*, n = 7; *Bibimys labiosus*, n = 1; and *Rattus rattus,* n = 1) were evaluated. However, none of the rodents showed evidence of SABV infection.

The factors facilitating SABV infection in an urban environment are even less understood. It is only known that the index patient worked in an office, had not traveled outside São Paulo State two months before becoming sick, and spent time in two different cities of São Paulo State with family and friends ten days prior to the symptoms. No family member or friend got sick[1]. A study investigating the activities performed by the patient a few days before the symptoms might help explain the conditions (mainly route of infection and contact with potential SABV hosts) that facilitated the infection. However, such a retrospective investigation is not feasible. Thus, studies investigating the potential host species of the SABV and serological studies should be conducted in the urban area where the first case of naturally acquired SABV infection was identified.

Perspectives on the ecology and surveillance of the arenaviruses

Infections linked to occupational exposure can be avoided by applying more stringent biosafety measures when handling the SABV. However, it is difficult to predict if naturally acquired infections will occur, since the ecology of the virus is poorly understood.

Taking into consideration the wide Brazilian biodiversity and the potential viruses existing within it, studies on viral ecology in the Brazilian ecosystem should be performed. This is important because only indentifying the pathogens, their hosts, and their potential to infect different species, will it be possible to prevent and combat new diseases and future potential epidemics that might affect humans[32]. Studies on zoonotic diseases may support strategies for controlling the circulation of pathogens between human and wild/domestic animals. However, studying the ecology of viral zoonoses and broad-spectrum surveillance are rather expensive because it generally involves a great number of professionals from various areas, survey of a large number of biological samples from human and non-human animals, and different methodologies of viral detection. Considering that developing countries are often most affected by tropical infectious diseases, conducting these studies might not be financially feasible. Thus, to develop public health strategies to detect adequately human and veterinary diseases and their zoonotic sources is essential. In addition, establishing simple and well-structured medical surveillance and reporting systems are important for the epidemiological control of viral diseases.

CONCLUSION

Figure 1 depicts the main events related to the history of the SABV. To date, four cases of SABV infection (two naturally acquired and two linked to occupational exposure in laboratories) have been reported in the literature. Little is known about the ecology of the SABV. In addition, the virus' reservoir species are unknown, although they are likely rodents. To date, the available data indicate that SABV is found naturally in Brazil; however, its occurrence in other countries cannot be ruled out. Current guidelines recommend handling the virus only in a biosafety level 4 environment. The antiviral drug ribavirin might be useful for the treatment of SABV infection in humans. The potential of the virus to cause an outbreak or epidemic is not known, but should not be overlooked by the scientific and medical communities.

Since many questions on the threat posed by the SABV, other viral pathogens, and recent outbreaks involving emerging viruses remain, studies on viral ecology (in particular in tropical countries) should be encouraged. Moreover, strategies to control potential outbreaks must be planned. Specific to the SABV, we strongly suggest that it is crucial to: I) identify the viral reservoir species; II) investigate the geographic regions of occurrence of the host/pathogen; III) identify routes of infection; IV) develop or identify antiviral treatments; V) establish rapid and accurate methods for diagnosis; VI) establish standards and recommendations for scientific research with this pathogen to ensure safety of researchers. Due to the scarce number of studies focused on SABV, to investigate this virus presents an opportunity for the discovery of important aspects of its biology, pathogenesis, and ecology.

Conflicts of interest

The authors declare that they have no conflict of interest.

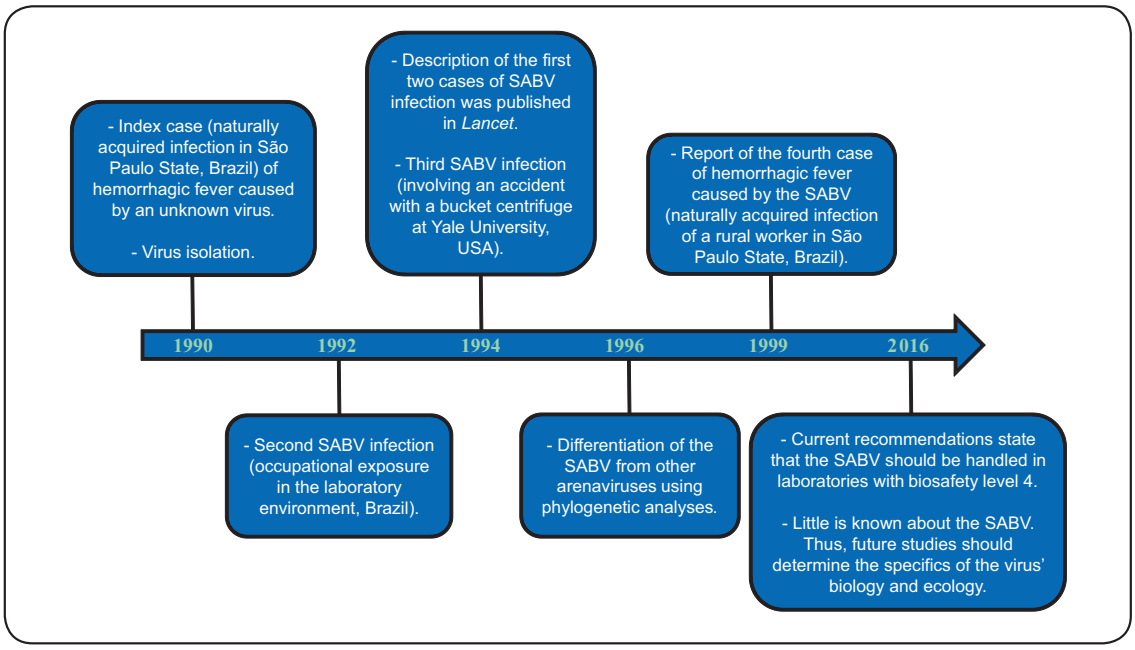

FIGURE 1 - Timeline of the main events related to the history of the Sabiá virus. SABV: Sabiá virus.

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CAPÍTULO IV

Rocio virus: an overview

Este capítulo apresenta o seguinte artigo de revisão publicado no periódico *Revista Peruana de Divulgación Científica en Genética y Biología Molecular (RDGBM)*:

Ellwanger JH, Kaminski VL e Chies JAB (2017) Rocio virus: an overview. RDGBM 1: 14-20.

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Artículo de revisión

Rocio virus: an overview
El Virus Rocío: Una visión general

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Resumen

El virus Rocío (ROCV) causó un brote epidémico de encefalitis viral en Brasil entre 1975 y 1980. En la actualidad este virus es considerado un agente emergente y representa una constante amenaza a la población brasilera, la cual en algún momento podría sufrir otro brote de infección por este virus. A pesar de la importancia de este patógeno, poco se sabe sobre sus aspectos biológicos. Así, con el objetivo de despertar el interés de la comunidad científica sobre este vírus, en la presente revisión describimos sus aspectos: epidemiológico, patológico, morfogenético y ecológico. Además algunos hechos históricos son relatados en este artículo.

Palabras clave: Enfermedades emergentes, Flavivírus, brote epidémico, virus Rocio, encefalitis viral, ecologia viral.

Abstract

Rocio virus (ROCV) was identified as responsible for an outbreak of human encephalitis in Brazil between 1975 and 1980. Currently, ROCV is considered an emerging virus and represents a constant threat to the Brazilian population, which at any moment may suffer from a new outbreak of encephalitis by ROCV. Despite the importance of this pathogen, little is known about its biological aspects. Aiming to draw attention to this virus, in this review we described epidemiological, pathological, morphogenetic, and ecological aspects about ROCV. Some historical facts regarding the virus are also presented in this article.

Key words: emerging diseases, Flavivirus, outbreak, Rocio virus, viral encephalitis, viral ecology.

Introducción

Natural environments are rich in biodiversity and potentially harbor a large number of pathogens, including unknown viruses. Some of these microbes have the capacity to infect humans, although generally use only wild animals as hosts. Unbalances and changes in the natural environment that approximate hosts or pathogen vectors to the human population act as facilitators to the emergence of new infectious diseases in humans (1-5). In addition, the simple fact of humans penetrate into wild natural environments where pathogens are naturally found facilitates infections from wild animals to humans. For example, a study (6) performed in the Netherlands showed that active forestry workers are at high risk to develop Hemorrhagic fever with renal syndrome and Lymphocytic choriomeningitis, both viral diseases. We can also assume that people became more susceptible to infections traveling to regions where an endemic disease occurs or visiting natural environments where vectors of pathogens are found.

Many viruses that until recently were absolutely unknown, are currently identified, classified, and with their ability to infect humans well recognized. This is the case of the Human Immunodeficiency Virus (HIV), discovered in the early 1980s and currently hosted by nearly 37 million people worldwide (7). Some viruses cause infections in a seasonal pattern, such as Influenza viruses (8). Other pathogens arise, cause few/isolated infections and then disappear, as is the case of the Sabiá virus (SABV). To date, only two natural infections by SABV have been reported, both in Brazil and with fatal outcomes (9-11). Little is known about the biological aspects of this pathogen. Similarly to the pathogens that cause isolated human infections, some viruses arise, cause outbreaks or epidemics, and then virtually no additional cases of new infections are recorded over a long time. Rocio virus (ROCV) is an example of pathogen that showed this infection behavior.
ROCV was identified and described in Brazil during an outbreak of human encephalitis (encephalitis by ROCV - enROCV). This outbreak began in the middle of the 1970s and ended at the beginning of the 1980s. ROCV can cause a serious pathological condition and is a potential causative agent of new outbreaks/epidemics in the Brazilian population. However, similarly to the SABV, little is known about ROCV. In addition, this pathogen can be considered a neglected virus by both scientific and public health authorities. Aiming to draw attention to this pathogen, in this article, we reviewed the biological aspects of the ROCV and synthetically present some historical facts about this virus.

Epidemiology and historical facts

ROCV belongs to the Flavivirus genus, as well as other well-known viruses, like Dengue, Saint Louis encephalitis and Yellow Fever (12). It is considered an emerging zoonosis in Latin America (13). Except the data accessed on experimental conditions, all information about ROCV were obtained through the analysis of an outbreak of human acute encephalitis that occurred in the Vale do Ribeira and Baixada Santista regions (São Paulo State, southeastern Brazil). Despite the outbreak only have been detected in 1975, it is likely to have already started in 1973. The end of the outbreak occurred in 1980 (12, 14). Five cases of infections by ROCV were registered outside the São Paulo State during the outbreak, all of them in Paraná, a state bordering São Paulo (15).
The outbreak of enROCV affected 1,021 people, caused approximately 100 deaths, and left sequelae in more than 200 individuals affected by the disease (12). The mortality rate of the disease was estimated at 13% (16). Adult males (aged 15-30 years) were the most severely affected (16, 17). Soon after the end of the outbreak, it was suggested that out-of-door exposure during routine work could be one of the causes for this group have been the most affected by enROCV (17)
ROCV was isolated and identified for the first time in 1975 from samples of the central nervous system (CNS) tissue of a 39-year old male who died from the disease. The name "Rocio" was given because it was the name of the village where the patient lived, located in the city of Iguape (São Paulo State, Brazil). Two sentinel mice exposed to the environment of the outbreak were infected by ROCV. Moreover, the virus was also detected in a rufous-collared sparrow collected in the same region (18). This data showed the circulation of the ROCV and its capacity to infect different animal species in the ecological system where the outbreak occurred.
After the end of the outbreak in 1980, little evidence indicated the circulation of the ROCV among Brazilian population. Tavares-Neto et al. (19) identified in 1984 the presence of antibodies against ROCV in a 12-year-old girl from the village of Corte de Pedra (Bahia State, Brazil). Iversson et al. (20) described serological findings (IgM antibodies) against ROCV in two children from the rural area of the Vale do Ribeira. Sera used for the analyzes were collected in 1987. Subsequently, Straatmann et al. (15) reported eight cases of humans with anti-ROCV antibodies from four cities of the Bahia State (Brazil). Although these data indicated the circulation of the ROCV in Brazil, the authors drew attention that these serological findings should be interpreted with caution. This prudence is needed due to the possibility of antigenic cross-reactivity with other flaviviruses. Recently, de Figueiredo and Figueiredo (21) cited two cases of ROCV infection (detected in 2010) in individuals from the city of Manaus (Amazon State, Brazil). Manaus is located at a distance greater than 2,000 km from the original enROCV outbreak region. Although scarce, these data indicate the circulation of the ROCV in different regions of Brazil after the end of the outbreak in 1980.
Few authors have focused on the historical aspects of the ROCV outbreak. Thus, data on these aspects are also scarce. However, Villela and Natal (22) performed a relevant work addressing the coverage of the media on the ROCV outbreak. The authors note that in face of the reports released by the print media about enROCV, tourists stopped going to the affected area. Consequently, a crisis occurred in the local market, causing revolt in the traders. Recently, Azevedo (23) made an important report for the Brazilian newspaper O Globo recalling historical facts about the outbreak. Interestingly, the journalist described reports from people who had a prominent role in the study and combat of the ROCV during the outbreak.

Vectors and transmission

Mosquitoes are the vectors of the ROCV. Different authors considered Aedes scapularis and Psorophora ferox as potential vector species of the virus (24-27). Laporta et al. (27) showed that people are highly exposed to bites by Aedes scapularis and Psorophora ferox in the region where the enROCV outbreak occurred. However, these species are present and can be considered potential vectors of the ROCV in other regions where there are no recorded cases of the disease, as the states of Goiás (28) and Rio Grande do Sul (29). Culex mosquitos may also be considered as potential vectors of the ROCV (17).

There is no evidence of other natural routes of ROCV transmission besides mosquito bites. Lopes et al. (16) drew attention to the fact that when assessing families living where the enROCV outbreak occurred, in 75% of cases only one family member got sick. The same authors described that there are no reported cases of infections among the medical staff who took care of the patients during the outbreak. Iversson et al. (30) also found no higher anti-ROCV antibodies prevalence among persons cohabiting with patients affected by enROCV as compared to other individuals. Such evidence indicates that there is no direct ROCV transmission from human to human.

The inability of ROCV to be transmitted between humans without the interference of specific vectors suggests that this pathogen is not able to sustain long epidemics. To cause a long epidemic, in addition to transmission from non-human animals to humans, many cycles of transmission between humans would also be necessary (31). Although the natural ROCV route of transmission appears to be restricted to mosquito bites, other possible routes of transmission should not be neglected. For example, early in the Zika virus epidemic in Brazil, it was believed that the virus was transmitted only by the bite of mosquitoes. However, currently it is known that other forms of transmission, such as sexual transmission, are also possible (32). Laboratory infections by ROCV were also reported (33). It is believed that transmissions in the laboratory environment may have been caused by aerosol, during the manipulation of samples with large viral loads in inappropriate biosafety conditions (23).

Structural and molecular characteristics

ROCV is morphologic and morphogenetic similar to other flaviviruses. Viral particles are spherical (34) and their size range from 34 to 43 nm (34, 35). In agreement with Harisson et al. (36), mature virus particles have a mean diameter of 39 nm. Using an animal model, Tanaka (35) showed that in the brain infected by ROCV, viral particles are found in the light of the reticular system of the cytoplasm and in the cisterns of the Golgi complex. Infected cells showed no mitochondrial changes. Moreover, the same author has found no evidence of the participation of the nucleon in ROCV replication. A complete list of ROCV proteins and their corres-pondent lengths can be found in the studies of Junglen et al. (37) and Medeiros et al. (38). Figueiredo et al. (39) described a Reverse Transcription – Polymerase Chain Reaction (RT-PCR) method to identify Brazilian flaviviru-ses, including ROCV. Posteriorly, Medeiros et al. (38) sequenced and characterized the entire ROCV genome. ROCV is a single-stran-ded and positive-sense RNA virus. The viral genome is composed of 10,794 nucleotides including an open reading frame of 10,275 nucleotides. This open reading frame is flanked by a 5' non-coding region of 92 nucleotides and a 3' non-coding region of 427 nucleotides. Interestingly, this was the first study to sequence and characterize the complete genome of a Brazilian Flavivirus. Baleotti et al. (40) performed a phylogenetic study of 15 strains of 10 Brazilian flaviviruses based on nucleotide and amino acid sequences of the *NS5* gene. In this study, the authors grouped the viruses into three main branches: (I) dengue, (II) Japanese encephalitis virus (JEV) complex, and (III) yellow fever branches. ROCV belongs to the JEV branch according to neighbor-joining and parsimony phylogenetic trees. Medeiros et al. (38) carried out multiple protein and phylogenetic analyses and reinforced the close relationship between ROCV and Ilheus virus (ILHV), as previously described by other authors (40, 41). However, despite such close relationship, data published by Medeiros et al. (38) confirmed that ROCV is a distinct pathogen from ILHV.

Infection and pathogenesis

As well as other flaviviruses, ROCV can cross the blood-brain barrier and cause encephalitis (42-44). The virus incubation period is 7-14 days. Young men are the individuals most affected by the disease (12). Signs, symptoms, and sequelae of the ROCV infection are described in Table 1.

Table 1. Signs, symptoms, and sequelae of a ROCV infection*.

General signs and symptoms	Encephalitis signs / neurologic symptoms (generally appear later)	Sequelae
abdominal distention, aerophobia, anorexia, coma with respiratory complications, falling, fever, headache, hyperemia of the oropharynx and conjunctivae, lacrimation, lassitude, lethargy, malaise, mastication, myalgia, nausea, photophobia, stupor, urinary retention, vomiting, weakness	blindness, consciousness alterations, convulsions, deafness, dysarthria, dyslalia, meningeal irritation, motor abnormalities (especially gait and impaired equilibrium), reflex disturbances,	disturbances in visual, auditory and olfactory acuity, dysarthria, dysphagia, memory disturbances, motor abnormalities (especially gait and impaired equilibrium), motor incoordination, paresthesia, sphincter incontinence, strabismus

*This table lists many signs or symptoms known or possible to happen during ROCV infection. However, signs/symptoms/sequelae do not necessarily occur together and/or in all patients (14, 16, 42, 44).

Rosemberg (45) studied eight human cases of enROCV. In the brain, the more damaged structures were thalamus, dentate nucleus, substantia inominata, brain stem, spinal cord, and basal nuclei. Gray matter was the most injured region. In the thalamus, a definitive loss of nerve cells was observed. Interstitial mononuclear infiltration, microglial proliferation, thalamic inflammatory necrosis, and perivascular lymphocytic cuffing were described amongst the pathological findings. It is important to highlight that, to the best of our knowledge, this was the first author to demonstrate the neuropathological findings of the infection by ROCV in humans, having already published preliminary findings in 1977 (46). Moreover, Harrison et al. (36) demonstrated that ROCV could cause changes beyond the CNS. In their study, using hamsters as an animal model, heart and pancreas were the organs most affected by the infection.

Infection and pathogenesis of the ROCV were addressed by few authors until now, although relevant results were obtained from these studies. For example, Barros et al. (43) evaluated the contribution of cytokines and nitric oxide (NO) to the outcome of infections by Brazilian flaviviruses and to the aspects of the replication of these pathogens, including ROCV, and their results suggested an absence of NO involvement in ROCV infectivity. Posteriorly, Dias de Barros et al. (44) using the Balb/C mouse strain found that ROCV induces neuronal degeneration and apoptosis in the CNS, and that this phenomenon was associated with an inflammatory process. In relation to neuronal death caused by ROCV, it is still debated whether neuronal death is caused directly by the viral replication or if it is the result of the inflammatory process induced by the viral infection (47). In a hamster model to study the persistence of the ROCV infection/pathogenesis, Henriques et al. (48) showed that ROCV can be found in viscera, brain, blood, serum, and urine. The virus was also detected by quantitative RT-PCR in the brain, liver, and blood (as long as three months after infection). In addition, the virus caused histopathological changes in the liver, kidney, lung, and brain. Viral antigens were detected in these organs up to four months after infection. Moreover, ROCV induced a strong immune response in the animals. These results indicate that ROCV affects different organs than the brain in a persistent way. However, these data should be interpreted with caution, since they came from an animal model (using intraperitoneal infection). It is not known whether these results would be similar in humans.

Chávez et al. (47) demonstrated in mice that the CC-chemokine receptor 5 (CCR5) and macrophage inflammatory protein (MIP-1 α) are important in the outcome of the ROCV infection. Shortly, infecting CCR5 and MIP-1 α knockout and wild-type mice with ROCV, knockout mice survived longer and had reduced brain inflammation as compared to the wild-type animals. Based on these data, the authors suggested that CCR5/MIP-1 α axis contributes to the migration of infected cells to the brain and affects ROCV pathogenesis.

Recently, Franca et al. (49) studied the immune response induced by ROCV using an experimental mice model. In this study, interleukin 33 (IL-33) signaling was essential to attenuate the development of the enROCV by downregulating the expression of nitric oxide synthase in the CNS.

Results obtained with the development of a vaccine against ROCV were published in 1980. The vaccine was tested in humans. However, the immunogenicity of the vaccine was not satisfactory (50). To date, there is no effective vaccine against ROCV. Figueiredo (51) pointed out that the development of a broad-reactive JEV complex vaccine offering protection to the ROCV and other viruses of the same complex is required.

Ecological aspects and surveillance

The amount of knowledge regarding ecological aspects about ROCV is scarce. For example, the reasons for the appearance and disappearance of the ROCV in the Vale do Ribeira are still a mystery (44). Since the end of the enROCV outbreak in the 1980s, some authors believed that besides mosquitoes acting as vectors, birds were also involved in the transmission cycle of the ROCV as natural hosts (25). This is quite likely because ROCV was found in a rufous collared sparrow (Zonotrichia capensis) in the country of Sete Barras (São Paulo State, Brazil) (18). Currently, it is still believed that wild birds are responsible for keeping the virus in the form of a naturally occurring zoonosis (44, 51).

Ferreira et al. (52) called attention to the fact that there are records of the enROCV in people who do not have left the area around their homes. The authors also presented data suggesting the circulation of the ROCV among wild birds in the Atlantic Forest region of the São Paulo State. According to these authors, the pathogen could move from São Paulo State to other Brazilian regions through migratory birds. The circulation of the virus through birds would explain the cases of the enROCV in individuals who were not exposed to high-risk areas of infection by ROCV, as highlighted by Figueiredo (51). This possibility makes the reemergence of the ROCV a permanent threat to the Brazilian population.

Importantly, other animals, besides mosquitoes and birds, can host and be involved in the transmission cycle of the ROCV. Two strains of the virus were isolated from sentinel mice exposed in the city of Cananéia (São Paulo State, Brazil) (18). Casseb (53) found a prevalence of 5.61% for ROCV antibodies in domestic herbivores in the Pará State. ROCV also circulates among water buffaloes (Bubalus bubalis) in Brazilian Amazon (54). In addition, horses seem to be important hosts of the ROCV. One equine seropositive for ROCV was found by Pauvolid-Corrêa et al. (55) in the Brazilian Pantanal region. Silva et al. (56) reported serological data suggesting that ROCV previously circulated among horses in different regions of Brazil (northeast, west-central, and southeast). Considering the presence of antibodies anti-ROCV in horses from different parts of Brazil, these authors stand out that other outbreaks of ROCV may be occurring without being detected.

Recently, Neves and Machado (57) warned about the re-emergence risk of the ROCV in Brazil and highlighted the importance of epidemiological surveillance of the ROCV circulation. We add that this surveillance must be carried out both in animals (wild or domestic) and humans. Khan et al. (58) developed a DNA microarray platform (SMAvirusChip) for screening a large set of viruses transmitted by small mammals and arthropods, including ROCV. Methods like this can be very useful for the early detection of the ROCV circulation in the population.

ROCV is classified as an emerging virus (51). The emergence or re-emergence of a pathogen among the human population is a complex event. For example, it is believed that the emergence of the HIV/AIDS in Africa has been caused by a series of ecological and social changes (59). Similarly, according to Pedroso and Rocha (5), the contributing factors to the emergence of the ROCV (among other diseases) were: ecological changes, economic development, and manipulation of land (classified as major factors). As specific factors, the authors quote agriculture, dams, deforestation and reforestation, changes in water ecosystems, floods and droughts, famine, and climate change.

Zoonoses are infectious diseases transmitted naturally between humans and non-human animals (wild or domestic) (60). Taking this definition into consideration, ROCV can be considered a zoonosis. In agreement with Slingenbergh et al. (60), the emergence of zoonoses and the spread of diseases are usually caused by human activity, being humans also responsible for prevention of such situations. For this, the fight against emerging diseases requires the effort of professionals from different areas (61). This is due to the complex and different socioecological factors that influence the dissemination of a pathogen among the human population. In other words, the emergence of diseases can be considered the result of disturbances in human, animal, and environmental health (62). In our point of view, ROCV is within this context. However, in order to prevent the possible re-emergence and spread of the ROCV among humans, the study and the better understanding of the basic ecological aspects of this virus are necessary (63).

Conclusion

The diversity of the Brazilian nature hides several pathogens. Most of them will probably never cause infections in humans. However, sometimes, due to socioenvironmental disturbances, some new infections, originally derived from wild or even domesticated animals, may emerge amongst human populations. This was the case of the ROCV, which emerged in Brazil in 1975 and caused an outbreak of human encephalitis. Due to the lack of knowledge about this pathogen, it is not possible to known with certainty which factors contributed to its emergence and to its subsequent "disappearance".

Morphogenetically, ROCV resembles other flaviviruses. Data obtained from experimental studies and through the analysis of samples from people who died of enROCV indicate that ROCV causes a very complex and serious pathological state, and this must be taken as an important alert to our health services. Measures for monitoring virus circulation between human and non-human animals are required. The development of a vaccine against ROCV is also essential to prevent a new outbreak of encephalitis among Brazilian population.

From a scientific perspective, the history of the ROCV is quite interesting. We believe that this overview will help to gather relevant information on the historical, pathological, epidemiological, morphogenetic, and ecological aspects about ROCV. Moreover, we hope that this study will help to raise the scientific community's interest about ROCV and to alert public health authorities regarding the importance of surveillance of this pathogen.

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CAPÍTULO V

Exosomes in HIV infection: A review and critical look

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Review

Exosomes in HIV infection: A review and critical look

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ABSTRACT

Exosomes are nanovesicles released into the extracellular medium by different cell types. These vesicles carry a variety of protein and RNA cargos, and have a central role in cellular signaling and regulation. A PubMed search using the term "exosomes" finds 67 articles published in 2006. Ten years later, the same search returns approximately 1200 results for 2016 alone. The growing interest in exosomes within the scientific community reflects the different roles exerted by extracellular vesicles in biological systems and diseases. However, the increase in academic production addressing the biological function of exosomes causes much confusion, especially where the focus is on the role of exosomes in pathological situations. In this review, we critically interpret the current state of the research on exosomes and HIV infection. It is plausible to assume that exosomes influence the pathogenesis of HIV infection through their biological cargo (primarily membrane proteins and microRNAs). On the other hand, evidence for a usurpation of the exosomal budding and trafficking machinery by HIV during infection is limited, although such a mechanism cannot be ruled out. This review also discusses several biological aspects of exosomal function in the immune system. Finally, the limitations of current exosome research are pointed out.

Contents

1. Introduction

An infection with the Human Immunodeficiency Virus (HIV) is today considered a chronic disease by some researchers, unlike three decades ago, when its poor prognosis meant that infected individuals only had a few year, or even months, of life left. The main achievement leading to the control of HIV infections has been the development of Highly Active Antiretroviral Therapy (HAART). Since then, recent

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studies have shown promising results that may effectively contribute to the fight against HIV. Antibodies, both against the host CD4 molecule and against the viral envelope, have been tested in humans and shown the capacity to reduce HIV viremia (Caskey et al., 2015; Caskey et al., 2017). The fusion protein eCD4-Ig was also effective in inhibiting HIV by binding to conserved regions of the HIV envelope glycoprotein; it could be considered as a potential agent for the development of a vaccine against the virus (Gardner et al., 2015). C-C chemokine receptor type 5 (CCR5) blockage is another important strategy for preventing cellular infection by HIV (Zhou et al., 2015). Furthermore, the CRISPR/Cas-9 genomic editing technology represents another promising strategy that could be used as a way to combat HIV, since it allows the inactivation of the viral genetic material integrated into the cellular genome (Ebina et al., 2013; Liao et al., 2015; Zhu et al., 2015).

All these methodological strategies are highly beneficial in the fight against the virus and, as such, very stimulating for researchers in the field of HIV and Acquired Immune Deficiency Syndrome (AIDS). Together, (I) the use of potential antibodies and vaccines, (II) the advances in our understanding of HIV biology and viral interaction with the host immune system, (III) the use of different strategies for prevention and treatment, in addition to (IV) the development of new approaches to attack the virus in its viral reservoirs suggest that in the near future, it will be possible to control the infection and perhaps to eradicate the AIDS pandemic. However, various methodological challenges still exist, mainly around our understanding of viral reservoir biology and the potential development of therapies focused on it (reviewed in Barouch and Deeks, 2014). It seems clear that future breakthroughs will depend on unraveling previously unexplored aspects of the interplay between the virus and the immune system.

Given intrinsic and extrinsic human diversity, the response to HIV infection and HAART will always vary between individuals. For example, there are individuals in which HIV evolves rapidly to AIDS (Olson et al., 2014). At the other extreme are the so called Elite Controllers, who, in spite of seropositivity, have an undetectable viral load and maintain their immune functions near normal levels, even over 10 years after seroconversion (reviewed in Poropatich and Sullivan, 2011). However, this apparent functional balance could be disrupted by various physiological events, unleashing AIDS. To gain a detailed understanding of HIV biology and the modulation of disease progression by individual or transient factors of the immune system, previously unstudied relationships between HIV infection and the immune system should be investigated.

Classical features of this relationship (such as viral replication, virus-cell interaction, and HIV-derived immune activation) have already been extensively explored. However, there are still blank spots with the potential to provide important insights into the control of the HIV infection by the immune system. Similarly, little is known about how the immune system behaves differently from those situations in which the virus overrides various arms of the immune system.

A topic of growing interest in immunology is the influence of exosomes and other extracellular vesicles (EVs) on the course of human diseases. Concerning HIV, much effort has focused on understanding the relationship between the course of HIV infection and exosome release, as well as on the potential therapeutic use of these nanovesicles in different strategies to combat HIV. In this review, we will present and discuss current knowledge about the relationship between exosomes and HIV infection. In addition to available experimental data, we also discuss hypothesis papers that present a summary of, or otherwise significantly contribute to, the subject addressed here. Finally, we present an overview of the role of exosomes from biological fluids in the course of HIV infection, as well as new insights into the potential role of these nanovesicles in HIV therapy. Because most studies focused on HIV were performed with HIV type 1, in this article we chose to always refer to the virus as “HIV”.

2. Exosomes: basic aspects and their relationship with HIV

The EVs that are currently known as exosomes were first described in the 80s, when they were found in red blood cells (Allan et al., 1980), neoplastic cell lines (Trams et al., 1981), and reticulocytes (Harding et al., 1983; Johnstone et al., 1987; Pan et al., 1985). Over the next decade, exosomes received little attention, until they returned to the forefront of research when their presence was described in B lymphocytes and dendritic cells (Raposo et al., 1996; Zitvogel et al., 1998).

Today, it is known that exosomes are nanovesicles released by different cell types, alongside other types of circulating EVs that occur in mammals, such as microvesicles/ectosomes and even apoptotic bodies. What distinguishes exosomes from other types of EVs is that they are derived from multivesicular bodies (MVBs), i.e. they are of an endocytic origin. Exosomes are spherical, with a diameter of 30 to 100 nm. They are rich in endosome-associated proteins, such as the tetraspanin tumor susceptibility gene 101 protein (TSG101) and ALIX (both associated to multivesicular body biogenesis), as well as other proteins of the tetraspanin family (reviewed in Mincheva-Nilsson and Baranov, 2010). Based on proteomic data, Kowal et al. (2016) suggested a classification of different EV subtypes. According to this classification, the tetraspanins CD63, CD9, and CD81 may be considered the best exosome markers to date.

In addition to membrane-associated proteins, exosomes carry a myriad of different molecules in their lumen, including proteins, mRNAs, microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). Exosomes are secreted by a wide variety of cells. After their release into the extracellular space, exosomes may break down, releasing their content into the extracellular space, or remain intact, able to interact with target cells over short or long distances. Such interactions may involve direct fusion with the plasma membrane and the discharge of exosomal contents into the cytoplasm, or the entrance into the cells through different types of endocytosis, where both membrane and lumen components are internalized. In addition to host cargo, exosomes are likely to transport pathogen-derived cargo (reviewed in Madison and Okeoma, 2015). Exosomes are present in every body fluid investigated to date, including amniotic fluid (Bretz et al., 2013), breast milk (Näslund et al., 2014), bronchoalveolar lavage fluid (Levänen et al., 2013), cerebrospinal fluid (Street et al., 2012), malignant ascites (Bretz et al., 2013), plasma (Baranyai et al., 2015), saliva (Zlotogorski-Hurvitz et al., 2015), semen (Madison et al., 2014), synovial fluid (Skriner et al., 2006), urine (Hiemstra et al., 2014), and vaginal fluid (Smith and Daniel, 2016). Fig. 1 shows a representation of common exosome components and summarizes their most important characteristics.

The diversity of the possible exosomal cargo translates into a wide variety of biological functions. Arguably, intercellular communication and transport of biomolecules are among the most important of these functions. Cell receptors, adhesion molecules, cytokines, and other cell signaling molecules may be transported from one cell to another by exosomes, thus assigning, even if transiently, new functions to the recipient cells. Moreover, RNAs carried by exosomes may modulate gene expression in recipient cells. Of particular interest is the immunomodulatory role of exosomes. Depending on the cellular origin or even physiological situation, exosomes may negatively or positively stimulate the immune system (reviewed in Robbins and Morelli, 2014). For example, in the context of viral infections, exosomes containing hepatitis C virus (HCV) RNA were reported to activate plasmacytoid dendritic cells (Dreux et al., 2012). On the other hand, in cancer, exosomes can have an immunosupressive role (Rong et al., 2016). All of these biological features could be relevant in the context of HIV infection, where the virus takes over the cell machinery, exploiting several cell-derived mechanisms in its favor. Regarding the exosomal release pathway, several mechanisms have been suggested to be exploited by HIV, such as a) its release from the cell as an exosome, b) the release of its mRNA/miRNAs into exosomes, c) the release of virus particles surrounded by

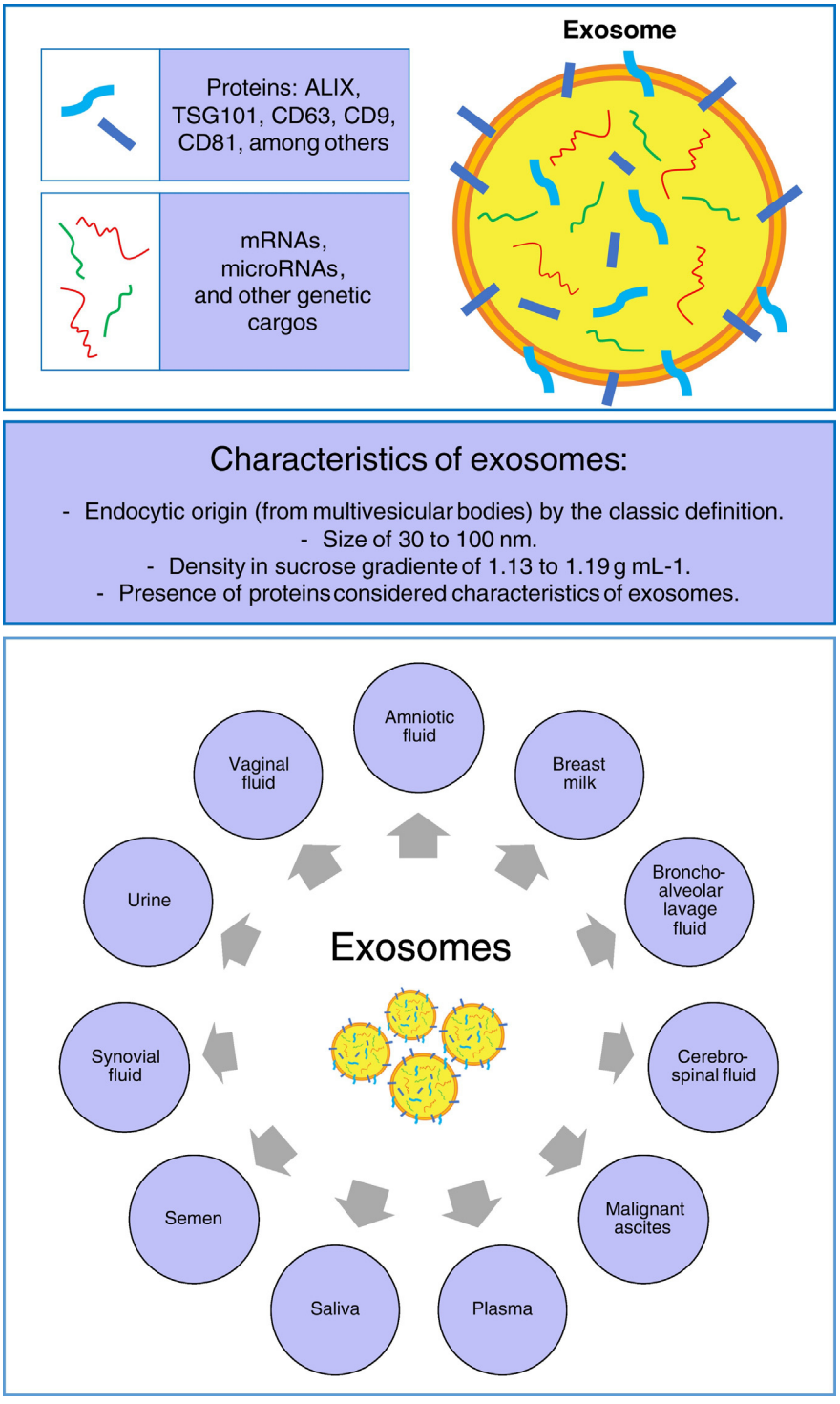

Fig. 1. Schematic representation of an exosome, its principal components and characteristics, as well as the biological fluids where exosomes are found. See text for references.

exosomes, d) the release of viral proteins in exosomes, potentially facilitating the infection of neighboring cells, e) the interference of HIV proteins with the exosomal release pathway, potentially enhancing or inhibiting the release of exosomes, or modifying their content. On the other hand, exosome release by some body fluids and cell types was proposed by some groups to have a protective role in HIV infection. Each of these topics will be addressed here.

The knowledge on exosomes and other EVs in human biology is evolving rapidly and increasingly reflecting great complexity. Progresses in biomarker discovery, vesicle isolation procedures, exosome cell biology and even the organization of EV nomenclature in the last years are changing our understanding of exosome and EV biology. This progress extends to the field of HIV research. Nevertheless, a retrospective analysis of the literature on EVs and the role of exosomes in HIV infection must take into account several different factors:

(I) EV nomenclature has evolved over time, and different research groups have used (and even continue to use) different definitions of "exosome";
(II) Different surface markers have been used by different groups to characterize exosomes, some of which are present in other types of EVs, such as ectosomes/microvesicles. To date, there is

no definitive marker for exosomes;

(III) Several different methods of exosome isolation have been employed, and none of these methods, which are mostly based on density gradient centrifugation, guarantee a perfect purification of exosomes from other vesicles and viral particles;

(IV) Elements once believed to be exclusive of the MVB/exosome transport machinery, such as ALIX and TSG101, have later been found to be involved in ectosome/microvesicle release, leading to the questioning and reinterpretation of early results in the exosome field.

In order to sidestep the possible confusion caused by the use of different definitions of exosomes, we tried, whenever possible, to reinterpret available findings in the light of the currently most accepted definition of exosomes, i.e. vesicles of an endocytic origin from MVBs. Furthermore, whenever relevant, we have given our point of view on the findings discussed here in the light of what is currently known about the methods of exosome isolation and characterization, pointing out the limitations of the available data and suggesting directions for future studies on exosomes.

3. Limitations of exosome isolation methods

Due to the phenotypic characteristics of exosomes, the isolation and purification of these nanovesicles represents a methodological challenge in itself. It can be performed in a series of centrifugation steps involving the use of filters and density gradients, and an increasing variety of commercial kits are available to facilitate the process. Exosome isolation and purification should be followed by an evaluation of protein markers to confirm the nature of the obtained material (Mathias et al., 2009; Théry et al., 2006). The direct visualization of exosomes is only possible in electron microscopy, where they often exhibit a 'cup-shaped' morphology (Gennebäck et al., 2013), an artifact of the electron microscopy preparation procedure. Moreover, the similarity between exosome and retrovirus particles introduces an additional level of complexity to the isolation and study of these vesicles in the context of HIV infection. A common approach is to rely on density gradient protocols in order to separate exosomes from viral particles, followed by an enzymatic assay to test for the presence of Acetylcholinesterase (AChe), claimed to be present in exosomes. However, AChe is currently considered a marker of ectosomes/microvesicles rather than exosomes, as shown by several proteomic studies available in Exocarta (http://www.exocarta.org/) and Vesiclepedia (http://www.microvesicles.org/). Clearly, what seems to be an effective method of separating HIV from EVs of similar size, still lacks effectiveness in the separation of different EV subpopulations. Further improvements in available separation methods will be crucial to advance our understanding of the role of exosomes and other types of EVs in the pathophysiology of HIV infection.

4. Could HIV usurp the exosome budding machinery for the production of viral particles?

It has long been known that HIV infects T cells and macrophages that express the CD4 receptor and the CCR5 or CXCR4 co-receptors. The possibility that HIV could hijack the exosome machinery in those cells in order to give rise to viral particles arose as an attractive explanation for the ability of HIV and other retroviruses to infect cells independently of Env or receptor engagement, and to thrive in the presence of otherwise healthy immune systems (Gould et al., 2003). In 2003, Gould and colleagues postulated the *Trojan exosome hypothesis*, which stated that "retroviruses use the preexisting, nonviral exosome biogenesis pathway for the formation of infectious particles, and the preexisting, nonviral pathway of exosome uptake for a receptor-independent, Env-independent mode of infection" (Gould et al., 2003). In their work, the authors presented evidences supporting the idea that HIV (and other retroviruses, such as the Human T-cell Leukemia Virus, HTLV) could use the machinery of the "exosome" biogenesis to promote virus budding and thus infect other cells. In agreement with this hypothesis, Nguyen et al. (2003) observed in a study of primary macrophages that viral particles could be immunoprecipitated with antibodies against "endosome markers", including CD63 and CD81. Also, Kramer et al. (2005) showed that HIV particles from lysed macrophages presented infectious activity and also precipitated efficiently with antibodies against "endosome components". The results from these two studies suggested that, in macrophages, HIV could bud into endosomes before being released into the lumen by the fusion of the endosomal bodies with the plasma membrane. However, later studies questioned these early results: in 2007, two independent teams of researchers used immunolabeling (Deneka et al., 2007) and immunolabeling-independent approaches (Welsch et al., 2007) and found that the assembly and release of HIV did not involve the endosomal pathway and most likely occurred at the plasma membrane. In a very recent study of mutant viruses unable to promote virus scission from the cell, Nkwe et al. (2016) observed that HIV assembly and budding in monocyte-derived macrophages was specifically targeted at those intercellular plasma membrane-connected compartments that were once mistakenly regarded as MVBs.

Another fact supporting the hypothesis of an exosome machinery hijack by HIV related to the recruitment of TSG101, a component of the Endosomal Sorting Complexes Required for Transport (ESCRT), which is required for virus budding, by HIV Group-specific antigen (Gag) protein. Early studies of TSG101 associated the protein with endosome/MVB formation, leading to the – reasonable – hypothesis that HIV would hijack an MVB process to promote budding. However, in 2007, members of the ESCRT machinery were found to be recruited to the cell midbody during cytokinesis, thus revealing a much broader role for the ESCRT machinery in cell biology (Carlton and Martin-Serrano, 2007). In 2012, Nabhan et al. described a novel virus-independent mechanism that mediates vesicle budding at the plasma membrane and that also recruits TSG101 (Nabhan et al., 2012). This budding mechanism is dependent on the interaction of TSG101 with ARRDC1, an accessory protein localized at the plasma membrane. All these findings shifted the notion of membrane budding as a virus-driven cell anomaly to a normal process for the generation of EVs at the plasma membrane, which is exploited by viruses in order to mediate their release from host cells. Overall, while available data cannot rule out the possibility of an alternative way of HIV release "as an exosome", there is currently very little evidence to support this hypothesis.

In addition to the existing confusion due to the lack of knowledge about the EV release machinery, there are the problems related to the EV nomenclature itself. Booth et al. (2006) reported that Jurkat cells, a common lymphocytic tumor lineage, secrete HIV Gag protein through "exosomes". However, by "exosomes", the authors meant EVs originating from endosome-like domains of the plasma membrane containing tetraspanins, rather than MVBs. Accordingly, the direct budding of exosomes from the plasma membrane would be characterized as "immediate exosome biogenesis", which is different from the "delayed mode of exosome biogenesis", in which exosomes are derived from MVBs. This broad definition of an exosome, based on vesicle size rather than subcellular origin, did not find much echo in the scientific community, which subsequently adopted terms such as "ectosomes" or "microvesicles" to refer to structures released from the plasma membrane. Later, it became clear that tetraspanins are enriched at the viral exit site and are incorporated in viral particles (reviewed in Thali, 2009).

5. Could HIV exploit exosomal release by surrounding itself with exosomes to escape immune surveillance?

An alternative hypothesis on the exploitation of the exosome pathway by HIV was suggested by Kadiu et al. (2012). This group directly observed viral particles associated to groups of exosomes in cell culture

supernatants from infected monocyte-derived macrophages (MDMs). Strikingly, a preparation of exosomes/microvesicles from infected MDMs containing 50-fold less viral HIV-1p24 presented comparable infectivity in MDM culture fluids in comparison to a viral preparation from the same source. These data suggested that, when surrounded by exosomes/microvesicles, HIV could accelerate the processes of infection and dissemination. Of note, the exosome preparation containing viral particles was not able to induce infection in HeLa cells lacking CD4, thus supporting the hypothesis of exosomes/microvesicles as facilitators of HIV receptor-dependent infection. In this work, the immunoisolation of different markers were performed in an attempt to discriminate microvesicles from exosomes. However, none of the different combinations of antibodies used can perfectly discriminate between the two subpopulations, despite the fact that, by electron microscopy, the size of the vesicles associated to the HIV particles was compatible with the size of exosomes. Further studies are needed to clarify the relative importance of each EV subtype in the facilitation of HIV infection. Also, the mechanisms underlying this facilitation remain to be determined.

6. Influence of exosomes in the immune system and HIV infection: focus on Nef

It is long known that the Nef protein is an important contributor to the progression of HIV infection, mainly due to its crucial role in HIV replication (Cheng-Mayer et al., 1989) and infectivity. The first report relating Nef to vesicle release was made by Guy et al. (1990). Many years later, the relationship between exosome release and HIV-Nef, as well as their involvement in the pathogenesis of AIDS, returned to be the object of interest by several groups. Nef was consistently reported to increase EV release and to be itself secreted in EVs. However, which type of EV is concerned by these findings, is still unclear. Campbell et al. (2008) described that Nef-transfected HEK293 cells could secrete vesicles containing Nef-GFP into the extracellular medium, as confirmed by electron microscopy. Muratori et al. (2009) observed that Nef accelerated endocytosis and exocytosis in Jurkat cells, as well as stimulating the release of "microvesicle clusters" in these cells in a budding-like process seemingly different from the classical exosome release mechanism. Budding structures were >500 nm in diameter and contained several dozens of small vesicles (as an aside, the authors also described the release of MVB-like structures from these cells, but claimed that these structures were more common in cells stimulated with PHA. No images of structures resembling MVB-release in Nef-stimulated Jurkat cells were published to allow verification). Those small vesicles remained together after release and could be found attached to the membrane of bystander cells. Microvesicle clusters release was confirmed as a broad phenomenon occurring in blood CD4 lymphocytes from HIV patients – notably, up to 29% of isolated CD4 T cells in patients with high viral load presented microvesicle clusters release. Nef transfer between cells was described as cell contact-dependent and involving two mechanisms, microvesicle transfer and trogocytosis. Raymond et al. (2011) observed that almost the totality of the secreted Nef in the plasma is associated to $CD45^+$ EVs (CD45 is reported to be associated with exosomes and microvesicles). The levels of Nef did not seem to correlate with viral load or CD4 cell counts. Lenassi et al. (2010) claimed that Nef was found in MVBs and secreted in exosomes. In their work, it was possible to observe a clear co-localization of Nef and CD63 in HeLa.CIITA cells. However, in lymphoblastic cell lines, such as Jurkat and Sup-T1, confocal microscopy images provided, in our view, poor evidence of the co-localization of Nef with MVBs. Likewise, a recent study by Luo et al. (2015) did not find any evidence of exosome-mediated Nef transfer in either Jurkat or 293T cells. The authors observed that, in 239T cells, Nef was present in vesicles containing AChe enzyme (which is associated to microvesicles, as stated earlier), but not in vesicles devoid of AChe. Overall, while several groups refer to the EVs involved in Nef release as "exosomes", there is stronger evidence for an association of Nef with vesicle release processes occurring at the plasma membrane than with true exosomes.

The cellular mechanisms associated with EV-mediated Nef secretion and their implications for HIV pathogenesis have been explored by several groups. Ali et al. (2010) described the presence of sequences at the aminoterminal region of the Nef protein that are important for vesicle secretion of Nef. Shelton et al. (2012) found that mortalin (HSPA9) is important for the secretion of Nef in "exosomes". Since mortalin is found both in preparations of microvesicles and in ectosomes, according to the Vesiclepedia database, these data do not clearly indicate the origin of the Nef-containing vesicles. Despite debatable evidence of exosome-mediated Nef transfer, available data implicate Nef in the augmentation of membrane trafficking processes in infected and non-infected cells. This increase in membrane traffic processes, including the exosomal pathway, could have a detrimental effect on immune cells and their functions. It remains to be clarified whether the budding EVs described by Muratori et al. (2009) are true exosomes or another type of vesicle.

The effect of EV-Nef on recipient cells has been assessed by several groups. Nef secreted by EVs may promote apoptosis in $CD4^+$ T cells (Lenassi et al., 2010) and cell lines (Raymond et al., 2011), which would contribute to the immune deficiency characteristic of AIDS. Nef-mediated induction of exosome-based cellular communication could stimulate quiescent $CD4^+$ T lymphocytes. This cell stimulation would be beneficial to HIV proliferation (Arenaccio et al., 2014a; Arenaccio et al., 2014b). Moreover, exosomes from HIV-positive patients could induce immune activation of T $CD4^+$ and T $CD8^+$ lymphocytes (Konadu et al., 2015). Conversely, exosomes derived from T cells present a potential immunosuppressive activity (Nazimek et al., 2015). In addition, in vitro evidence suggests that exosomes from HIV-infected cells could reactivate latent HIV reservoirs in a process mediated by the enzyme ADAM17, which induces the release of TNFα from target cells, thus reactivating a latent infection (Arenaccio et al., 2015). Exosomal Nef transfected to microglial cells was reported to disrupt the integrity and permeability of a brain blood barrier model and were thus suggested to contribute to neuroimmune pathogenesis (Raymond et al., 2016). Another study conducted by Khan et al. (2016) found that the levels of "exosome"-packaged Nef protein and mRNA were higher in the circulation of patients with HIV-associated dementia than in those without dementia, and that those vesicles were capable to induce both Nef and Beta Amyloid protein (which is commonly found in plaques of Alzheimer's disease and HIV patients with dementia) in SH-SY5Y neuroblastoma cells (Khan et al., 2016). Importantly, antibodies against Nef were able to prevent the uptake of Nef exosomes in those cells, suggesting that these antibodies could be of great help in treating the neurological manifestations associated with HIV. According to these data, the association between Nef and exosomes seems to have some influence on HIV-associated brain disorders.

In addition to its inherent ability to elicit its vesicular self-export from infected cells, Nef was also reported to interfere with the content of vesicles. It has been shown that CD4 molecules could be present on the surface of exosomes (De Carvalho et al., 2014), which would cause the interaction of HIV with exosomes and subsequently decrease the viral interaction with $CD4^+$ T cells. Nef could promote HIV infection by reducing the presence of CD4 molecules on the surface of exosomes, thus decreasing the interaction of the viral particles with these nanovesicles and favoring the interaction of HIV with $CD4^+$ T cells (De Carvalho et al., 2014). Several studies have reported that Nef interferes with the exosomal RNA content in infected cells. This issue will be discussed in detail in the next section.

7. Exosomes, HIV, and RNAs

Exosomes are a well-known means for the intercellular transport of a range of RNA species, including mRNAs, lncRNAs and small RNAs such as miRNAs. The identification of HIV RNA sequences in "exosomes" has

been claimed by Cabezas and Federico (2013) in a study of the lymphoma-derived U937 cell lineage. The phenomenon was driven by specific sequences in the HIV genome and seemed to be inversely correlated to HIV packing into viral particles. However, the authors failed to demonstrate any infectivity associated with HIV RNA-bearing "exosomes". Moreover, the abovementioned work of Luo et al. (2015) demonstrated that, in protocols based on differential centrifugation followed by ioxidanol gradient centrifugation, the fractions displaying AChe activity are, at best, a mix of vesicles with different phenotypes (Luo et al., 2015). Thus, the viral RNA claimed to have been found in exosomes may, in fact, have been inside ectosomes/microvesicles budding at the plasma membrane instead. Clearly, more work is needed to define the type of EV in which the viral RNA is incorporated. In addition, the functional effect of the HIV RNA delivery by exosomes/microvesicles, in the absence of a reverse transcriptase in the receiving cell, should also be investigated.

MiRNAs have been extensively studied in exosomes. As reviewed by Madison and Okeoma (2015), miRNAs with the potential to confer protection against HIV observed in exosomes include miR-28, miR-29a, miR-29b, miR-125b, miR-149, miR-150, miR-198, miR-223, miR-324, miR-378, and miR-382. Conversely, viral miR88, viral miR99m, and viral miR-TAR, all observed in exosomes from HIV-1 infected cells, could enhance HIV infection. Recently, Roth et al. (2016) described 38 miRNAs present in exosomes from HIV-infected macrophages but not in exosomes from uninfected cells. These data indicate that HIV can interfere with the miRNA content of exosomes. However, considering the scarcity of studies in this field, the diversity of miRNAs from both host and virus with the capacity to interfere in HIV infection must be much higher than reported. The effect of different miRNAs on different cell types can also be variable.

Some examples of miRNAs that influence the interaction between HIV and the immune system in an exosome-mediated manner must be highlighted. HIV-derived RNA-TAR was found in exosomes derived from virus-infected cells, predominantly in a pre-miRNA form, and it was shown that these RNAs could inhibit apoptosis of recipient cells by down-regulating the expression of pro-apoptotic proteins Bim and Cdk9 (Narayanan et al., 2013). The same group later demonstrated that the TAR RNA in exosomes from HIV-infected cells was able to modulate the gene expression of pro-inflammatory cytokines, such as IL-6 and TNF-β, in human primary macrophages, possibly influencing the inflammation observed in HIV-infected patients (Sampey et al., 2016).

Host-derived exosomal miRNAs regulate important genes involved in HIV pathogenesis. On the other hand, the viral protein Nef can suppress miRNA-mediated gene silencing (Aqil et al., 2013), deregulate the expression of miRNAs, and even interfere with the release of these molecules by exosomes (Aqil et al., 2014). Additionally, Aqil et al. (2015) demonstrated in a transcriptome analysis that there were four mRNAs exclusively retained in U937 cells expressing Nef. These mRNAs coded for genes involved in chromatin modification and gene expression – MECP2, HMOX1, AARSD1, and ATF2. Moreover, their targeting miRNAs were found to be exocytosed in exosomes. Conversely, three mRNAs involved in apoptosis and fatty acid transport were secreted in exosomes released from Nef-expressing cells – AATK, SLC27A1, and CDKAL. In this case, their targeting miRNAs were retained in Nef-expressing cells (Aqil et al., 2015). These data demonstrate that there is a complex relationship between Nef, mRNAs and miRNAs, a link that may be mediated by exosomes.

The action of exosomal miRNAs is not restricted to immune cells. HIV-Tat protein induces the secretion of miR-29b through astrocyte-derived exosomes that have the potential to negatively regulate the expression of platelet-derived growth factor (PDGF)-B, resulting in decreased neuronal viability (Hu et al., 2012). This finding highlights the possible involvement of exosomal miRNAs in the neurodegeneration caused by HIV.

In order to evaluate the possible effects of the exosomal miRNA content, it is important to explain how these nanovesicles interact with cells of the immune system during HIV infection. The direct influence of these miRNAs on the virus is a topic that has been little explored and should be investigated in other functional studies.

8. Protective action of exosomes against HIV infection

Some findings have pointed to a protective role of exosomes against the progression of HIV infection. It has long been known that CD8 T cells are able to control HIV replication in infected CD4 cells in a non-cytolytic, MHC-independent manner. Tumne et al. (2009) observed that exosomes derived from $CD8^+$ T lymphocytes have a non-cytotoxic suppressive effect on HIV replication in vitro (both R5 and X4 types). Such "antiviral exosomes" were non-toxic to target cells and inhibited the transcription of the HIV LTR promoter, seemingly through an unknown protein moiety present at the surface of exosomes. This mechanism seemed independent of exosome internalization and was dependent on STAT1 signaling, a component of the interferon-mediated signaling pathway (Tumne et al., 2009). This finding may help explain the well-known non-cytotoxic suppression of HIV replication shown by $CD8^+$ T cells. However, the exosome-associated protein(s) involved in this suppression remain to be elucidated. Likely, there are several cell types that produce HIV suppressor EVs, since structures with suppressive activity are found in different biological fluids, as discussed in Section 10.

9. The roles of exosomal cytokines and chemokines in HIV infection

Cytokine delivery by exosomes and microvesicles is thought to underlie the signaling function of these structures. In a recent study, Konadu et al. (2015) isolated 21 cytokines and chemokines of exosome/microvesicle origin from plasma and compared their levels between infected and non-infected patients. They found the levels of all cytokines and chemokines measured in the "exosomal" fraction of the isolate to be significantly higher in patients than non-infected subjects (Konadu et al., 2015). Of note, the association of many of these cytokines and chemokines with exosomes/MVs still lacks confirmation by mass spectrometry or other protein validation methods. Nevertheless, the increased levels of these important immune molecules in the EV fraction of HIV patients highlight the potential importance of an exosome-based delivery mechanism for the course of HIV infection and other pathological situations, which should be addressed by future studies.

10. Exosomes/EVs from different biological fluids and HIV infection

In this section, we draw together the results of studies addressing the different biological features of EVs from semen, breast milk, urine, and saliva with regard to HIV infection. Since it is difficult to determine the sub-cellular origin of the vesicles assessed in those fluids, we will refer to them in this section collectively as EVs. EVs from human semen seem to have an important antiretroviral activity. Madison et al. (2014) showed that EVs isolated from semen of healthy men are able to inhibit viral replication upon incubation with different strains of HIV. It is believed that the defective viral replication is caused by an EV-mediated disruption of the reverse transcriptase activity. It is interesting to note that the antiretroviral activity was specifically observed in semen-derived EVs, but not in EVs isolated from blood (Madison et al., 2014). Later, the same group described that EVs isolated from semen of healthy men also had the potential to inhibit HIV transmission by vaginal cells and were able to disrupt the viral replication at the vaginal epithelium (Madison et al., 2015). These findings reinforce the importance of studying the antiretroviral activity of exosomes and other vesicles derived from semen in order to assist in the development of new therapies against HIV. Studies performed with EVs isolated from breast milk also provided interesting results on HIV inhibition. EVs isolated from breast milk were reported to present a modulatory activity on the immune system (Admyre et al., 2007). Näslund et al. (2014)

observed that EVs isolated from human breast milk of healthy donors inhibited the HIV infection of monocyte-derived dendritic cells and viral transfer to $CD4^+$ T cells. It is believed that the protection against infection occurs because these EVs bind to the DC-SIGN receptor, competing with the virus and inhibiting the cell infection by HIV, a process that also protects against the subsequent infection of $CD4^+$ T cells. Similar to what was observed in the study of Madison et al. (2014), the antiviral activity was not present in plasma-derived EVs. These findings indicate the presence of exosome-carried molecules with antiretroviral activity in some, but not all, biological fluids of healthy individuals.

Apart from blood, semen and breast milk, which are of natural interest concerning their great importance in HIV transmission, exosomes and other EVs have been evaluated in other body fluids. Saliva, for instance, is easy to collect and has been suggested as a potential source of EVs (Machida et al., 2015; Michael et al., 2010). In this context, the analysis of exosomal proteins derived from saliva could be used to monitor the expression of molecules that positively or negatively influence HIV infection progression, as well as medical conditions related to AIDS. In a study of heroin users, Dominy et al. (2014) presented evidence that HIV infection could influence the release of proteins from salivary glands and oral epithelium by EVs. The study suggested that EVs from saliva have their protein cargo modified by HIV infection. Besides, EVs present in urine have been identified as effectors of innate immunity (showing great amounts of innate immune proteins) and presented antimicrobial activity. For example, urinary EVs inhibited the growth of *Escherichia coli*, both commensal and pathogenic strains, indicating a possible system defense of the host urinary tract (Hiemstra et al., 2014). However, to our knowledge, no reports investigating the relationship between EVs derived from urine and HIV infection are available to date. Despite a number of limitations, the use of urinary exosomes and other EVs may represent an alternative to regular blood samples taken to monitor the progression of infection in HIV-positive individuals. Moreover, urinary EVs could be used as a valuable tool for research in the field investigating the relationship between HIV and kidney disease (reviewed in Dimov et al., 2009). Likewise, EVs from vaginal fluid are emerging as a protective factor against HIV infection (Smith and Daniel, 2016).

In spite of the interesting results mentioned above, much work remains to be done on the role of exosomes and other EVs in body fluids and tissues. By exploring exosomes from other biological fluids, especially cerebrospinal fluid, which is strictly related to the immunoprivileged environment of the brain, we could help explain how the transport and regulation of immunomodulatory molecules occurs in this environment during the HIV infection, thus contributing to our understanding of HIV-related brain manifestations.

11. Insights into the potential role of exosomes in HIV therapy

Exosomes containing antiviral proteins are regarded as possible relevant allies in HIV therapy (Khatua et al., 2009). In addition, the use of exosomes as vectors of therapeutic vaccines seems promising (Lattanzi and Federico, 2012) and should be explored as another strategy against HIV. In this context, the secretion of a LAMP/Gag chimera (which has immunomodulatory activity) by exosome-like vesicles was shown by Godinho et al. (2014).

Classically, exosomes are regarded as potential vectors of therapeutic nanomolecules and nanosystems. The use of engineered exosomes carrying immunogenic molecules has proven experimentally feasible in mice (Di Bonito et al., 2015). Thus, therapeutic molecules could be directed to viral reservoirs by exosomes, assigning exosomes as a very significant tool in combating the HIV infection.

The use of exosomes as biomarkers stands out in the literature. As previously stated, exosomes derived from easily accessible body fluids, such as saliva and urine, as well as from canonical body fluids, i.e. blood, semen, and breast milk, could be used to monitor the progression of HIV infection and associated pathologies of HIV-positive patients. However, several issues need to be clarified to enable this. It is necessary to better characterize the molecules associated with exosomes derived from each biological fluid and to understand how each of them correlates with different physiological and pathological states. For example, Hubert et al. (2015) described a greater amount of exosomes in the plasma of ART-naïve HIV-positive patients in comparison to uninfected control individuals and elite controllers. However, the profile and the action of exosomes released from different cells types in response to HAART regimens are not yet known. We believe that the identification of the proteins associated with exosomes from different cell types will allow the evaluation of the cellular responses in the different drug regimens used in HAART.

Based on current evidence, it is plausible to assume that exosomes have the potential to become an important ally in the fight against HIV infection, allowing the development of strategies for the maintenance of a healthy immune system in HIV-positive individuals. An exploration of the antiretroviral activity of exosomal molecules will deepen our understanding of viral replication mechanisms. These issues must primarily be addressed through proteomic and lipidomic studies, as well as by a thorough characterization of the non-coding RNAs present in exosomes. Functional studies should also be encouraged, especially those focused on the inhibitory potential of the exosome on viral replication.

12. Conclusions and perspectives

The influence of exosomes on HIV is still poorly understood. In a general way, exosomes can modulate immune responses and may affect HIV pathogenesis, playing a relevant role in HIV pathophysiology. This seems to be mediated by the exosomal cargo, which comprises mainly membrane proteins and non-coding RNAs. The current state of the field can be summarized as follows:

(I) A lack of distinction between exosomes and other EVs creates much confusion in the literature. The labeling of other types of vesicles as "exosomes" makes the comparison of results from different studies very difficult. Besides, a range of experiments were performed using a mixture of exosomes with other types of EVs;
(II) Over the years, mounting evidence has left little room for the hypothesis of HIV exploiting the MVB/exosome export machinery for its release (thought to occur especially in infected macrophages), although it is clear that the virus may explore components that are shared between exosome and vesicles budding at the plasma membrane;
(III) An alternative hypothesis of the exploitation of exosome/EV release by HIV is a camouflage of the viral particles by surrounding themselves with exosomes, which could enhance the infectivity of viral particles by facilitating their contact with target cells;
(IV) Nef plays an important role in increasing import/export processes in infected cells and regulating exosomal cargo. Nef is long known to be secreted, and a body of work sustains that most of it is secreted in EVs. Although most of the experimental evidence implicates ectosomes as the primary source of secreted Nef, exosomes could also play a role. Nef secreted in EVs was suggested to have a range of effects on target cells, such as induction of apoptosis and, at a neurological level, Beta-Amyloid induction and the disruption of the blood-brain barrier;
(V) HIV induces the expression of several types of RNA, such as vRNA-TAR, that are incorporated in exosomes and have been reported to have important functions in target cells, suggesting an important role in HIV infection. Conversely, host miRNAs carried by host exosomes could exert a protective effect against HIV pathogenesis. The viral protein Nef was described to influence miRNA export in exosomes/retention in Nef-expressing cells, thus influencing gene expression;
(VI) The identification of the biomolecules transported by exosomes

and the elucidation of their immune regulatory effect in HIV-positive individuals will provide new insights into the role of these immunomodulatory nanovesicles in AIDS pathogenesis;

(VII) Exosomes from different cell sources play different roles in HIV pathogenesis. Whereas exosomes/EVs from infected cells may promote viral replication and the dissemination of infection, exosomes/EVs from uninfected tissues or cells could protect the immune system against the virus, as described in CD8 cells. The direction of the action depends on the cargo, the type of their cell of origin, and the interaction with viral proteins;

(VIII) Exosomes from semen and breast milk have a potential anti-HIV activity. The biomonitoring of HIV/AIDS progression could be performed through the evaluation of exosomes derived from different biological fluids;

(IX) Engineered exosomes could be used as vectors for therapeutic molecules and nanosystems.

Finally, in order to understand how exosomes/EVs and their cargos interact with HIV and modulate the immune system during the viral infection, isolation and characterization methods must be improved, standardized and fully described. An ability to clearly determine which type of vesicle is involved in Nef transfer, for example, could lead researchers to search for ways to specifically block the pathway responsible for its release as a means to control viral spread. Research on exosomes/EVs and viral infections is an incipient field. However, we believe that within a few years, many challenges will be overcome and the use of exosomes for disease monitoring and controlled drug delivery during HIV infection will be widespread. HIV seems to control cell export mechanisms in many different ways. While virus export and secretion of Nef seems to be a cell membrane-driven process, viral and host RNA export are more likely associated to the exosomal pathway. However, many gray areas exist and current knowledge on the exploited pathways is limited by the difficulty in discriminating exosomes from ectosomes/microvesicles. We believe that by clearly establishing which aspects of HIV are associated to microvesicles and which are associated to exosomes, we can develop a better understanding of the infection and thus provide novel ideas to combat it effectively.

Conflicts of interest

The authors declare that they have no conflict of interest.

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CAPÍTULO VI

Exosomes are possibly used as a tool of immune regulation during the dendritic cell-based immune therapy against HIV-I

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Exosomes are possibly used as a tool of immune regulation during the dendritic cell-based immune therapy against HIV-I

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ABSTRACT

Dendritic cell (DC)-based immune therapy (IT) against HIV showed variable results. It is known that different factors influence host response to DC-IT. Exosomes derived from DC are regulators of the immune system. In this context, here we hypothesize about the role of the DC-derived exosomes on the DC-IT response. Based on data from RT-PCR array genes expression (focused on the *TSG101* gene, an exosome marker) and flow cytometry experiments of a DC-IT against HIV-1 clinical trial, we hypothesize that: During the DC-IT exosomes are used as an additional tool for immune system modulation. In addition, we believe that a low release of exosomes can be more beneficial for the DC-IT response than a high release of exosomes. Our data reinforce the concept that exosomes can act as an immune regulatory tool, however not in a generalized manner, but in a highly precise way. Our hypothesis is based in preliminary experimental data, thus, it should be tested using experimental and functional strategies involving a great number of patients. Once the hypothesis confirmed, the immunomodulatory role of the exosomes during DC-IT must be considered as an important factor in the (I) evaluation, (II) modulation, and (III) success of DC-IT against HIV.

Introduction

Different protocols for HIV-1 treatment using dendritic cell (DC)-based immune therapy (IT) showed variable results (reviewed in [1,2]). At present, 13 clinical trials, using DC-IT to fight HIV infection, have been concluded. The most promising findings were achieved with the protocol tested by Lu et al. [3] with 8 out 18 patients showing a good response to the treatment (decrease of the viral load allied to the achievement of stable T CD4+ cell numbers) at least for 12 months after the IT.

It is well known that DC-IT induces persistent cellular responses to control viral replication through an (I) autologous, (II) safe and (III) well-tolerated protocol. However, different factors influence the host response to different strategies of DC-IT. The host genome, as well as the viral one, could affect the success of the therapy [4–7], but probably many other factors related to time of infection, chronic inflammation, and the consequent immunologic cells impairment could interfere with the response to DC-IT. In this context, we highlight the potential role of DC-derived exosomes in such scenario.

Exosomes are nanovesicles released into the extracellular medium by different cell types (for example DC, other immune cells, and neoplastic cells) that can be internalized by other cells. Exosomes carry a variety of molecules including proteins, lipids, mRNAs and microRNAs. All of these components attribute to exosomes various physiological functions in several biological systems. Among them, the role of the exosomes as modulators of the immune system stands out (reviewed in [8]). Depending on the physiological situation, exosomes may carry a variety of molecules that have the ability to inhibit or stimulate the immune system through different pathways, such as cytokine modulation and chemokine transport. In the presence of pathogens, such as HIV, the transport of molecules by exosomes can be modified. For example, in HIV-positive (HIV+) patients, the concentration of cytokines and chemokines in exosomes from plasma are much higher than the one found in exosomes from HIV-negative controls [9].

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The exosomes derived from dendritic cells have been described as important regulators of the immune system. For instance, it was reported that they can: (I) Transport functional peptide-Major Histocompatibility Complex (MHC) complexes between dendritic cells, contributing to the initiation of the primary adaptive immune responses [10]; (II) Stimulate CD8+ cytotoxic T lymphocytes responses and antitumor immunity [11]; (III) Induce and modulate antigen-specific humoral immunity [12]; (IV) Induce protective immunoglobulin responses against bacterial infection [13]; (V) Contribute to natural killer cell activation and proliferation [14]; (VI) Be used in immunization strategies [15]; (VII) Promote immune response against neoplastic cells [16]; (VIII) Induce allergic immune responses [17].

It was also observed that the DC maturation status influences the content of exosomes produced by those cells. Thus, differences in the role of the exosomes derived from immature DC (iDC) as compared to exosomes derived from mature DC (mDC) have been described. Exosomes from mDC are more potent antigen-specific T cell activators than exosomes from iDC [18,19]. In addition, depending on the maturation stage, DC interact differently with HIV particles. iDC can capture HIV and then transmit the virus to T cells [20], while mDC promote the trans-infection of the virus within the lymph node (reviewed in [21]).

Motivation and data used for the study

It was recently hypothesized that the profile of "good" or "weak/transient" responders to DC-IT may depend on the quality of DC obtained *in vitro* from HIV+ patient's peripheral blood monocytes [22]. Considering that exosomes could affect the activation state of DC, and considering that, to date, no data are available about the importance of exosomes in response to DC-IT, the findings obtained in the above-mentioned study were revised in order to verify the existence of any relationship between exosomes and DC-IT.

Data used in the present study derive from RT-PCR array gene expression and flow cytometry experiments described in Pontillo et al. [22]. Briefly, biological samples were obtained from 6 individuals (males; 31.3 ± 7.6 years) out of 19 HIV+ Brazilian patients submitted to DC-IT clinical trial at the Laboratory of Medical Investigation/LIM-56 (Faculty of Medicine, University of Sao Paulo, Brazil). Written informed consent was obtained according to the protocol of the "Hospital das Clínicas" Ethical Committee (CAPPesq) (number 0791/09, 04 November 2009). The expression of eighty-four genes involved in anti-HIV response was analyzed in monocytes and monocyte-derived DC, differentiated and stimulated *in vitro* according to Lu et al. [3]. Differential gene expression was evaluated in monocytes, iDC, iDC pulsed with aldrithiol-inactivated autologous HIV-1 for 4 h (4 h-DC), iDC pulsed with virus and incubated with maturation cocktail for 14, 24 and 48 h (14 h-, 24 h- and mDC), compared to monocytes. Differentiation and activation state of iDC and mDC were measured using common surface markers (CD11c, HLA-DR, CD80, CD86, CD40) through flow-cytometry.

Preliminary findings during a DC-IT against HIV-1

According to Pontillo et al. [22], clustering analysis of Fold Change (FC) values for the 84 genes in all DC time-points of the 6 HIV+ individuals allow the segregation of patients' samples in two independent groups (called A, $n = 3$ including patients with the majority of genes down-regulated and B, $n = 3$ including patients presenting the majority of genes up-regulated). DCs from group A patients presented a general down-regulation of anti-HIV response genes, while an up-regulation was observed in DCs from group B individuals, however this difference apparently did not correlate with the phenotypic profile of DC, clinical data (plasma viral load – PVL and CD4+ T lymphocyte counts) or response to IT (ΔPVL), even if a higher mean levels of CD4+ T lymphocytes during IT has been observed in group A compared to B patients.

Among the 84 genes analyzed, the exosome marker *TSG101* resulted oppositely modulated in DC from group B and group A patients at all the considered time points (Table 1), being strongly down-regulated in A-DC versus monocytes and up-regulated in B-DC. The expression of *TSG101*, similarly to the other genes, is not modulated during monocyte-to-DC differentiation, but possibly depends on the initial monocyte expression, although group B monocytes expressed lower levels of the gene compared to group A monocytes (FC = 2exp-4), even if this difference did not achieve statistical significance ($p = 0.079$).

Considering the role of the exosomes on the immune response, it is possible that patients from group A presented a lower expression of *TSG101* due to the necessity of transport of immune regulatory molecules in a highly targeted and precise way. Besides, the down-regulation of *TSG101* could contribute to the maintenance of CD4+ T lymphocytes levels during IT.

The hypothesis

The *TSG101* gene (11p15.1) encodes a protein of the same name, which is considered as a marker of exosomes [23–26]. Thus, an increased *TSG101* expression can be associated with increased release of exosomes. On the other hand, the down-regulation of this gene can be linked to a decreased release of exosomes.

As discussed above, it is widely accepted that exosomes can modulate the immune system by transporting molecules with capacity to inhibit or activate the immune response. The DC-IT may be considered as a situation in which the immune system is activated with a consequent complex intercellular interaction occurring between molecules with immunomodulatory activity.

According to the results here discussed, it is possible that, during DC-IT, the release of exosomes is stimulated as an additional tool of immune regulation, explaining the findings in relation to the increase in the *TSG101* expression during DC-IT in some patients (group B). On the other hand, it is possible that the down-regulation of *TSG101* observed in patients from group A contributes to a better response to the DC-IT since a trend to higher levels of CD4+ T lymphocytes is observed during DC-IT in patients from this group. In addition, the use of this phenomenon (regulation of exosome release) seems not to be related to the DC maturation stage.

Therefore, we hypothesize that, during the DC-IT, exosomes are used as an additional tool for modulating the immune system. According to our preliminary observations, low release of exosomes would be more beneficial for the DC-IT response than a high release of these nanovesicles. This hypothesis reinforce the idea that exosomes can be used as an immune regulatory tool, probably not in a generalized manner, but in a highly precise way, modulated and directed to specific targets. A schematic representation of this proposal is presented in the Fig. 1.

Discussion and consequences of the hypothesis

It is possible that exosomes act as an important tool to regulate the immune system during DC-IT. However, an association between a better response to therapy with a lower release of exosomes seems to exist. This is a quite interesting finding since it indicates that the regulatory pathways mediated via exosomes should occur subtly and in a well directed way, being not a widespread uncontrolled phenomenon. Moreover, this idea support the

Table 1
Expression data of *TSG101* in DC used for immunization of HIV+ patients.

DC maturation stage	Group A (n = 3)		Group B (n = 3)	
	FC (FR)	p-value	FC	p-value
iDC	0.05 (−20.00)	**0.040**	3.89	0.053
4 h-DC	0.06 (−16.67)	**0.038**	3.53	**0.031**
14 h-DC	0.03 (−33.00)	**0.007**	3.45	0.079
24 h-DC	0.06 (−16.67)	**0.043**	3.90	**0.047**
48 h-DC	0.06 (−16.67)	0.103	8.66	0.168

Fold-change (FC) as well as t test p-values are reported for *TSG101* modulation in DC compared to monocytes. Fold-regulation (FR) is indicated when necessary. Values considered statistically significant are shown in bold.

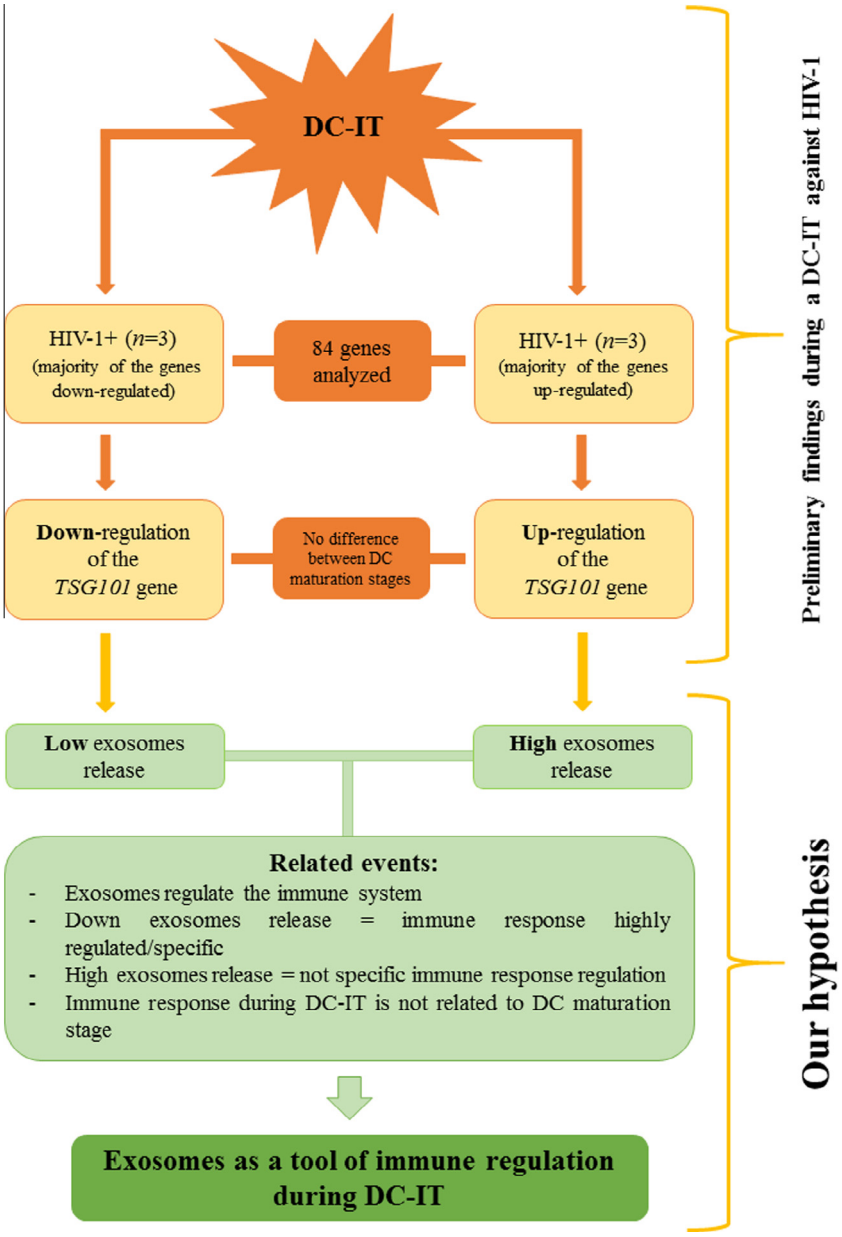

Fig. 1. Schematic representation of summary results and hypothesis.

role of exosomes as tools of transport and regulation of biomolecules in a targeted, stable and highly regulated manner [27]. Although there is evidence that the profile of the released exosomes is related and can be modified according to the DC maturation stage [18,19], our data indicate that there is not a significant influence of this association in relation to the DC-IT response.

Our hypothesis leads to four main consequences: (I) Strengthen the role of the exosomes as a modulation tool of the immune system; (II) Indicates that the differences in the DC maturation stages

do not influence the immune modulation mediated by exosomes; (III) Suggests that the type of patient's response to DC-IT involves the level of exosomes release, since the down-regulation of *TSG101* is associated with a better response to the DC-IT (at least in relation to the levels of CD4+ T lymphocytes); (IV) Reinforces the suggestion that exosomes can be used as biomarkers, as supported by different authors [28–30].

How to test our hypothesis

We propose the following strategies to test our hypothesis:

I. The replication of the analyzes here described on a larger number of individuals treated with DC-IT; The best scenario would require the recruitment of patients from all 13 clinical trials involving DC-IT.
II. The isolation of exosomes (from plasma) of patients with up- and down-regulated genes of interest and the characterization of the proteins carried by them;
III. The analysis of a greater number of gene markers of exosome release;
IV. The establishment of correlations between the proteins carried by exosomes and genes differently expressed between patients with up- and down-regulated genes;
V. The development of functional studies in order to identify a possible relationship, not yet detected, between the DC maturation stages with the release of exosomes, focusing on their cargo of biomolecules with potential for regulating the immune system.

Conclusions and perspectives

Our preliminary data indicate that during the DC-IT, exosomes are used as an additional tool for modulating the immune system. However, a low release of exosomes seems to be more beneficial for the DC-IT response rather than a high release of exosomes. Additionally, the release of exosomes with the capacity to modulate the immune system is probably not related to the DC maturation stage. Despite, it should be noted that these observations are derived from a preliminary study in which few patients were evaluated.

As prospects aiming the comprehension of the role of exosomes in DC-IT, we would suggest: (I) The testing our hypothesis using experimental and functional strategies; (II) Whether the hypothesis is corroborated, we must consider the immunomodulatory role of the exosomes during DC-IT as an important factor in the evaluation, modulation, and success of this kind of therapy against HIV.

Conflict of interest statement

No conflicts of interest to declare concerning this work.

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CAPÍTULO VII

Increased IL-8 levels in HIV-infected individuals on ART – A potential hallmark of chronic inflammation

Este capítulo apresenta o seguinte artigo de dados submetido para publicação no periódico *Journal of Infection and Public Health*:

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Increased IL-8 levels in HIV-infected individuals on ART – A potential hallmark of chronic inflammation

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Abstract

Background: Currently, HIV-infected (HIV+) individuals on antiretroviral therapy (ART) have a life expectancy very similar to that observed in the general population. However, chronic ART-controlled HIV infection is linked to increased frequency of diseases traditionally observed in old age. Also, HIV+ individuals show a chronic inflammatory state even when viral replication is suppressed by ART. Late ART initiation is a contributing factor to chronic inflammation in HIV infection. For this reason, one of the current objectives of HIV infection therapy is to reduce inflammation. Therefore, the identification of inflammatory markers in HIV+ individuals on ART is essential. In line with this goal, the aim of this study was to assess the levels of pro-inflammatory cytokines (IL-1β, IL-8, and IL-12p70) in the plasma of HIV+ individuals who initiated ART after immunosuppression ($CD4^+$ T cells counts <350cell/mm^3).

Methods: We stratified HIV+ individuals according to two extreme phenotypes: Slow Progressors (SPs; individuals with at least 8 years of infection before ART initiation [median: 9 years; 25%-75% percentile: 9-11 years]) and Rapid Progressors (RPs; individuals who needed to initiate ART within 1-4 years after infection [median: 1 year; 25%-75% percentile: 1-3 years]). We also evaluated cytokine levels in HIV-uninfected individuals (control group).

Results: We found increased IL-8 levels (median: 5.13pg/mL; SPs and RPs together) in HIV-infected individuals on ART as compared to controls (median: 3.2pg/mL; p=0.04). No association of IL-8 levels with the progression profile (slow or rapid progressor) or with $CD4^+$ T cell counts at sampling was observed.

Conclusions: IL-8 is a general marker of chronic inflammation in HIV+ individuals on ART, independently of $CD4^+$ T cell counts or of the potential progression profile of a given individual. If this finding is confirmed, IL-8 may be considered as a possible target for novel therapies focused on reducing inflammation in chronic HIV infection.

Keywords: AIDS, HIV, Interleukin 1β, Interleukin 8, Interleukin 12, Inflammation.

Introduction

Nearly forty years after the description of the first Acquired immunodeficiency syndrome (AIDS) cases in the medical literature [1], progression to AIDS in HIV-positive (HIV+) patients can now be successfully controlled through the use of combined antiretroviral therapy (ART). However, HIV+ patients on ART face a reduced life expectancy and are afflicted by several health problems due, in part, to a state of chronic inflammation [2]. Indeed, it is consensus that a pro-inflammatory state remains active even after ART initiation probably in the majority of patients [3, 4]. In this sense, HIV infection should be considered as including a chronic inflammatory disease [4]. Since HIV life cycle is suppressed by ART in these patients, the chronic inflammatory state observed in such patients is maintained by factors secondary to HIV replication, including reduced immunoregulatory function, co-infections, microbial translocation, and impaired liver function [2-4]. Residual HIV replication also contributes to the increased inflammation observed in individuals on ART [5].

Important multifunctional effector molecules of the immune response, cytokines are also responsible for leukocyte recruitment to inflammation sites. Cytokines are involved in the control of homeostasis and also in the pathogenesis of various diseases, such as neurodegenerative disorders, cancer, and viral infections [6]. Moreover, cytokines can favour residual HIV replication and participate in the maintenance of viral reservoirs [7]. Thus, cytokines can be involved in the maintenance of the chronic inflammatory state observed in HIV+ individuals even after viral suppression. Specifically, interleukin 1β (IL-1β), interleukin 8 (IL-8), and interleukin 12 (IL-12) are candidates to play a role in HIV-associated inflammation due to their pro-inflammatory characteristic [8-11].

Initiating ART early after HIV infection contributes to the maintenance of immune competence, limits the viral reservoirs, and reduces inflammation [7, 12]. Thus, currently, a major goal of HIV therapy is to reduce inflammation *per se* [12]. However, to achieve this goal in the clinical practice is still a challenge, once there is no consensus or sufficient evidence on which therapeutic strategies should be applied in order to reduce the chronic and systemic inflammation. Also, there is no consensus on the best anti-inflammatory agents that should be offered to HIV+ individuals. Therefore, identifying altered inflammatory markers in patients on ART is essential to indicate potential targets for anti-inflammatory therapies. Based on the above-mentioned scenario, the aim of this study was to evaluate the peripheral plasma levels of the pro-inflammatory cytokines IL-1β, IL-8, and IL-12 in HIV+ individuals who initiated ART after immunosuppression (meaning individuals with $CD4^+$ T cells counts <350cell/mm^3).

Methods

Individuals, plasma samples, and ethical aspects

Plasma samples selected to compose this study are derived from the sample repository of HIV+ individuals with different profiles of clinical progression established in the Laboratory of Immunobiology and Immunogenetics from UFRGS (*Universidade Federal do Rio Grande do Sul*, Porto Alegre, Brazil). Plasma samples derived from systemic blood were obtained between 2011 and 2013, and were stored at -80°C. Detailed characteristics of the patients who compose this repository as well as the criteria used to the classification of the progression state were described in previous studies of our group [13, 14]. Samples of control individuals (HIV- uninfected volunteers, *n*=9) came from this same repository. For this study, HIV+ individuals (*n*=23) showing two profiles of clinical progression were selected: Slow Progressors (SPs, individuals with at least 8 years of infection before ART initiation [median: 9 years; 25%-75% percentile: 9-11 years]) and Rapid Progressors (RPs, individuals who initiated ART within 1-4 years after infection [median: 1 year; 25%-75% percentile: 1-3 years]). All HIV+ individuals included in this study initiated ART with $CD4^+$ T cells counts <350cell/mm^3 and were on ART at the date of the blood collection used for cytokine quantifications.

Table 1 details the basic clinical and demographic characteristics of the controls and HIV+ individuals evaluated in this study. Ethnic classification was performed based on skin color self-declaration. $CD4^+$ T cell counts "at sampling" refer to the closest measurement (available in medical records) to the date of blood collection used for cytokine quantification in this study. All volunteers signed informed consent forms developed according to the Resolution No. 466 from *Ministério da Saúde* [15]. This study was approved by the Ethics Committees of *Hospital Nossa Senhora da Conceição* (Porto Alegre, Brazil, protocol number: 002964-20.69/10-5) and *Universidade Federal do Rio Grande do Sul* (Porto Alegre, Brazil, protocol number: 30491714.0.0000.5347).

Cytokines quantification

IL-1β, IL-8, and IL-12p70 levels were quantified in all plasma samples by flow cytometry (FACSAria III, BD Biosciences, San Jose, CA, USA) using a BD Cytometric Bead Array (CBA) Human Inflammatory Cytokine kit (Catalog No. 551811, BD Biosciences). Of note, IL-12p70 is the bioactive form of IL-12 [16]. Although this CBA kit also allows the evaluation of IL-6, IL-10, and TNF, these markers were not included in the analysis because they have already been evaluated in a previous study of our group [13]. CBA analysis was performed according to the manufacturer's instructions. The FACSDiva software (BD Biosciences) was used to acquire the samples and generate the raw data. Next, the FCAP Array software (BD Biosciences) was used to evaluate raw data and express cytokine levels in pg/mL.

Statistical analysis

The statistical analyzes were performed using WINPEPI [17] and GraphPad Prism 5.01 softwares (GraphPad Software, Inc., San Diego, CA, USA). All graphs were plotted using GraphPad Prism. Sex and ethnicity ratios (categorical data) were compared between groups using Fisher's exact test. All quantitative data were checked for normality (Gaussian distribution). Unpaired t test (for data with normal distribution) and the non-parametric Mann-Whitney U test (for data without normal distribution) were applied for comparisons involving two groups. The non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test was used for the analysis involving three groups. For the correlation analyses, we applied the Spearman's correlation test (for data without normal distribution) and Pearson's correlation test (for data with normal distribution). A p-value ≤ 0.05 was set as statistically significant.

Results

No statistically significant differences were observed between HIV+ individuals and controls regarding age and sex distribution ($p>0.05$, Table 1). On the other hand, the groups differed in relation to ethnicity ($p=0.004$, Table 1), although it is important to point out that there are no reports in the literature concerning different IL-8 levels according to ethnicity. Table 2 shows the clinical data of HIV+ individuals stratified according to Slow Progressors (SPs) and Rapid Progressors (RPs). The progression time of HIV infection was clinically and statistically different between these sub-groups, as expected ($p<0.0001$, Table 2), and no difference regarding other parameters was observed ($p>0.05$, Table 2). This indicates that the HIV+ individuals stratification based specifically on the progression time of HIV infection was effectively performed.

Regarding cytokines quantification, in the majority of samples, IL-1β and IL-12 showed values below the detection limit, resulting in median values of 0pg/mL (data not shown). Therefore, both cytokines were excluded from the analysis, in line with the strategy adopted elsewhere [18]. On the other hand, circulating IL-8 levels were detected in controls (median: 3.2 pg/mL; 25%-75% percentile: 2.29-4.96 pg/mL) and HIV+ individuals (median: 5.13 pg/mL; 25%-75% percentile: 4.26-6.48 pg/mL). Therefore, IL-8 data was compared between the groups and included in the correlation analyzes.

First, we assessed potential differences regarding IL-8 levels between controls, HIV+ SPs, and HIV+ RPs. Although the HIV+ RPs sub-group showed higher IL-8 levels as compared to the other groups, no statistically significant differences were observed between these three groups using the Kruskal-Wallis test ($p=0.0645$, Figure 1) followed by Dunn's multiple comparisons test ($p>0.05$ in all comparisons between groups). Thus, concerning the analysis involving IL-8, both HIV+ SPs and HIV+ RPs individuals were pooled in a single group (HIV+ group). Next, we evaluated whether there

was a difference regarding IL-8 levels between the control group and the HIV+ group. In this analysis, IL-8 levels were statistically significant higher in the pooled HIV+ group as compared to controls (p=0.0442, Figure 1B).

Finally, a correlation analysis considering IL-8 levels and $CD4^+$ T cell counts at sampling was performed. This analysis included only HIV+ group and was initially performed without stratifying the individuals according to SPs and RPs (Figure 2A). Subsequently, the analysis was performed stratifying individuals as HIV+ SPs (Figure 2B) and HIV+ RPs (Figure 2C). No statistically significant differences were observed in these analyses (all p-values >0.05). Of note, correlation analyzes considering IL-8 levels and $CD4^+$ T cell counts before ART was also performed, although no statistically significant differences were observed (data not shown).

Discussion

Measurement of peripheral cytokine levels in HIV+ individuals who initiated ART with $CD4^+$ T cell counts below 350cell/mm^3 revealed increased circulating IL-8 levels as compared to healthy controls, a result which corroborates previous data from Haissman et al. [19], Matsumoto et al. [20], and Meddows-Taylor et al. [21]. Interestingly, increased circulating IL-8 levels were linked to increased risk of death in HIV+ individuals [22, 23] although, in our study, this finding (i.e. the increased circulating IL-8 levels) was independent of the progression profile, being observed both in slow as well as in rapid progressors. In line with our results, Matsumoto et al. [20] found no association of IL-8 levels with disease state or with the use of systemic drugs in HIV+ patients. On the other hand, Haissman et al. [19] observed that individuals with severe immunodepression ($CD4^+$ T cell counts <200cells/µL) had higher IL-8 plasma levels than those with $CD4^+$ T cell counts >200cells/µL, and HIV-associated $CD4^+$ T cell depletion was linked to increased levels of circulating pro-inflammatory cytokines [24]. Moreover, some authors reported a negative correlation between IL-8 levels and $CD4^+$ T cell counts [19, 25]. Differently, in our study no direct correlation between IL-8 plasma levels and $CD4^+$ T cell counts at sampling was observed, suggesting that circulating IL-8 levels in the context of HIV infection can be affected by several distinct factors besides the total number of $CD4^+$ T cells. Nevertheless, we cannot rule out a potential influence of low $CD4^+$ T cell counts in our results, since all HIV+ patients presented $CD4^+$ T cell counts <350cells/mm^3.

In accordance with our findings, Wada et al. [26] observed significantly higher circulating IL-8 levels in HIV+ men on ART with suppressed viral load in comparison to HIV-uninfected men. Furthermore, in a study evaluating HIV-infected children, increased plasmatic IL-8 levels were found in progressors (authors have pooled slow and rapid progressors) and ART nonresponders as compared to long-term nonprogressors and ART responders, respectively [25]. However, according to Meddows-Taylor et al. [27], HIV uninfected children already present high IL-8 levels and such difference may impact the progression of HIV infection. A reduction in circulating IL-8 levels after

administration of ART was observed in adults by Haissman et al. [19] and in children by Pananghat et al. [25]. The high levels of cytokines evaluated before and during ART were suggested as good predictors of mortality and morbidity in the context of HIV infection [3, 28]. In general, inflammatory cytokine levels decrease after ART initiation and such decrease vary according to the combination of antiretrovirals used [28].

Nevertheless, results that do not support increased IL-8 levels in the context of HIV infection have also been reported. As showed by Hober et al. [29], plasmatic IL-8 levels were similar in HIV+ individuals as compared to controls. Furthermore, Jacobs et al. [30] did not detect differences in circulating IL-8 levels between HIV-uninfected women and groups of HIV+ positive women with different progression profiles (elite controllers, uncontrolled viral replication, and ART-derived suppressed viremia). Although there are some findings suggesting that IL-8 can inhibit HIV replication *in vitro* [31, 32], most of the data points to the opposite direction. HIV-gp120 up-regulates IL-8 in monocytes [33] and HIV-Tat protein induces IL-8 production by T cells [34]. In astrocytes, IL-8 production can be stimulated by HIV-gp120 [35], HIV-Tat [36], and HIV-Vpr [37]. HIV-infected patients with impaired cognitive skills showed higher IL-8 levels in cerebrospinal fluid as compared to HIV+ individuals with no impaired cognitive skills [38]. In addition, IL-8 may favour HIV replication and transmission [39-41]. Taking together, these findings strongly support an effect of IL-8 in HIV pathogenesis. Indeed, there is a set of evidence indicating that IL-8 mediates neuroinflammatory processes observed in HIV+ patients. Thus, IL-8 is a potential target for the development of new therapeutic strategies focused on HIV-related neurodisorders. In this context, the blockage of IL-8 receptors may be an interesting path to follow [42]. However, only by studying the effects of IL-8 blockade in humans and in animal models will be possible to understand the actual impact of IL-8 on HIV pathogenesis [9]. An interesting approach could also rely on strategies for the reduction of the chronic inflammation state present in HIV+ patients through the use of a more general immunosuppressive therapy. In this sense, a recent study has shown that rosuvastatin (10 mg/day) is effective for reducing IL-8 and IL-12 levels (both inflammation markers) in HIV+ individuals on ART [43].

The information regarding co-infections of the individuals evaluated in our study is limited (data not shown). However, it is necessary to consider that chronic co-infections or comorbidities commonly found in HIV+ individuals may further aggravate the IL-8-mediated inflammation. For example, Krarup et al. [44] reported increased IL-8 levels in bronchoalveolar lavage fluid of HIV-infected individuals with bacterial pneumonia. HIV/*Mycobacterium tuberculosis* [21] and HIV/*Plasmodium falciparum* [18] co-infections were also linked to increased circulating IL-8 levels. Moreover, in a cooperative way, HIV-gp120 and HCV-E2 (an HCV envelope protein) promote IL-8 up-regulation [45], indicating that HIV/HCV co-infection may have an important impact on IL-8-mediated inflammation.

Considering other pro-inflammatory cytokines, it is known that IL-12 induces a Th1-type response, acting against infections [16], and IL-1β has a close involvement in HIV-associated inflammation [46]. However, IL-1β and IL-12 levels in most of our samples were below the detection limit of the assay employed. Similarly, these cytokine levels were below the detection limit in a study that evaluated different circulating cytokines in the context of HIV/*Plasmodium falciparum* infections [18]. According to Freeman et al. [28], plasmatic IL-1β levels are commonly undetected in chronic HIV-infected patients. In line with this information, ART can reduce the levels of IL-1β [8]. These factors may explain, at least in part, the lack of IL-1β detection in our samples obtained from HIV+ individuals on ART.

Some limitations of this study should be discussed. First, the controls available for this study correspond to nine individuals, which were sampled and stored at -80ºC, under the same conditions of all other samples. This lead to a reduced number of controls although minimized the chance of differences in the cytokine levels due to different storage times (as mentioned in methods, plasma samples used in this study were collected between 2011 and 2013). Second, circulating systemic cytokine levels may differ from the levels found in specific physiological microenvironments [8], and inflammatory chemokines are mostly expressed at high levels when there is a damaging stimulus [47]. On the other hand, the chronic inflammatory condition observed in HIV infection can be represented by systemic inflammatory markers, and elevated systemic levels of cytokines may indicate the presence of multiple inflammatory microenvironments [28]. Third, the groups addressed in this study were not homogenous regarding ethnicity. This point should be taken into consideration since the ethnic background was associated to differences in cytokine levels in the context of HIV infection by Shebl et al. [24], although concerning specifically IL-8 levels, no differences were observed by these authors. Fourth, only individuals who initiated ART with $CD4^+$ T cell counts <350cells/mm^3 were included in this study, which can limit the interpretation of our results.

On the other hand, our study has important strengths that must be highlighted. Since 2013, the public health policies in Brazil recommend ART administration as soon as possible after the diagnosis of HIV positivity [48]. Therefore, the individuals evaluated in this study present clinical conditions related to infection and progression which are now rare and very difficult to obtain in new studies involving HIV+ populations. For example, HIV+ individuals who initiated ART showing $CD4^+$ T cell counts <350cell/mm^3 (as evaluated in our study), or individuals with different progression profiles (SPs or RSs), will rarely be found in Brazil for a new study focused on inflammation markers.

Conclusions

Our results indicate that circulating IL-8 levels are significantly increased in HIV+ individuals on ART. This finding reinforces the role of IL-8 as a hallmark of the chronic inflammation observed in the HIV+ population, complementing previous studies of our group [13, 14]. Besides, increased IL-8

levels were independent of the progression profile (slow or rapid progressor) and of the $CD4^+$ T cell counts. Of note, all individuals evaluated in this study initiated ART with $CD4^+$ T cell counts <350cell/mm^3. It is possible that sustained increased IL-8 levels are at least partially due to the prolonged immunosuppression experienced by HIV+ individuals before initiating ART. If this hypothesis is confirmed, initiating ART as soon as possible after HIV infection will also bring benefits to maintaining circulating IL-8 at normal levels.

Finally, it is not clear whether high levels of circulating IL-8 is a consequence of HIV infection, a contributing factor to HIV pathogenesis, or both. Further studies applying different analysis strategies are important to reveal more information on the roles of IL-8 in the chronic inflammation observed in HIV+ individuals.

Conflicts of interest

We have no conflict of interest regarding this study.

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Table 1. Characteristics of the individuals evaluated in this study.

Characteristic		Control group (*n*=9)	HIV+ group: SPs+RPs (*n*=23)	*p*-value
Age, years [a]		33 [27-49.5]	42 [35-47]	0.3513 [c]
$CD4^+$ T cell counts (nadir, before ART) [a]		-	204 [95-269]	-
Viral load, log (nadir, before ART) [a]		-	4.53 [3.5-5.08]	-
$CD4^+$ T cell counts (at sampling) [a]		-	482.5 [274-802] [b]	-
Sex	Male, n (%)	3 (33.33%)	5 (21.7%)	0.654 [d]
	Female, n (%)	6 (66.67%)	18 (78.3%)	
Ethnicity	Caucasoids, n (%)	9 (100%)	10 (43.48%)	0.004 [d]
	Non-caucasoids, n (%)	0 (0%)	13 (56.52%)	

a, values expressed in median [25%-75% percentile]. b, based on *n*=18. c, Unpaired t test. d, Fisher's exact test. SPs, slow progressors. RPs, rapid progressors.

Table 2. Clinical characteristics of the HIV+ individuals stratified as slow and rapid progressors.

Characteristic	HIV+ SPs (*n*=10)	HIV+ RPs (*n*=13)	*p*-value
Time of progression, years	9 [9-11]	1 [1-3]	<0.0001 [b]
Age, years	42 [35-45.5]	42 [34.5-51.5]	0.8340 [c]
CD4 counts (nadir, before ART)	211.5 [89-284]	175 [108.5-267]	0.9843 [c]
Viral load, log (nadir, before ART)	3.83 [3.0-5.12]	4.8 [3.97-5.05]	0.2653 [c]
CD4 counts (at sampling)	599 [233-743] [a]	464 [311.5-984.5] [a]	0.5850 [c]

All values are expressed in median [25%-75% percentile]. a, based on *n*=9. b, Mann-Whitney U test. c, Unpaired t test. SPs, slow progressors. RPs, rapid progressors.

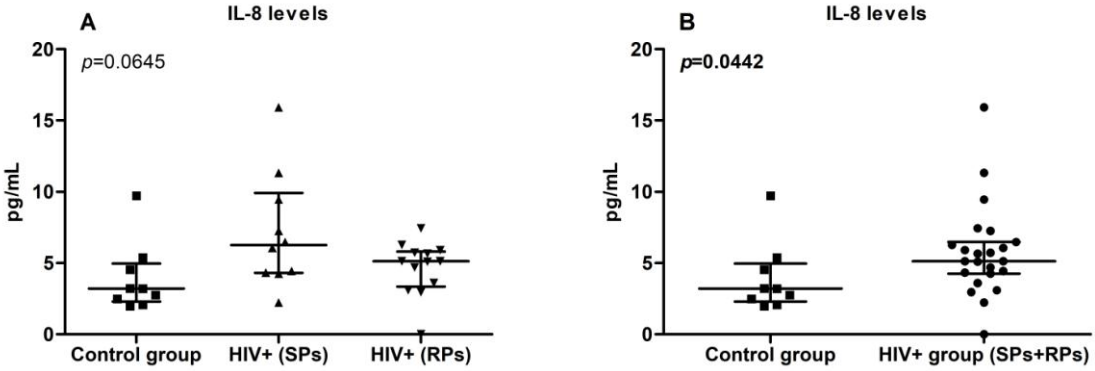

Figure 1. Comparison of IL-8 levels between groups. A: The groups were compared between them using the Kruskal-Wallis test (*p*-value indicated in the graph) followed by Dunn's multiple comparisons test (all *p*-values >0.05). **B:** HIV+ group (all HIV+ individuals included in this study: SPs and RPs) was compared to the control group using the Mann-Whitney U test (*p*-value indicated in the graph). Values are expressed in median with interquartile range. SPs, slow progressors. RPs, rapid progressors. Statistically significant *p*-value is shown in bold.

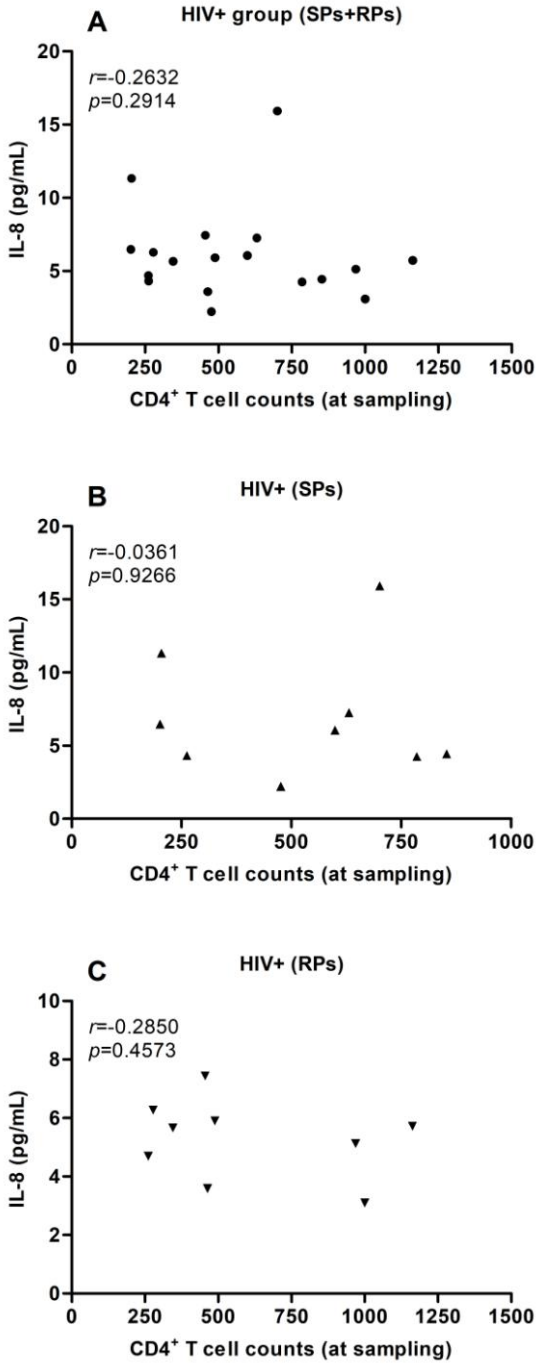

Figure 2. Correlation analysis between IL-8 levels and CD4$^+$ T cell counts at sampling. A: Data from HIV+ group (all HIV+ individuals included in the study: SPs and RPs). **B:** Data from HIV+ (SPs) group. **C:** Data from HIV+ (RPs) group. Data were analyzed using the Spearman's correlation test (A) and Pearson's correlation test (B and C). SPs, slow progressors. RPs, rapid progressors. Values of *p* and *r* are indicated in the graphs.

CAPÍTULO VIII

Immunogenetic studies of the hepatitis C virus infection in an era of pangenotype antiviral therapies - Effective treatment is coming

Este capítulo apresenta o seguinte artigo de revisão publicado no periódico *Infection, Genetics and Evolution*:

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Review

Immunogenetic studies of the hepatitis C virus infection in an era of pan-genotype antiviral therapies - Effective treatment is coming

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ABSTRACT

What are the factors that influence human hepatitis C virus (HCV) infection, hepatitis status establishment, and disease progression? Firstly, one has to consider the genetic background of the host and HCV genotypes. The immunogenetic host profile will reflect how each infected individual deals with infection. Secondly, there are environmental factors that drive susceptibility or resistance to certain viral strains. These will dictate (I) the susceptibility to infection; (II) whether or not an infected person will promote viral clearance; (III) the immune response and the response profile to therapy; and (IV) whether and how long it would take to the development of HCV-associated diseases, as well as their severity. Looking at this scenario, this review addresses clinical aspects of HCV infection, following by an update of molecular and cellular features of the immune response against the virus. The evasion mechanisms used by HCV are presented, considering the potential role of exosomes in infection. Genetic factors influencing HCV infection and pathogenesis are the main topics of the article. Shortly, HLAs, MBLs, TLRs, ILs, and IFNLs genes have relevant roles in the susceptibility to HCV infection. In addition, ILs, IFNLs, as well as TLRs genes are important modulators of HCV-associated diseases. The viral aspects that influence HCV infection are presented, followed by a discussion about evolutionary aspects of host and HCV interaction. HCV and HIV infections are close related. Thus, we also present a discussion about HIV/HCV co-infection, focusing on cellular and molecular aspects of this interaction. Pharmacogenetics and treatment of HCV infection are the last topics of this review. The understanding of how the host genetics interacts with viral and environmental factors is crucial for the development of new strategies to prevent HCV infection, even in an era of potential development of pan-genotypic antivirals.

1. Introduction

As a multifactorial condition, susceptibility to viral infections will depend on both genetic and environmental factors. In this sense, the study of the genetic aspects that confer susceptibility to viral infectious diseases is a highly complex task, since even individuals with very similar genetic backgrounds may be differently exposed to viruses. Variability in frequency and route of exposure to pathogens will always limit and confound the observations made in the context of genetic susceptibility studies. Besides, one must take into consideration the dynamic aspect of viral infections. In this context, a continuous co-evolutionary process takes place, with the selection of both new variants inside the viral population, and more resistant hosts.

To understand how and why a given human genetic variant could interfere on virus susceptibility or on disease outcome, we will first review some clinical and immunological aspects of the hepatitis C virus (HCV) infection. Specific mechanisms of immune evasion adopted by HCV will also be mentioned, as will be discussed the potential involvement of exosomes in virus infectivity. Finally, after focusing on the genetic aspects of both viruses and host, recent advances in HCV treatment and pharmacogenetic approaches will be highlighted.

2. Clinical aspects of HCV infection

HCV is one of the main causes of chronic liver disease in industrialized and developing countries. HCV is primarily transmitted through parenteral exposure to blood and other body fluids. After infection, acute hepatitis with jaundice occurs in one-fifth of persons,

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while the remaining cases go unnoticed. Spontaneous resolution occurs in 15 to 45% of persons in the first six months of the infection. The remaining patients develop chronic hepatitis C (CHC) with variable course, some of them progressing to cirrhosis (20–30%) and even to hepatocellular carcinoma (HCC) (1–4%). The natural history of CHC is greatly influenced by host, viral, and environmental factors (Lingala and Ghany, 2015).

After the initial infection, HCV replicates preferentially in the host liver cell cytoplasm. This cell-specific infection is attributable to HCV interaction with surface molecules present in the hepatocytes, such as heparan sulfate proteoglycans (Fan et al., 2017; Morikawa et al., 2007). However, a broad clinical spectrum of extrahepatic complications and associated diseases (such as non-Hodgkin's lymphoma, lymphoproliferative disorders, etc.) has already been detected in HCV carriers, indicating that other cells probably support viral replication in all phases of the infection (Blackard et al., 2006a). Moreover, HCV RNA and/or proteins have been detected in peripheral blood mononuclear cells (PBMC), B and T lymphocytes, monocytes/macrophages, dendritic cells, and other extrahepatic tissues of infected individuals (Fujiwara et al., 2013). More recently, PBMC were identified as a HCV replication site (Di Lello et al., 2014). In the liver, HCV does not have direct cytopathic effect and this organ presents a persistent infection, which depends on the viral replication and continuous cell-to-cell propagation, without immune response to viral antigens by T lymphocytes (Hoofnagle, 2002).

The time course of the acute phase has been difficult to define once symptoms are mild and patients frequently do not seek care. Recently infected individuals (who were accompanied after clinical referral, prison surveillance or community outbreak with seroconversion), usually presented symptoms like a self-limited flu (Grebely et al., 2014), with only a few patients presenting more severe manifestations, such as jaundice, abdominal pain, anorexia, dark urine, and other typical hepatitis symptoms. Acute hepatitis C can be prolonged and even severe, but a fulminant course is extremely rare and it mainly occurs in association with superinfection in hepatitis B virus (HBV) carriers (Sagnelli et al., 2014). HCV has a high replicative capacity in the beginning of the acute phase, reaching serum titers of 10^5–10^7 international units (IU)/ml few days after infection (Hoofnagle, 2002). The human host quickly reacts by the stimulation and expression of interferon (IFN) α, β, γ, and λ genes, in an attempt to restrict viral replication. After a delay of four to eight weeks, HCV-specific T cells are recruited to the liver in the second or late acute phase. It lasts four to ten weeks, being this a unique opportunity to eliminate HCV spontaneously. The viral level can decrease, fluctuate, or become intermittent until the achievement of a complete viral clearance or progression to chronic infection (Horner and Gale, 2013).

In the transition from the acute to the chronic phase, patients present intermittently low HCV RNA levels for a period of one week to two months (Glynn et al., 2005), followed by a ramp phase lasting eight to ten days with an exponential increase of the viral particles in the blood. In two months, viral and serum alanine aminotransferase (ALT) levels reach a high-titer plateau, indicating hepatocyte injury and necrosis. Generic symptoms and jaundice appear in this period, although most patients are still asymptomatic. Further, anti-HCV antibodies production increases and viremia decreases before the development of CHC (Hajarizadeh et al., 2013). This progression is marked by the continuous replication of the virus in the patients blood, a situation that averages 75 to 85%. Spontaneous resolution after six to twelve months is unusual in CHC (Bulteel et al., 2016). Consequently, CHC is defined as the persistence of HCV RNA in the blood for more than 6 months after the onset of the acute infection (Westbrook and Dusheiko, 2014).

CHC has usually a long, clinically silent period, with a slow progression to severe liver damage. Clinical manifestations usually appear 10 to 40 years after infection, and patients usually remain viremic, and therefore transmitters, for this long period of time (Yen et al., 2003). HCV RNA and ALT levels can fluctuate markedly along this whole period. Usually, one-quarter of the CHC patients has undetectable HCV-RNA and normal ALT levels, but most individuals have serum ALT levels higher than normal, without correlation with disease activity (fibrosis, cirrhosis, etc.). These patients have also few (if any) symptoms, the most common being an intermittent fatigue, with right upper quadrant pain (in the liver), nausea, and poor appetite as well as other possible symptoms (Hoofnagle, 2002).

Basic histopathological findings in CHC can be separated into inflammatory and fibrotic components and reflect the severity of disease (activity or “grade”) and cumulative damage (fibrosis or “stage”), respectively. Fibrosis progression rates are extremely variable and can be influenced by host, viral, and environmental factors. The rates of progression are not linear and may vary according to fibrosis stages (Westbrook and Dusheiko, 2014).

Cirrhosis develops in 20 to 40% of the CHC patients, with clinical manifestation only in a late stage, including symptoms of portal hypertension or hepatic insufficiency. Once cirrhosis is established, the disease progression remains unpredictable: it can remain indolent for many years or result in hepatocellular carcinoma (HCC), hepatic decompensation, and death. There is a 1 to 5% annual risk of HCC and a 3 to 6% of hepatic decompensation (variceal hemorrhage, ascites, encephalopathy). After an episode of decompensation, the risk of death is between 15 and 20% in the following year (Thein et al., 2008). External and host factors can accelerate these clinical manifestations. Alcohol consumption and the presence of co-infections such as HBV and human immunodeficiency virus (HIV) favor the progression of the disease. Other factors that may contribute to the occurrence of hepatic complications include immunosuppression, insulin resistance (diabetes), non-alcoholic steatohepatitis, schistosomiasis, hemochromatosis, smoking, host genetic factors, iron and aminotransferase levels, and factors such as diet and toxic contaminants (Westbrook and Dusheiko, 2014).

3. Immunological aspects of HCV infection

3.1. Innate immune response: molecular and cellular mechanisms

The innate immune response is the first line of defense against viral infections, such as HCV infection. This antiviral response is controlled by type I IFNs, which are predominantly produced by plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs) (Montoya et al., 2002). IFNs are cytokines responsible for the activation and regulation of different cell subsets of the innate immunity, such as natural killer (NK) cells (Hart et al., 2005). The pathways that drive the production of these cytokines are frequently dependent on endosomal toll-like receptors (TLRs) signaling through the recognition of viral RNA or DNA, and on cytoplasmic viral RNA or DNA sensor molecules, such as retinoic acid inducible gene-1 (RIG-1) and melanoma differentiation antigen 5 (Mda5) (Hart et al., 2005; Yoneyama et al., 2004). Both pathways converge in the activation of key transcription factors: nuclear factor-kappaB (NF-κB) and interferon regulatory factor (IRF) 3 and 7, whom control the antiviral defense (Honda and Taniguchi, 2006). Also, TLRs expressed on the cell surface or into phagosomes/endosomes of local innate immune cells, sense pathogen-associated molecular patterns (PAMPs), such as viral nucleic acids, and trigger a robust cytokine production, therefore recruiting circulating immune cells to the site of infection (Kawai and Akira, 2006).

The most important signal transduction pathway for IFNs involves the Jak-STAT (signal transducers and activators of transcription) molecular mechanisms (Darnell et al., 1994). Type I IFNs bind to the same cell surface receptor (IFNAR) and activate the receptor-associated tyrosine kinases Jak1 and Tyk2. Then, the kinases phosphorylate a single tyrosine residue and activate STAT1 and STAT2, being the activated STATs translocated to the nucleus, where they bind specific DNA elements in the promoter region of interferon-stimulated genes (ISGs) (Shuai et al., 1993). In HCV infection, hepatocytes sense the HCV

genome via RIG-1 and Mad5, which results in the induction of over 300 antiviral ISGs, as well as the secretion of type I and type III IFNs (Cao et al., 2015; Heim and Thimme, 2014; Saito et al., 2008; Saito and Gale, 2008). Mda5 contributes to the induction of ISGs around 2 days after HCV infection, while RIG-I signaling is triggered earlier (Cao et al., 2015). In hepatocytes where HCV establishes efficient replication, almost all viral proteins abrogate antiviral innate immunity by inhibiting both RIG-1/Mda5 and IFN-Jak-STAT signaling. Duong et al. (2004) observed in liver biopsies from patients with CHC and in a mouse model that HCV inhibits the transcriptional activation of the ISGs induced signaling via of the Jak-STAT pathway by up-regulating a protein phosphatase 2A (PP2A), hypomethylation of STAT1, and increased protein inhibitor of activated STAT1 (PIAS1) association (Duong et al., 2004).

An infection by HCV triggers different cellular mechanisms of innate immune response. For example, functional Kupffer cells, are liver resident macrophages which constitute 80–90% of total body macrophages (Ishibashi et al., 2009), are competent to sense danger signals via TLRs and trigger the release of inflammatory cytokines/chemokines that will be used against the HCV infection (Saha and Szabo, 2014). Also, monocyte-derived macrophages are linked to fibrosis and chronic inflammation. For instance, it was observed that TLR4 plays an important role in hepatic stellate cells activation and fibrogenesis during HCV infection (Guo and Friedman, 2010). Dendritic cells (DCs) play a predominant role in the production of type I and III IFNs via TLRs signaling activation during HCV infection. In this context, myeloid dendritic cells (mDCs) senses HCV Core and NS3 proteins through TLR2 (Szabo and Dolganiuc, 2005). TLR3, also expressed in certain types of mDCs, recognizes HCV dsRNA which results in the production of IL-1b, IL-6, and IL-12 (Zhang et al., 2013a). mDCs are the main responsible for the production of type III IFN (IFN-λ) in response to synthetic ligands or HCV proteins, via TLR3, a molecule significantly expressed in this cell subset (Zhang et al., 2013a). Furthermore, pDCs produce high amounts of type I IFN through TLR3, 7 and 9 signaling after exposure to HCV particles or HCV-infected cells (Takahashi et al., 2010).

NK cells are critical in the HCV infection once they are enriched in the human liver, secrete chemokines/cytokines such as TNF-α and IFN-γ, and can communicate with pDCs and mDCs (Gerosa et al., 2005). NK cells contribute to the HCV suppression via IFN-γ secretion or removal of infected cells (Tseng and Klimpel, 2002; Zhang et al., 2013b). Furthermore, NK cells are activated by cytokines in acute HCV infections and contribute to disease pathogenesis and to the infection outcome (Yoon et al., 2009). NK cells function is controlled by a balance between activating and inhibitory receptors, such as the inhibitory killer cell immunoglobulin-like receptors (KIRs), the activator natural cytotoxicity receptors and NKG2D (Lanier, 2005). Thus, the cross-talk between NK cells and DCs can be affected in HCV infection by abnormal NKG2A-expression. For example, a study observed an increased expression of NKG2D on NK cells. IFN-γ production and also cytotoxicity by NK cells were higher in HCV-infected individuals in the acute phase as compared with healthy controls (Amadei et al., 2010).

Recent works elucidated an additional mechanism by which infected cells spread viral products in the form of extracellular vesicles and manipulate immune cells. For instance, Kupffer cells sense HCV–PAMPs derived in the form of exosomes or other forms of extracellular vesicles secreted from infected hepatocytes (Schorey et al., 2015) (more details, see exosomes topic of this review).

3.2. Adaptive immune response: molecular and cellular mechanisms

Different components of the adaptive immune system are involved in the viral clearance, including antibody and T cell responses (Thimme et al., 2002). HCV-infected individuals produce neutralizing antibodies during the acute infection (Logvinoff et al., 2004). However, only a small fraction of such antibodies has a significant antiviral activity and, noteworthy, HCV control and clearance have also been observed in the absence of neutralizing antibodies, even in hypoglobulinemic individuals (Heim and Thimme, 2014). Furthermore, functional studies have shown that spontaneous clearance of HCV infection is associated with strong and sustained $CD4^+$ and $CD8^+$ T cell responses that target multiple epitopes within different HCV proteins (Lechner et al., 2000; Missale et al., 1996; Thimme et al., 2002). In this sense, HLA alleles have been widely studied in the outcome of HCV infection (Ali et al., 2010; Neumann-Haefelin et al., 2006).

The efficiency of virus-specific CD4 mediated responses can be subverted towards persistent viremia during the acute and chronic HCV infection (Bowen and Walker, 2005), highlighting the significant role of $CD4^+$ T cell responses in the clinical outcome definition. Thus, acute resolving HCV infection is characterized by strong Th1/Th17 responses with a specific expansion of IL-21-producing CD4 T cells and increased IL-21 plasma levels (Kared et al., 2013). Conversely, viral persistence was associated with lower frequencies of IL-21-producing $CD4^+$ T cells, decreased proliferation and increased expression of inhibitory receptors Tim-3 (T cell immunoglobulin and mucin-domain-containing-molecule-3), programmed death 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) on HCV-specific $CD8^+$ T cells (Golden-Mason et al., 2009; Kared et al., 2013; Radziewicz et al., 2008; Radziewicz et al., 2009). Indeed, several studies described dysfunctional HCV-specific $CD4^+$ T cell responses and a higher frequency of suppressive $CD4^+CD25^+$ T cells in chronic infection (Boettler et al., 2005; Cabrera et al., 2004; Rushbrook et al., 2005; Semmo et al., 2005). Also, a hallmark of chronic HCV infection is the frequency of functionally impaired virus-specific $CD8^+$ T cells, characterized by their inability to proliferate or secrete antiviral cytokines, such as IFN-γ (Klenerman and Thimme, 2012). In individuals with chronic HCV infection, $CD8^+$ T cell exhaustion was characterized by an upregulation of PD-1 and low expression of CD127 (Radziewicz et al., 2007). All these molecular and cellular mechanisms of the innate and adaptive immune responses for spontaneous clearance or persistent viremia of HCV depend on host genetic, immunological, and viral factors.

3.3. HCV: mechanisms of viral immune evasion

Viral genomes can be maintained in host cells by limited gene expression and several mechanisms allow them to evade the host immune response. For example, the genome size of RNA viruses is limited and mutation is an important escape mechanism due to the low fidelity of RNA polymerases. As mentioned, by limiting gene expression, viruses can escape from the host immune response. Nevertheless, viruses need to replicate and infect other cells and such processes are associated with the production of antigenic proteins that make the virus vulnerable to immune control mechanisms that will warn the host of the presence of an invader (Alcami and Koszinowski, 2000). Thus, evasion should start simultaneously to infection. When viruses enter a cell, a series of molecules (PAMPs) will be recognized by the host immune system, signaling a viral infection. PAMPs can be components of infection or replication (such as single-stranded or double-stranded RNA molecules), and are recognized by proteins that serve as PAMP receptors, recruited by specific TLRs or nucleic-acid binding proteins. The host launches an (innate) immune defense against the HCV infection that, in turn, attempts to evade the host response through a multifaceted process that includes (I) signaling interference, (II) effector modulation, and (III) continuous genetic variation. These evasion strategies, eventually, end up by promoting persistent infection and spread of HCV (Gale and Foy, 2005).

In addition to mutations, it is widely accepted that recombination plays an important role in the evolution of RNA viruses by creating genetic variation through the exchange of nucleotide sequences between different genomic RNA molecules (Moreno et al., 2009). Regarding HCV, recombination has already been reported in populations from different geographic locations (Echeverría et al., 2015). Additionally, it is important to consider the occurrence of intra patient

recombination, also called intra quasispecies recombination (Moreno et al., 2006). Thus, HCV is capable of successfully complete all the steps necessary to produce a recombinant viral form, i.e. simultaneous infection of a cell by different viral strains, replication of both viral genomes, strand shift by the viral RNA polymerase without disturbing the correct reading frame, and encapsidation followed by the release of the recombinant genomes as viable viral particles (González-Candelas et al., 2011).

Viral infections activate the IRF-3 through two independent signaling pathways: engagement of TLR 3 by dsRNA; and through the RIG-I (Li et al., 2005; Yoneyama et al., 2004). Since viruses entry activates these signaling pathways, the ability to control the response or the amplification of such signals represents an excellent strategy for viral evasion. Important points for HCV evasion from the host immune system are found in the PAMP-responsive pathways: disrupting signaling cascades involved in the induction of IFN synthesis, subverting Jak-STAT signaling to limit the expression of interferon stimulated genes (ISGs), or directly blocking the antiviral activities of these same molecules (Li et al., 2005). HCV protein NS3/4A is a functional antagonist of the host response induced by dsRNA and is an essential viral protease with important roles for the establishment and maintenance of both HCV infection and HCV evasion. This protease causes specific proteolysis of Toll-IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF or TICAM-1), an adaptor protein linking TLR3 to kinases. TLR3 is responsible for the activation of IRF-3 and NF-κB, transcription factors important in antiviral defenses. When TRIF is cleaved by NS3/4A, a reduction on TRIF abundance occurs, consequently leading to inhibition of signaling through the TLR3 pathway before its bifurcation to IRF-3 and NF-κB (Foy et al., 2003).

RIG-I is a DEx/D-box RNA helicase belonging to a small family of helicases involved in host response signaling (Yoneyama et al., 2004). The viral disruption of RIG-I or TLR3 signaling leads to various consequences: (I) it attenuates two major pathways of IFN production in hepatocytes (Li et al., 2005); (II) there is a break in the amplification and duration of the PAMP signaling imposed by NS3/4A, produced by some factors including RIG-I, TLR3, TRIF which are responsive to IFN (Foy et al., 2003); (III) it promotes alterations in antigen presentation, leading to inefficient activation of cytolytic T cells and an inability of the adaptive immune response to clear HCV-infected hepatocytes since MHC components of antigen processing and presentation are themselves ISG products (Shoukry et al., 2004; Der et al., 1998; Su et al., 2002). The blockade of virus-induced NF-κB activity regulates the expression of a variety of chemokines and cytokines (Foy et al., 2005), such as IL-1, which mediates antiviral actions against the HCV (Zhu and Liu, 2003).

Another evasion mechanism involves the activity of microRNAs (miRNAs). HCV infection up-regulates miR-21 expression, which in turn suppresses HCV-triggered type I IFN production, promoting the virus replication. miR-21 targets two important factors in the TLR signaling pathway, myeloid differentiation factor 88 (MyD88) and IL-1 receptor-associated kinase 1 (IRAK1), which are involved in HCV-induced type I IFN production. Thus, upregulation of miR-21 during HCV infection negatively regulates IFN-α signaling through MyD88 and IRAK1 (Chen et al., 2013).

NK cells are also important in the control of viral infections by cytolytic and cytokine-producing effector functions (Guidotti et al., 1999; Tay and Welsh, 1997). According to some data, NK cells could be the primary target for HCV evasion once NK cells activity can be directly inhibited by the virus, allowing the establishment of a replicative advantage prior to the induction of specific immune responses (Golden-Mason and Rosen, 2006). This idea is anchored in the fact that NK cells activity can be directly inhibited by the binding of recombinant HCV envelope protein to the CD81 receptor of NK cells (Crotta et al., 2002).

In another strategy to evade the host immune system, HCV particles might directly bind to lipoproteins or incorporate lipoprotein components, such as lipids and apolipoproteins, either through their interaction with the blood of infected patients or with virus producer cells (Dao Thi et al., 2011). The association of HCV with lipoproteins facilitates virus entrance into target cells. Indeed, the LDL receptor (LDLR) has been shown to internalize HCV associated with LDL and VLDL in various human cell types in vitro, leading to infection (Agnello et al., 1999; Andre et al., 2002; Monazahian et al., 1999). It was suggested that lipoproteins associated with the virus are critical for the infectivity of serum HCV and could provide protection against antibody-mediated neutralization, perhaps shielding the viral surface glycoproteins. Also, evidence suggests that HDL stimulates HCV cell entry at a post-binding stage, which reduces the time window during which neutralizing antibodies (nAbs) can bind to and neutralize the virus (Dreux et al., 2006). Thus, lipoproteins may help the virus to escape from the recognition by the host immune system and its subsequent neutralization by two main mechanisms: first, the virus association with LDL and VLDL provides protection against antibody neutralization by masking epitopes on viral surface glycoproteins; and, second, HDL accelerates viral entry, which limits the exposure of the virus to nAbs (Di Lorenzo et al., 2011). Of note, the glycans on viral-derived glycoproteins are produced by the host cellular machinery, thus they are often recognized as 'self' by the immune system. Consequently, glycans associated with viral envelope proteins decrease the immunogenicity of viral particles by shielding important epitopes, thus protecting HCV against antibody neutralization (Balzarini, 2005; Zhang et al., 2004).

Many enveloped viruses have evolved mechanisms to move between cells without diffusing through the extracellular environment. This mode of transmission shields the virus from the innate and adaptive immune effector mechanisms thus facilitating rapid viral dissemination (Sattentau, 2008). HCV has been shown to spread via direct cell-to-cell transfer. The first report of in vitro cell-to-cell spread of HCV was from infected human lymphoblastoid B cells to human hepatoma-derived cells (Timpe et al., 2008; Valli et al., 2007). Studies also support the idea that virus particles may be transmitted directly between cells (Brimacombe et al., 2011; Timpe et al., 2008; Valli et al., 2006; Witteveldt et al., 2009). Alternatively, exosomes were also suggested to be involved in virus infectivity, spread, and escape of the immune system. Examples of immune evasion mechanisms used by HCV are shown in Fig. 1.

3.4. Exosomes in virus infectivity, spread, and escape of the immune system

Exosomes are extracellular nanovesicles of endosomal origin released from various cell types. They are found in amniotic effusions, blood, bronchoalveolar lavage fluid, breast milk, malignant ascites, synovial fluid, and several other biological fluids. The basic functions of exosomes are intercellular communication and traffic of biomolecules. Proteins and different types of RNAs are the main cargos transported by exosomes (Shahabipour et al., 2017). The transport of mRNA and miRNAs via exosomes highlights its functional activity since genetic cargos released by one cell into exosomes can be delivered to another cell without losing their functional activities (Valadi et al., 2007). Exosomes seem to affect the infection capacity of different viruses, such as HIV, Human T-cell lymphotropic virus (HTLV), Human papilloma virus, and Epstein-Barr virus (Anderson et al., 2016).

Masciopinto et al. (2004) reported the transport of RNA and proteins from HCV by exosomes, suggesting that such vesicles could facilitate HCV infection. The traffic of HCV-RNA via exosomes was experimentally demonstrated by different methodological approaches (Dreux et al., 2012; Longatti et al., 2015; Zhao et al., 2017) although Chen et al. (2015) argued that most HCV-RNA and virions are actually transported by other types of microvesicles rather than by exosomes. However, further studies have shown that HCV secretion from host cells can be mediated by mechanisms of exosomes release (Elgner et al., 2016; Shrivastava et al., 2016; Tamai et al., 2012).

Interestingly, Ramakrishnaiah et al. (2013) observed in vitro HCV transmission mediated by exosomes causing productive infection. HCV

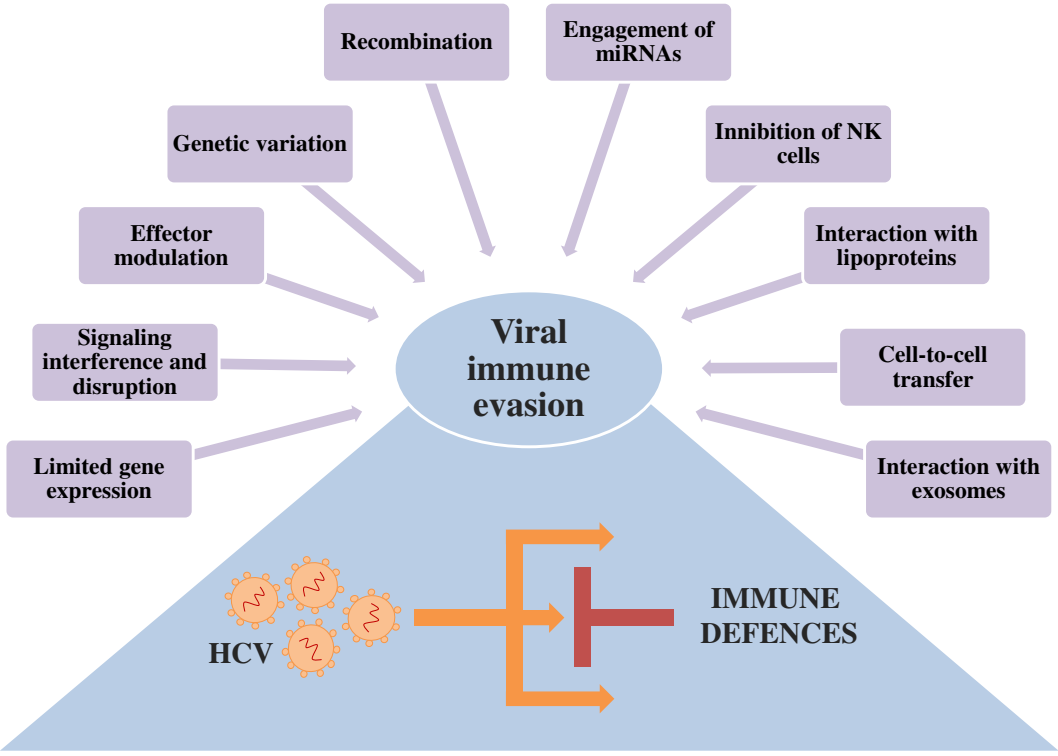

Fig. 1. Examples of immune evasion mechanisms used by HCV. See text for references.

transmission via exosomes from the blood of HCV-positive patients was also reported by Bukong et al. (2014). A direct demonstration of the HCV-exosome association was given by Liu et al. (2014), who observed HCV particles inside exosomes using electron microscopy. HCV traffic mediated by exosomes is considered a potential form of immune system evasion (Dreux et al., 2012; Longatti et al., 2015). However, the release of HCV via exosomes seems to occur only in a minor viral population (Lai et al., 2014).

However, the relationship between exosomes and HCV is not always advantageous to the virus. According to Giugliano et al. (2015), exosomes can show anti-HCV activity. Moreover, Qian et al. (2016) have been shown inhibition of HCV infection by exosomal miRNAs. It is of clinical relevance to identify which exosomal cargos have anti-HCV activity, as well from which cells they are released. Thus, it will be possible to develop therapies to inhibit HCV infection based on engineering exosomes loaded with therapeutic and/or anti-viral molecules. For example, the delivery of iRNA via exosomes was already suggested for hepatitis treatment (Pan et al., 2012).

During HCV-associated disease, exosomes also mediate intercellular communication in acute and chronic hepatitis C (Devhare et al., 2017). In addition, they have an important role in the disease progression. Welker et al. (2012) observed an increase of CD81 in exosomes of HCV-infected patients. This increase was associated with inflammatory activity and fibrosis, markers of disease severity. Most of the studies published to date try to elucidate the interactions between viral components and exosomal molecules.

Finally, it is important to clearly identify the genetic cargos carried by exosomes during the HCV infection, as well as to understand how these cargos act on the HCV lifecycle. This knowledge will allow the development of strategies to negatively regulate the virus replication or to disrupt other stages of the virus lifecycle. It is also not yet clear how viral components, mainly viral RNA or viral proteins, interact with exosomes during cell entry, viral budding, and cell-to-cell traffic. Thus, functional studies will be essential to understand the role of exosomes and other microvesicles in HCV infection and pathogenesis.

4. Host genetic factors influencing HCV infection and pathogenesis

4.1. Susceptibility to HCV infection

The investigation of host genetic variants such as single nucleotide polymorphisms (SNPs) is quite important to understand the role of distinct genotypes on HCV susceptibility. According to Singh et al. (2007), different alleles from classical and non-classical HLA molecules impact the HCV infection and disease, although ethnic diversity, complex immune-regulatory mechanisms, and variations in the studies design and methodologies make difficult to find a general association between any classical HLA allele and HCV infection (Singh et al., 2007). Besides, SNPs in several other non-HLA genes were reported as important in the context of the HCV infection.

Considering the non-classical HLA genes, Cordero et al. (2009) found that allele C of the *HLA-G* + 3142 C/G SNP (rs1063320) was protective against HCV infection. In addition, in African-derived individuals co-infected with HCV and HIV, the ins/ins genotype from *HLA-G* 3′ untranslated region (UTR) 14 bp ins/del polymorphism (rs66554220) was more frequent in co-infected individuals than in those infected only with HIV (da Silva et al., 2014). Moreover, several *HLA-G* polymorphisms and one specific haplotype were more frequent in HCV-infected Europeans as compared with uninfected controls (Catamo et al., 2017). Since HLA-G expression correlates with the variants evaluated, these data highlight that differences in HLA-G expression might affect susceptibility to HCV infection. Of note, in adult healthy individuals *HLA-G* has a restricted expression pattern that includes some subpopulations of regulatory T cells, which could explain a modified host susceptibility to HCV. Other classes of *HLA* genes have also been addressed in the context of the HCV infection. For example, in Chinese population, the *HLA-DMA* C/T (rs1063478), *HLA-DOA* A/G (rs2284191), *HLA-DOB* A/G (rs7383287) (Huang et al., 2014), and *HLA-DQ* T/C (rs2856718) SNPs were involved in the susceptibility to HCV (Yue et al., 2015).

Considering the HCV-host cell interaction, different gene families

related to the production of cell surface receptors and immunological response stand out as modulators of susceptibility to HCV infection. Recently, Budzko et al. (2016) reported that *Immunoglobulin Lambda Like Polypeptide 1 - IGLL1*, *Myeloid/Lymphoid or Mixed-lineage Leukemia; translocated to, 4 - MLLT4*, also known as *adherens junction formation factor* (*AFDN*), *3-Phosphoinositide Dependent Protein Kinase 1 - PDPK1*, and *Protein Phosphatase 1 Regulatory Subunit 13 Like - PPP1R13L* are genes involved in host-virus interaction and that variants of these genes could influence HCV infection susceptibility.

The risk of HCV infection of a given individual is highly dependent on cytokine gene expression and, therefore, on their genetic variants. In a meta-analysis, Lu et al. (2010) reported *IL-10* -592 A/C SNP (rs1800872) as a risk factor for HCV infection in Asians, although this result does not apply to Europeans. Since IL-10 is an important immunomodulatory cytokine, variants in the *IL-10* gene can potentially interfere with the establishment of a balance between anti- and pro-inflammatory cytokines, potentially explaining the altered susceptibility to HCV infection (Afzal et al., 2011). Other cytokine genes and their variants also stand out as risk modulators to HCV infection. CT and TT genotypes from *IFNL4* C/T SNP (rs12979860; formerly referred by some authors as a polymorphism from *IL28B* gene), CT and TT genotypes from *TGF-β*-509 C/T SNP (rs1800469), and AG and AA genotypes from *TNF-α*-308 G/A SNP (rs1800629) are associated with susceptibility to HCV infection. These associations possibly involve variations in cytokine production (Pasha et al., 2013). Also, the genetic background associated with high IL-12 production is linked to protection against HCV infection (Hegazy et al., 2008). Of great importance, among the different cytokine genes, the region encompassing *IL-28B*, *IFNL3* and *IFN4* stands out in the context of HCV susceptibility (Jin et al., 2014). In short, the studies abovementioned indicate that variations affecting these cytokine production levels impact on the susceptibility to HCV infection.

The role of TLRs on HCV susceptibility, previously discussed, is evidenced by genetic studies with different populations. Evaluating the variants *TLR7* C/G (rs179016), *TLR7* C/G (rs5743733), and *TLR7* A/G (rs1634323) in a high-risk population from China, Xue et al. (2015) associated an elevated risk of HCV infection with CCA and GGA haplotypes, respectively among women and men. Recently, Al-Anazi et al. (2017) evaluated eight *TLR3* polymorphisms in a Saudi Arabian population. The *TLR3* GG genotype (from rs78726532) was associated with susceptibility to HCV infection. Also recently, Valverde-Villegas et al. (2017) showed that in African-derived individuals from Brazil *TLR9* AA genotype (from rs352140) was associated with susceptibility to HCV and HIV co-infection.

Other molecules related to the innate immune response were also evaluated through immunogenetic approaches. For instance, it was suggested that mannose-binding lectin (MBL) acts as an anti-HCV molecule, performing a protective role in the first stages of infection. This view is supported by the increased frequency of *MBL2* wild-type alleles in controls compared to HCV-infected individuals, when evaluating three polymorphisms in the first exon of *MBL2* gene, at codons 52, 54, and 57 (Segat et al., 2007). On the other hand, *MBL* alleles (MBL*A, MBL*B, MBL*C, and MBL*D) were not associated with susceptibility to HCV infection in Brazilians from the Amazon region (Vallinoto et al., 2009). Bevilacqua et al. (2009) investigated the role of some genes associated in adults with HCV infection (*HLA-DRB1*, *MBL2*, *TNF-α*, *IFN-γ*, and *IL-10*), but in that case, focused on mother-to-child transmission. Looking at *HLA-DRB1*, HLA mismatch between mother and child was a protective factor against HCV infection. The same authors suggested that alloreactive immune responses can be a protective factor against HCV vertical transmission.

HLA, MBL, TLRs, and cytokine gene families and their variants have relevant roles in the susceptibility to HCV infection (see Table 1). In this sense, it is clear that the genetic background of different populations affects the risk of HCV infection. Genetic susceptibility to infection will depend on (I) the expression pattern of susceptibility genes (for example, those that modulate cytokine production); (II) the presence of specific polymorphisms in susceptibility genes in a given population; (III) the interaction between the products of different polymorphic genes; (IV) the sex of the individual; (V) the ethnic background; (VI) gene-environment interactions.

4.2. Host genetics and disease progression

According to Matsuura and Tanaka (2016), variants in *IFNLs*, *HLA-DQ*, *HLA-DR*, *PNPLA3*, *RNF7*, *MERTK*, *TULP1*, *MICA*, and *DEPDC5* genes regulate the progression of HCV-associated disease, from the HCV infection until the development of hepatocellular carcinoma, and passing through chronic hepatitis and liver cirrhosis, considered to be intermediary steps of the disease. However, other genes and their variants also have important effects on the HCV pathogenesis. Among these other genes and variants, cytokine genes are prominent.

In a meta-analysis focused on the *IL-18* -607 C/A (rs1946518) and -137 G/C (rs187238) SNPs, Yang and Liu (2015) observed an association between rs187238 and chronic HCV-associated hepatitis. Fakhir et al. (2016) evaluated the association between the variants *NF-κB1*-94Ins/DelATTG (rs28362491) and *NFκBIA* 3′UTR 2758 A/G (rs696) with the outcome of HCV infection in 343 patients with persistent HCV infection, 78 resolvers, and 138 controls. The Ins/Ins genotype of *NF-κB1* SNP was associated with high risk for advanced liver disease, although rs696 failed in association with either HCV resolution or progression. Furthermore, the *NF-κB1* Ins allele was associated with higher HCV loads. In the same year, Bader El Din et al. (2016) investigated the impact of *TNFα*-308 G/A and *TGFB1*-509 C/T SNPs on hepatic fibrosis progression in HCV-infected Egyptians. The combined unfavorable *TNFα* GA/AA and *TGFβ1* CT/TT genotypes were linked to abnormal liver function and they were frequent among high activity and late fibrosis HCV-infected individuals, suggesting that both SNPs, in synergy, modulate the progression of hepatic fibrosis.

The following SNPs, *IL28B/IFNL4* C/T (rs12979860), *TGF-β1*-509 C/T (rs1800469), *TNF-α*-308 G/A (rs1800629), and *IL-10*-1082 G/A (rs1800896) were evaluated in HCV-infected Egyptians by Pasha et al. (2013). *IL28B/IFNL4* CT and TT, *TGF-β1* CT and TT, and *TNF-α* AG and AA genotypes were linked to disruption in cytokine production, resulting in resistance to combined antiviral therapy. Interestingly, no association was found between the *IL-10* rs1800896 and response to treatment (Pasha et al., 2013). The role of *IL-10* variants on HCV disease progression was evaluated in different populations. Overall, variations in this gene have a relevant impact on HCV-related diseases. Świątek-Kościelna et al. (2017) analyzed SNPs in the *IL-10* promoter region, including -1082 A/G (rs1800896), -819 C/T (rs1800871), -592 C/A (rs1800872), and one SNP in the 3′ UTR region, + 4529 A/G (rs3024498), and their relationship with severity of liver disease and outcome of HCV-related therapy (pegylated interferon alpha and ribavirin, combined). The study was performed with Polish patients with HCV-related chronic hepatitis. The -592 C allele was associated with mild hepatic inflammation. The -819 C allele was related to sustained virological response (SVR). Furthermore, the ACCA haplotype and the intermediate IL-10 producer ACC haplotype were linked to SVR and nonrelapse. Differently, da Silva et al. (2015) found no association between -1082 A/G, -819 C/T, and -592 C/A SNPs and SVR in Brazilians. However, -1082 GG and AG genotypes were more frequent among HCV-infected patients showing advanced stages of fibrosis and cirrhosis. Also in Brazilian HCV-infected individuals, Ramos et al. (2012) studied the following SNPs: *IL-10* -592 C/A, -819 C/T, and -1082 A/G; *IL-4* + 33 C/T; *INF-γ* + 874 T/A; TNF-α -238 and -308; *IL28B/IFNL4* C/T (rs12979860) and T/G (rs8099917). The *IL-10*-1082 G, *IL-4* + 33 C, *IL28B/IFNL4* C (rs12979860), and *IL-28B* T (rs8099917) alleles were associated with spontaneous viral clearance.

As previously stated, the region encompassing the *IFNLs* genes stands out with crucial impact on HCV-related disease progression. According to the meta-analysis performed by Sato et al. (2014), *IL-28B*

Table 1
Genes involved in susceptibility to HCV infection and/or HCV-related disease progression according to studies included in this review.[a]

Gene	Involvement in susceptibility to HCV infection	References	Involvement in HCV-related disease progression	References
BTNL2			X	(Urabe et al., 2013)
C6orf10			X	(Urabe et al., 2013)
DEPDC5			X	(Matsuura and Tanaka, 2016)
EGF			X	(King et al., 2014)
HLA-DMA	X	(Huang et al., 2014)		
HLA-DOA	X	(Huang et al., 2014)		
HLA-DOB	X	(Huang et al., 2014)		
HLA-DQ	X	(Yue et al., 2015)	X	(Matsuura and Tanaka, 2016; Urabe et al., 2013)
HLA-DRB1	X	(Bevilacqua et al., 2009)	X	(Matsuura and Tanaka, 2016; Urabe et al., 2013)
HLA-G	X	(Catamo et al., 2017; Cordero et al., 2009; da Silva et al., 2014)		
IFNLs	X	(Jin et al., 2014; Pasha et al., 2013)	X	(de la Fuente et al., 2017; Fischer et al., 2016; Kamal et al., 2014; King et al., 2014; Matsuura and Tanaka, 2016; Pasha et al., 2013; Patin et al., 2012; Ramos et al., 2012; Sato et al., 2014)
IGLL1	X	(Budzko et al., 2016)		
IL-4			X	(Ramos et al., 2012)
IL-10	X	(Lu et al., 2010)	X	(da Silva et al., 2015; Ramos et al., 2012; Świątek-Kościelna et al., 2017)
IL-12B	X	(Hegazy et al., 2008)		
IL-18			X	(Yang and Liu, 2015)
MBL2	X	(Segat et al., 2007)		
MERTK			X	(Matsuura and Tanaka, 2016; Patin et al., 2012)
MICA			X	(Matsuura and Tanaka, 2016)
MLLT4	X	(Budzko et al., 2016)		
NF-κB1			X	(Fakhir et al., 2016)
PDPK1	X	(Budzko et al., 2016)		
PNPLA3			X	(King et al., 2014; Matsuura and Tanaka, 2016; Patin et al., 2012)
PPP1R13L	X	(Budzko et al., 2016)		
RNF7			X	(Matsuura and Tanaka, 2016; Patin et al., 2012)
TGF-β1	X	(Pasha et al., 2013)	X	(Bader El Din et al., 2016; Pasha et al., 2013)
TNF-α	X	(Pasha et al., 2013)	X	(Bader El Din et al., 2016; Pasha et al., 2013)
TULP1			X	(Matsuura and Tanaka, 2016; Patin et al., 2012)
TLL1			X	(Matsuura et al., 2017)
TLR3	X	(Al-Anazi et al., 2017)		
TLR7	X	(Xue et al., 2015)		
TLR9	X	(Valverde-Villegas et al., 2017)	X	(Fischer et al., 2016)

[a] Importantly, new genes linked to susceptibility to HCV infection and HCV-related disease progression are constantly described.

polymorphisms modify the natural course of the chronic HCV-related disease. Specifically rs12979860 and rs8099917 SNPs, respectively, were associated with severe fibrosis and inflammation, highlighting the role of these SNPs/genotypes as promoters of HCV-related complications. Nevertheless, in high-risk Egyptians exposed to HCV, the *IL-28B/IFNL4* CC genotype (from rs12979860) promoted early multispecific T cell responses, a feature that could help in viral clearance (Kamal et al., 2014). Looking at the same polymorphism, non-CC genotype was associated with increased risk of clinical deterioration in patients from the USA with HCV-related cirrhosis (King et al., 2014). However, evaluating Caucasians that received a first liver transplantation for HCV-related cirrhosis or for alcoholic cirrhosis, de la Fuente et al. (2017) did not found a significant association between rs12979860 and hepatocarcinogenesis, but the T allele and the TT genotype frequencies were high in patients with HCV-related cirrhosis. Although no association was observed with hepatocarcinogenesis, most studies indicate that the *IL-28B* gene is crucial in other HCV-associated outcomes. For example, it plays an important role in persistent infection, inflammatory steatosis, and fibrosis. Conversely, *MICA* and *DEPDC5* appear to be important genes in hepatocarcinogenesis (Matsuura and Tanaka, 2016). A genome-wide study involving HCV-positive European descendants pointed apoptosis regulatory genes as important influencers of fibrosis development (Patin et al., 2012). However, taking into consideration the role of apoptotic events in tumoral biology, apoptosis-controlling genes might also influence the hepatocarcinogenesis. In conclusion, the role of *IFNLs* in HCV-associated diseases seems to be related to intermediary steps of the disease.

Some genetic variations could be used for HCV disease prevention or clinical follow-up. Evaluating the *TLR9*-1486 T/C (rs187084), *TLR9*-1237 T/C (rs5743836), and the *IFNL4* (rs12979860) SNPs, Fischer et al. (2016) identified an association between the C allele of the *TLR9*-1486 variant with spontaneous HCV clearance in European women. The T allele of the *IFNL4* rs12979860 had a similar influence on viral clearance. These authors suggested that genotyping *TLR9*-1486 T/C in combination with *IFNL4* rs12979860 might be a tool to identify patients at high risk to develop chronic HCV-related complications (Fischer et al., 2016). Similarly, a genome-wide study pointed to an association between the *TLL1* A/T SNP (rs17047200) and development of hepatocellular carcinoma in Japanese patients showing SVR (Matsuura et al., 2017). Based on this, Matsuura et al. (2017) suggested *TLL1* A/T SNP genotyping to identify patients at risk for hepatocellular carcinoma. Also involving the Japanese population, Urabe et al. (2013) performed a genome-wide study including individuals with HCV-related liver cirrhosis and individuals with CHC. Their results suggested multiple genetic variations in the MHC region to be used as biomarkers for CHC monitoring.

Similarly to HCV susceptibility, cytokine genes, mainly *IL-10* and *IL-28B*, as well as TLR genes stand out as modulators of HCV-associated diseases progression (see Table 1). Genotyping of SNPs associated with disease development may be used as biomarkers/monitoring tools of individuals with susceptibility to faster disease progression or predisposition to HCV-related clinical complications. The understanding of the variation of these genes is essential to identify individuals at high risk to develop HCV-associated complications. Moreover, in the future,

it will soon be possible to adjust anti-HCV therapies according to the individuals genotype in order to reach a better response to the treatment.

5. Viral factors affecting infection

HCV is an enveloped, positive-sense single-strand RNA virus, from the genus *Hepacivirus* and family Flaviviridae. The RNA genome has approximately 9600 nucleotides in length with one continuous open reading frame (ORF) flanked by nontranslated regions (NTRs) at 5′ and 3′ ends. The 5′NTR has a highly conserved region, and forms the internal ribosome entry site (IRES), a tridimensional structure that directs cap-independent translation of the viral polyprotein. The ORF contains 9024 to 9111 nucleotides (according to the HCV genotype) and encodes a single polyprotein (~3000 amino acids) processed by viral and host proteases to generate a total of ten viral proteins: Core - E1 - E2 - p7 - NS2 - NS3 - NS4A - NS4B - NS5A - NS5B (in order). The first three proteins (Core, E1, and E2) are located at the amino terminus of the polyprotein and form the virions, while the remaining ones (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) act in the viral replication. The Core protein is a component of the viral capsid, while E1 and E2 are envelope glycoproteins that mediate host cell entry. The nonstructural (NS) proteins are involved in many steps of the HCV lifecycle: p7 protein is an ion channel protein (viroporin); NS2 and NS3 are autoproteases, which catalyze the polyprotein cleavage; NS3, NS4A, NS4B, NS5A, and NS5B are the major components of the HCV RNA replication complex. All these NS proteins are also involved in the virus assembly (Kim et al., 2016).

Due to error-prone RNA polymerase and the lack of proofreading mechanisms, HCV presents an extensive genetic heterogeneity with a high degree of RNA sequence variability. Phylogenetic analysis of partial HCV sequences recovered from a large number of patient isolates demonstrated that HCV can be classified into six major genotypes with several subtypes (Simmonds et al., 1993). These six genotypes (numbered from 1 to 6) encompass all the epidemiologically important HCV variants identified worldwide. This was later confirmed based on analysis of full-length HCV sequences with the proposition of a consensus classification (Simmonds et al., 2005), although a seventh major genotype was reported in few individuals in Central Africa more recently (Murphy et al., 2015). With the advances in sequence analysis techniques, there is an enormous increase in the number of published HCV sequences, confirming the existence of 67 subtypes (Smith et al., 2014). Basic morphogenetic aspects of HCV are shown in Fig. 2.

Furthermore, viral RNA sequence diversity is also observed within infected individuals. HCV circulates as a quasispecies, a mixture of closely related but distinctly different genomes. The viral genomes of a quasispecies typically differ by 1 to 3% and it is the result of mutations that accumulate over time during infection or mutations that are present from the onset of the infection (due to simultaneous transmission of multiple viral species). A new dominant HCV sequence can result from the accumulation of mutations over time and/or from the selection of a preexisting minor viral quasispecies. Some of these mutations enable HCV to replicate more efficiently and help the virus evade the host immune responses or antivirals (Farci, 2011; Jardim et al., 2009).

The six main genotypes are disseminated around the world with differences in the geographical distribution. An international survey demonstrated the following order of global prevalence, excluding data from Oceania: genotype 1 (46.2%), genotype 3 (30.1%), genotype 2 (9.1%), genotype 4 (8.3%), genotype 6 (5.4%) and genotype 5 (0.8%) (Messina et al., 2015). It is noteworthy that genotypes/subtypes distribution is changing due to the reduction of transfusion-associated transmission, modifications in the transmission routes (intravenous drug abuse has a major role in recent HCV transmissions) and migration from regions with a different genotype distribution. Some subtypes (1b, 2a, and 2b) are typically found in elderly populations with an epidemiological profile probably resulted from iatrogenic spread several decades ago, including transfusions; other subtypes (1a and 3a) are linked with widespread intravenous drug abuse (Simmonds, 2013).

HCV genetic heterogeneity has implications in spontaneous clearance, diagnosis, and treatment. Although some authors found no association with the HCV subtype and spontaneous clearance, other studies suggest that serum RNA from HCV genotype 1 or 1b are more likely to clear spontaneously than other genotype infections (Harris et al., 2007; Rolfe et al., 2011). Also, HCV genotype 3 patients are more likely to have liver steatosis (Negro, 2006). However, the impact on the long-term outcome of the HCV infection by different genotypes seems to be minimal. It is also well established that HCV genotype is associated with response to IFN-based treatments (patients infected with genotype 1 and 4 respond poorly to treatment compared to patients infected with genotypes 2 or 3), with implications in the period of the therapy (Pawlotsky et al., 2015). More recently, HCV genotype 3 has become “the most difficult to treat” with the novel IFN-free direct-acting antiviral (DAA)-based treatments (Goossens and Negro, 2014).

The quasispecies nature of HCV has also implications in the natural history, response to antiviral therapy, and effectiveness of the vaccine candidates. The great potential of HCV in introducing functional genome changes have been experimentally shown to promote escape from neutralizing antibodies and cellular immune responses (Ball et al., 2014). It is also associated with the outcome of acute HCV infection (Farci et al., 2000) and affects the viral population following reinfection, for example, after liver transplantations (Pérez-del-Pulgar et al., 2015). Importantly, this HCV heterogeneity found in an individual patient can contribute to viral escape from DAA, with variants evolving from preexisting resistant associated substitutions or developing “de novo” during the treatment (Pawlotsky, 2016).

6. Evolutionary aspects of host-HCV interaction

As previously discussed, the high error rate of RNA-dependent RNA polymerase and the pressure exerted by the host immune system has driven the evolution of HCV towards the development of a global diversity that revealed the existence of seven genetic lineages, characterizing the genotypes from 1 to 7. Genotypes 1 to 6 of HCV contain a series of more closely related subtypes that typically differ from each other by at least 15% in nucleotide positions within the coding region (Echeverría et al., 2015; Smith et al., 2014). Mutation at the nucleotide level seems to be the main cause of genetic variation in RNA viruses, such as HCV. These mutations are primarily generated by an error-prone, non-proofreading RNA-dependent RNA-polymerase which directs the replication of the virus genetic material (González-Candelas et al., 2011; Simmonds, 2004).

6.1. HCV in the context of quasispecies

The mutation rate of HCV, estimated at 10^{-4} substitutions per site and round of replication (Bartenschlager and Lohmann, 2000), is among the highest for RNA viruses including retroviruses (Kim et al., 1996), and would seem to be high enough to generate all the genetic variation found in this virus. It was suggested that, for RNA viruses, low replicative fidelity generates a diverse population of variants. Although several of these variants are commonly less fit, they may take over if an unexpected change in environment, such as immune pressure, occurs, shifting the corresponding fitness landscape. On the contrary, a homogeneous population, generated by high replicative fidelity, would lack this flexibility and might be less successful in the dynamic host environment. Support for this hypothesis has been provided by different experiments (Arnold et al., 2005; Pfeiffer and Kirkegaard, 2005). Due to this feature and to the high replication rate of HCV, a large number of different but closely related viral variants are continuously produced during infection. As previously discussed, these circulate in vivo as a complex population commonly referred as a quasispecies (Biebricher and Eigen, 2005; Chambers et al., 2005; Domingo et al., 1998; Domingo

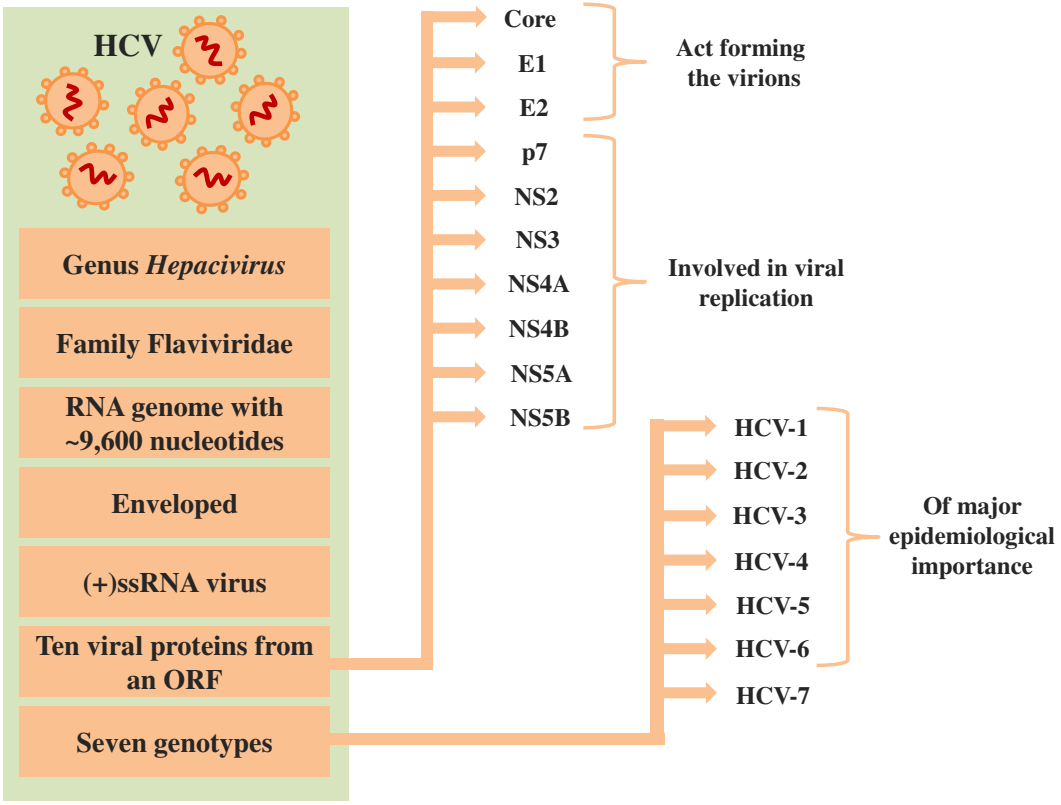

Fig. 2. Basic morphogenetic aspects of HCV.
This figure was created based on information described by Murphy et al. (2015), Kim et al. (2016), and Simmonds et al. (2005).

et al., 2005; Feliu et al., 2004; Laskus et al., 2004; Martell et al., 1992). Although variations in the viral genome are products of random base substitutions, there is evidence indicating that humoral immune responses might mediate quasispecies selection, by exerting selective pressure against the predominant strain. This results in the generation of new minor variants that will eventually become more prevalent. Later the immune system will recognize and exert pressure on the new dominant variant and new mutants will be selected (Forns et al., 1999).

6.2. Persistent HCV infection and cell resistance

A 6-month in vitro experiment with different phases employed an established system to propagate infectious HCV particles during the persistent phase of infection, and demonstrated that both the virus and the host cell evolve during persistent infection (Zhong et al., 2006). The experiment was based on a genotype 2a JFH-1 strain of HCV (Wakita et al., 2005; Zhong et al., 2006) and Huh-7/scr and Huh-7.5.1 cell lineages, with the virus acquiring increased specific infectivity and concomitantly the host cells becoming resistant to HCV infection (Zhong et al., 2006). The experiment launches important observations regarding to the persistent HCV infection with interesting results about the enhancement of HCV infectivity during persistent infection, the change in buoyant densities of viral particles, the identification of genetic mutations in chronic-phase virus, emergence of HCV-resistant cells, the inhibition of HCV RNA replication, and the loss of cellular CD81 expression during persistent infection.

7. HCV and HIV co-infection

HIV and HCV are transmitted through similar routes: percutaneous exposure to blood, sexual intercourse, and mother-to-child transmission. However, HCV is ~10 times more infectious than HIV through percutaneous blood exposures (Gerberding, 1994). In this sense, injecting drug users represent the main cause of HCV/HIV co-infection in most European cities (Wenz et al., 2016; Wiessing et al., 2011). Also, sexual transmission and mother-to-child transmission of HIV are more efficient when compared to HCV transmission, although the efficiency of HCV transmission can increase due to HCV/HIV co-infection (Eyster et al., 1991; Hershow et al., 1997).

HIV or HCV infection seems to reciprocally influence their natural course of infection. The biological mechanisms are not fully understood, however, an increased risk of accelerated disease progression; higher HCV replication; decreased rate of HCV clearance after an acute infection; increased microbial translocation from the gastrointestinal tract; increased fibrosis; diminished response to antiviral therapy for HCV; and death are among the facts frequently observed in co-infected individuals (Benhamou et al., 1999; Chen et al., 2014; Focà et al., 2016; Sulkowski et al., 2007). Also, HCV may also impact HIV disease progression. For instance, high HCV-RNA levels correlate with accelerated HIV disease progression (Piroth et al., 1998).

HCV/HIV co-infected individuals have higher TGF-β1 and HCV-RNA levels than HCV monoinfected persons, suggesting that HIV enhances TGF-β1 expression and HCV replication in vivo (Blackard et al., 2006b). Also, an in vitro study observed that exposure to HIV (or its envelope glycoprotein gp120) increases HCV replication and persistence in hepatocytes 2–3 fold through up-regulation of TGF-β1 (Lin et al., 2008). Indeed, it was observed that hepatocyte apoptosis is increased in the presence of HCV/HIV co-infected cells compared to HCV or HIV monoinfected cells, and that this increase is mediated by up-regulation of the TRAIL receptor 1 (DR4, death receptor 4) and 2 (DR5, death receptor 5) (Jang et al., 2011). Since CCR5 and CXCR4 are receptors expressed on activated hepatic stellate cells (HSCs), the main fibrogenic cell type in the liver, Tuyama et al. (2010) analyzed in vitro if HIV could infect HSCs. They observed that HIV isolates can infect primary human HSCs promoting HSC collagen I expression and production of monocyte chemoattractant protein-1 (MCP-1/CCL2), a pro-

inflammatory chemokine (Tuyama et al., 2010). Then, Kong et al. (2012) revealed that both CCR5- and CXCR4-utilizing HIV can infect hepatocyte cell lines, as well as primary hepatocytes. Notably, both HIV and HCV induce a production of cytokines and chemokines that regulate the immune response. The liver-resident macrophages (Kupffer cells) are able to sense the danger signal via TLRs and trigger the release of inflammatory chemokines such as CCL2 (Ansari et al., 2014). Thus, circulating monocyte-derived macrophages, cells that were already linked to fibrosis and chronic inflammation in a mouse model, are recruited into the liver in response to these chemokines (Karlmark et al., 2009).

In addition, in vitro experiments also revealed some molecular mechanisms related to the HCV/HIV co-infection. For example, a study showed that TNF receptor-associated factors (TRAFs) such as TRAF2 and TRAF5 interact with the HCV Core protein and, the HIV-1 Nef interacts with HCV Core. The activation of TRAF (2, 5, 6), mediated by HIV-1 Nef and HCV Core, enhances the activation of NF-κB and increased HIV-1 replication in MDMs (Khan et al., 2013). Other study indicated that TNF-α induced activation of the HIV long terminal repeat (LTR) in hepatocytes. However, HIV LTR activity was suppressed in hepatocytes in the presence of HCV Core and the suppressive effect persisted in the presence of TNF-α (Sengupta et al., 2013). Furthermore, both HIV and HCV can contribute to liver damage, since they trigger the production of reactive oxygen species (ROS). The HCV NS3 protein activates Nox2 protein of phagocytes and trigger dysfunction of T and NK cells and apoptosis. Nox2 protein leads to increased generation of ROS and other reactive species that can prompt oxidative stress to the nearby cells (Choi and Ou, 2006; Thorén et al., 2004). In in vitro systems, H_2O_2 promotes the replication of HIV, and antioxidants such as *N*-acetyl-cysteine have the opposite effect (Schwarz, 1996; Staal et al., 1990). Roederer et al. (1991) showed that T cells with high intracellular glutathione levels were selectively depleted early during the progression of HIV infection (Roederer et al., 1991). These events can explain, in part, the higher and persistent depletion of peripheral and mucosal subsets of $CD4^+$ T cells observed in HCV/HIV co-infected when compared to HCV or HIV monoinfected individuals (Roe et al., 2009). Fig. 3 presents the impacts of HIV on HCV infection in co-infected individuals.

8. Pharmacogenetics and treatment

HCV treatment has been shown to improve liver histology and to reduce the incidence of HCC (Bang and Song, 2017; George et al., 2009). The standard of care used for chronic HCV infection until 2011 was treatment with pegylated interferon (PEG-IFN) and ribavirin (RBV) for 24 or 48 weeks, depending on the HCV genotype. However, in naïve patients infected with HCV genotype 1, this treatment gives a SVR (achieved when HCV RNA is no longer detectable in plasma at 6 months after treatment end) rate of about 50% (Manns et al., 2001). Besides, treatment was associated with considerable adverse effects, with half of the patients presenting flu-like symptoms such as fatigue, fever, and headache; and a third experiencing psychiatric side effects (depression, irritability, and insomnia) (Fried et al., 2002). In 2011, a new strategy was introduced in the treatment of chronic HCV infection by the development of DAAs. These small molecules are inhibitors of different viral proteins and changed radically the chronic HCV therapeutics scenario, improving both response rates and the tolerability of treatment. The first generation of DAAs (boceprevir and telaprevir) targeted the HCV NS3 protease. They raised the SVR (up to 75%), but had important deficiencies, particularly when combined with PEG-IFN and RBV, which limited eligibility to treatment (Hézode et al., 2014). A new generation of DAA agents opened an era of all-oral, pan-genotypic, IFN-free, short-period (8–24 weeks) regimens, with SVR exceeding 90% (Falade-Nwulia et al., 2017).

There are three classes of DAAs: protease (NS3/NS4) inhibitors (e.g., simeprevir, paritaprevir, faldaprevir, vaniprevir, asunaprevir, sovaprevir, grazoprevir), NS5A inhibitors (e.g., ledipasvir, ombitasvir, daclatasvir, velpatasvir, elbasvir), and RNA-dependent polymerase (NS5B) inhibitors [nucleoside analogues (e.g., sofosbuvir) and non-nucleoside (e.g., dasabuvir, beclabuvir)]. These DAAs can be used in

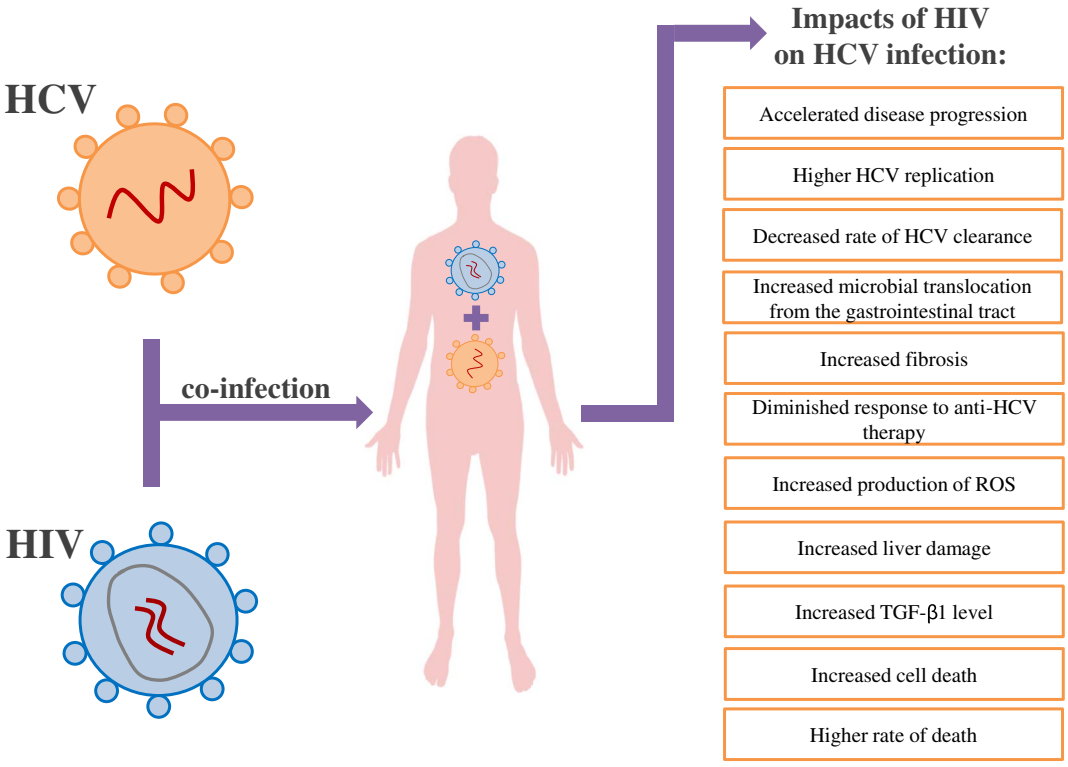

Fig. 3. Impacts of HIV on HCV infection in co-infected individuals. See text for references.

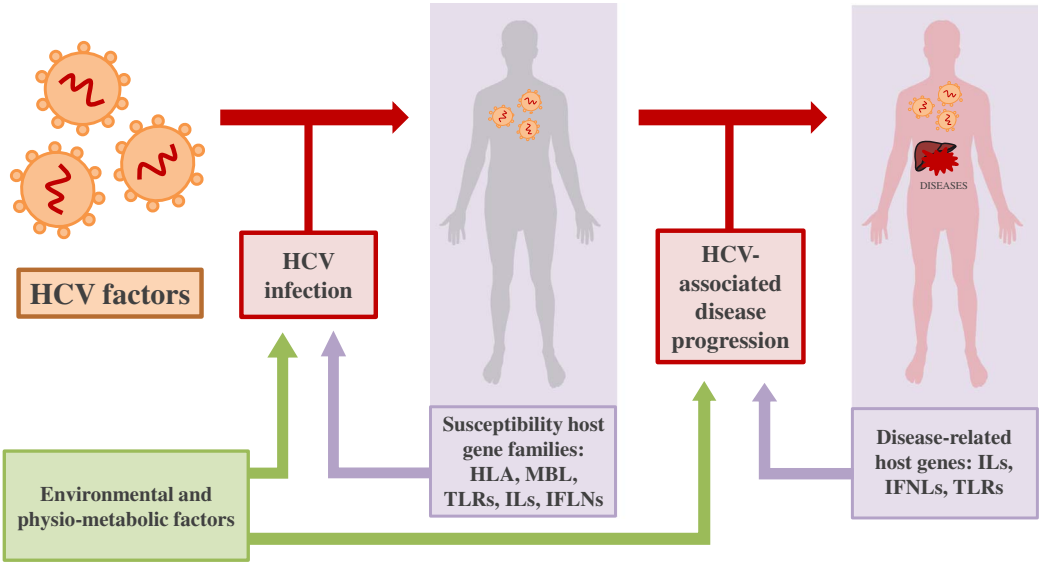

Fig. 4. Contributing factors to HCV infection and progression of HCV-associated diseases. Several genes of both the innate and the adaptive immune system modulate individual susceptibility to HCV infection after exposition. Also, HCV characteristics and environmental factors are determinants for the success of the infection. When the infection is established, progression to HCV-associated diseases is also modulated by host genetics. However, metabolic, environmental and also viral factors can influence disease progression. See text for references.

combination aiming to achieve potent inhibition of HCV replication and a concomitant high barrier to resistance, which means the ability to avoid selecting resistant HCV viral strains. DAAs have been evaluated in clinical trials regarding combinations, doses, period of treatment, and efficacy to specific viral genotypes. Moreover, nowadays there are multiple interferon-free, oral DAA regimens available for treatment of chronic HCV infection. Interestingly, the clinical efficacy of DAA therapy is high in both clinical trials and real-world settings (Welzel et al., 2017). However, DAA treatment in patients infected with HCV genotype 3 has presented lower SVR rates. In addition, these therapies are suboptimal in patients either with decompensated cirrhosis or with chronic kidney disease (Feld and Foster, 2016). With the advent of DAA regimens, it was expected that RBV use would be abolished, but the data indicate that it remains an important component in certain treatment regimens, especially in patients presenting more difficult to reach the cure. Recent data suggest that RBV is effective in increasing the genetic barrier to resistance, ultimately leading to a lower rate of relapse and greater SVR (Feld et al., 2017). Besides RBV, some regimens can still include PEG-IFN (Falade-Nwulia et al., 2017).

Genetic factors can influence the HCV treatment outcome. Gene-specific candidate-driven studies initially focused on genes that have impact on host response to HCV. With the progress in genotyping procedures, GWAS identified SNPs upstream of interferon-3 gene (*IFNL3*; formerly known as *IL28B*), in chromosome 19, strongly associated with spontaneous and PEG-IFN treatment-induced clearance of HCV infection (Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Rauch et al., 2010). As previously discussed, several studies confirmed the significant association between the CC genotype of the rs12979860 polymorphism (located approximately 3Kb upstream of *IFNL3*) and spontaneous and PEG-IFN treatment-induced clearance of HCV infection (Matsuura et al., 2014). In 2013, the *IFNL4* gene was discovered. It is controlled by a dinucleotide polymorphism, denoted *IFNL4* ΔG > TT (rs368234815) and located in exon 1 of *IFNL4*. The *IFNL4* ΔG allele creates a novel gene which encodes the INF-λ4 protein, moderately similar to INF-λ3. The alternative allele *IFNL4* TT does not create this protein. *IFNL4* ΔG > TT seems to be a better predictor of HCV clearance than previously identified SNPs (O'Brien et al., 2014; Prokunina-Olsson et al., 2013).

In the current era of IFN-free DAA therapies, the clinical relevance of *IFNL* genotypes diminished. However, *IFNL* polymorphisms were associated with treatment outcomes based on DAAs. It was reported a role of *IFNL* polymorphisms in response to DDA combination therapies with simeprevir/PEG-IFN/RBV (Fried et al., 2013), and sofosbuvir/PEG-IFN/RBV (Lawitz et al., 2013), and also in IFN-free therapies such as mericitabine/danoprevir (Chu et al., 2012), faldaprevir/deleobuvir/RBV (Zeuzem et al., 2013), sofosbuvir/RBV (Meissner et al., 2014), and simeprevir/sofosbuvir (Kwo et al., 2016).

GWAS also identified SNPs associated with anemia, a very common side-effect resulting from RBV-induced hemolysis. This adverse effect is dose-related and reversible, affecting up to 30% of patients, which requires close monitoring of hemoglobin and dose modification in up to 15% of patients (Thompson et al., 2010). However, dose reduction can affect treatment efficacy. Genetic variants in inosine triphosphate pyrophosphatase (*ITPA*) gene were associated with hemoglobin reduction after four weeks of treatment (Fellay et al., 2010). The *ITPA* gene is located on chromosome 20 and encodes an enzyme which is involved in purine metabolism, converting inosine triphosphate (ITP) to inosine monophosphate (IMP). The rs1127354 and rs7270101 *ITPA* SNPs were associated with reduced enzyme activity and with RBV-induced hemolytic anemia. Nevertheless the identification of SNPs, the exact mechanisms of RBV-induced hemolytic anemia is still not fully understood. Highly effective, oral DAAs have revolutionized the treatment of chronic hepatitis C. Currently the goal is to simplify the treatments with pan-genotypic regimens which use the same protocol for all patient populations. In this scenario, it is quite possible to imagine that, in a near future, the need for both virus and host genotyping will be eliminated.

9. Perspectives and a critical look

An important question emerges after all these previous considerations: What exactly is the role of genetic studies in an era of potential pan-genotype antiviral therapies? It seems to be exactly the same role they always had, but now in a different way. Actually, studies evaluating genetic factors of susceptibility to HCV infection will continue to be important in assessing individuals or groups of individuals that should receive special attention to combat, reduce or even avoid new infections. Furthermore, even in a scenario in which antiviral drugs are fully effective against all HCV types and subtypes, there will always be individuals who will not tolerate therapy due to adverse effects or who will respond abnormally to the antiviral therapy. Knowing the genes and genetic variants involved in the metabolism of antivirals or those that induce different responses to anti-HCV therapies will be essential for the continuous development of new antiviral drugs, with lower adverse effects and better tolerability for a wider range of individuals. It is also important to emphasize that host-HCV interaction is not a static process, and new genetic changes in both virus and host are forged as a result of this interaction. Monitoring these genetic changes is important

to recognize when new viral strains arise and to understand divergent progressions during HCV infections, even under antiviral therapy. Thus, we should focus our efforts on the development of pan-genotype therapies, but continue to evaluate virus types/subtypes as well as groups of populations in terms of susceptibility to HCV infection and progression to HCV-related diseases.

Moreover, virus-virus and virus-host interactions will occur in individuals co-infected with HCV genotypes or even with different viruses. Interactions between viral and host genetic factors occurs constantly, either directly (e.g. via iRNAs or miRNAs) or indirectly (e.g. mediated by evolutionary pressures). Furthermore, these interactions are modulated by environmental factors that cannot be disregarded in genetic studies. It should always be taken into account that environmental factors and other situations modulated by such factors (e.g. stress, nutrition, exposure to different pathogens) can affect both immune responses and gene expression.

10. Conclusion

Several genes of the innate and adaptive immune system modulate individual susceptibility to HCV infection and disease progression. Fig. 4 presents a schematic representation of some of the host and viral genetic factors discussed throughout the present review. Some of the important genes and molecules are highlighted, but these are not the only factors that determine infection and disease outcome. Environmental factors are determinants for the success of the infection. Interactions between viral and host genetic factors are also crucial to a successful treatment, although pan-genotypic DAA regimens are currently under development. Finally, the knowledge of the genetic factors that determine HCV infection susceptibility and/or disease outcome may be helpful to understand the behaviour, not only of this but of several other infectious diseases.

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CAPÍTULO IX

MicroRNA-related polymorphisms in infectious diseases - Tiny changes with a huge impact on viral infections and potential clinical applications

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REVIEW
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MicroRNA-Related Polymorphisms in Infectious Diseases—Tiny Changes With a Huge Impact on Viral Infections and Potential Clinical Applications

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MicroRNAs (miRNAs) are single-stranded sequences of non-coding RNA with approximately 22 nucleotides that act posttranscriptionally on gene expression. miRNAs are important gene regulators in physiological contexts, but they also impact the pathogenesis of various diseases. The role of miRNAs in viral infections has been explored by different authors in both population-based as well as in functional studies. However, the effect of miRNA polymorphisms on the susceptibility to viral infections and on the clinical course of these diseases is still an emerging topic. Thus, this review will compile and organize the findings described in studies that evaluated the effects of genetic variations on miRNA genes and on their binding sites, in the context of human viral diseases. In addition to discussing the basic aspects of miRNAs biology, we will cover the studies that investigated miRNA polymorphisms in infections caused by hepatitis B virus, hepatitis C virus, human immunodeficiency virus, Epstein–Barr virus, and human papillomavirus. Finally, emerging topics concerning the importance of miRNA genetic variants will be presented, focusing on the context of viral infectious diseases.

Keywords: microRNA, miR, polymorphism, hepatitis B virus, hepatitis C virus, human immunodeficiency virus, Epstein–Barr virus, human papillomavirus

INTRODUCTION

Viruses are found abundantly in the most diverse environments on earth (1). Some of the animal viruses are responsible for causing human infections. Viral diseases weaken humans individually and have important impacts on the environment, on the social organization and public health systems of populations worldwide. Historically, viruses are responsible for epidemics and outbreaks that impact all nations, being especially a burden in developing countries. Moreover, some viral diseases, such as the acquired immunodeficiency syndrome [AIDS, caused by human immunodeficiency virus (HIV)], affect the entire world, assuming a pandemic characteristic.

Many advances have been made in the combat against viral diseases. Vaccination and antiviral drugs development are examples of medical technologies effectively used against viruses. However, the number of people affected by viral diseases around the world is still alarming. The HIV pandemic

alone affects about 37 million people worldwide (2). Our knowledge about the pathogenesis of many viruses is still incipient. Similarly, the natural human defenses against pathogens or the immunogenetic aspects that determine, individually or in terms of a whole population, the degree of susceptibility or resistance to viral infections can still be greatly explored.

Within the context of the host genetics, this review will discuss the impact of microRNA (miRNA)-related polymorphisms on infections caused by hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, Epstein–Barr virus (EBV), and human papillomavirus (HPV). Taking into account the interaction of miRNAs with the epigenetic machinery (3, 4), this review will be relevant to readers interested in epigenetics, genetic polymorphisms, and/or viral diseases.

From this point onward the word "microRNA" will be abbreviated to "miRNA" when we are referring to miRNAs in a general way. However, some clarifications regarding the terminologies used in this review for specific miRNAs are important. miRNAs are named according to the species of which they were derived, indicating it before the prefix "miR," followed by the identification number of each miRNA (for example, hsa-miR-101 for *Homo sapiens* and mmu-miR-101 for mouse). The prefix "miR" is used to identify mature miRNAs and the prefix "mir" is used to identify precursor hairpins (5, 6). In this review, most cited miRNAs are human-derived mature miRNAs. Thus, the miRNAs quotation was standardized as follows: miR-101, miR-102, miR-103, for example. The few cases of miRNAs encoded by viral genes will be adequately indicated. Besides, in this review, the quotation of the polymorphisms was standardized according to the Single Nucleotide Polymorphism Database (dbSNP) of NCBI (https://www.ncbi.nlm.nih.gov/snp/), based on the reference SNP cluster (rs#) of each polymorphism. Importantly, some authors refer to the forward strand alleles of a given polymorphism while other authors, who studied the same polymorphism, refer to the reverse strand alleles. Although we have standardized the quotations of the SNPs according to the dbSNP, we respect the quotations of the alleles according to the original cited article. Thus, the reader should be aware of this aspect.

GENERAL ASPECTS OF miRNAs

MicroRNAs are small non-coding single-stranded RNA molecules of 19–25 nucleotides in length, well known by its important role in posttranscriptional regulation of gene expression (7). They are present in almost all eukaryotes, including humans, and regulate diverse biological processes in both physiological and pathological conditions (8–10). miRNAs were described to interfere in processes as distinct as cell proliferation and differentiation, apoptosis, or even in viral infections (11, 12). In such infections, the main focus of this review, miRNAs stand up as relevant mediators of the host response, and studies have demonstrated that these molecules can contribute to intracellular defense against the infection, to individual resistance to certain viruses, as well as control the survival, amplification, and modulation of cellular tropism of viruses. On the other hand, also viruses can produce miRNAs. Actually, they use the host cell machinery to generate their own miRNAs (10, 13, 14), which can, for example, to induce viral latency and decrease inflammatory responses, as well as to prevent cell apoptosis, contributing to the oncoviruses-related malignant transformation (15).

To understand how polymorphisms can influence the gene expression regulation by miRNAs, and even alter a given biological process, it is important to remember how miRNAs are generated. These molecules can be codified by independent genes or can be inserted in exons or introns from other genes. Briefly, in humans, miRNA biogenesis begins when they are transcribed by the RNA polymerase II as a primary transcript (pri-miRNA), consisting of a molecule encompassing 500–3,000 bases (see **Figure 1**). In the nucleus, the pri-miRNA is cleaved into pre-miRNA (60–70 nucleotides long) by a complex formed by the Drosha enzyme and its cofactor DGCR8 (DiGeorge syndrome critical region 8 protein) (7). After translocation from nucleus to cytoplasm, a process mediated by the molecule exportin-5 (Exp-5, a nuclear transport factor), pre-miRNAs are cleaved in a mature miRNA (19–25 bases long) by the Dicer/TRBP (transactivation response RNA-binding protein) complex. Next, the mature single-stranded miRNA and the Argonaut protein (AGO) constitute a multicomponent complex called RNA-induced silencing complex, which allows the binding to complementary sequences in the 3′ untranslated region (3′UTR) of a target mRNA, leading to translational repression or degradation of the mRNA (7, 16–18). The key binding point for miRNA–mRNA interaction is the seed region, located within nucleotides 2–8 from the 5′ end of the mature miRNA sequence (19, 20). In general, a partial complementarity of the mRNA 3′UTR to the miRNA seed sequence leads to translational inhibition, while a perfect complementarity results in mRNA degradation. A slightly distinct process occurs when the miRNA precursor is located in mRNA introns (see **Figure 1**). In this case, the pre-miRNA will be spliced out and then exported from the nucleus to the cytoplasm, bypassing the Drosha/DGCR8 complex, and then will follow the remaining aforementioned pathway (10).

Polymorphisms in miRNA genes can influence gene transcription, alter the processing of pri- or pre-miRNA, and affect miRNA–mRNA interactions. Moreover, such miRNA–mRNA interactions can also be either facilitated or hindered by polymorphisms located in the 3′UTR of the target mRNAs, by the generation or loss of miRNA-binding sites, for example (25). The effect of gene regulation by miRNAs is quite complex, since a certain miRNA can target several mRNAs, and conversely, a single mRNA can bind to distinct miRNAs, being the final effect determined by the joint action of, potentially, several miRNAs (26). Despite best known by their capacity to impair translational processes, decreasing the rates of protein expression, miRNAs, in some cases, can bind to 5′ untranslated regions, to exons, or even to DNA elements, leading to increased transcription or translation (27–29).

An important emerging research topic concerns the effects of polymorphisms in miRNAs and its target-sites in viral-associated diseases. Recent case–control and functional studies have pointed out to a role of such polymorphisms in susceptibility to viral infection, as well as in chronicity of the disease versus viral clearance, and even in viral-associated cancer development (30–33). Given the increasing interest in such processes and phenomena,

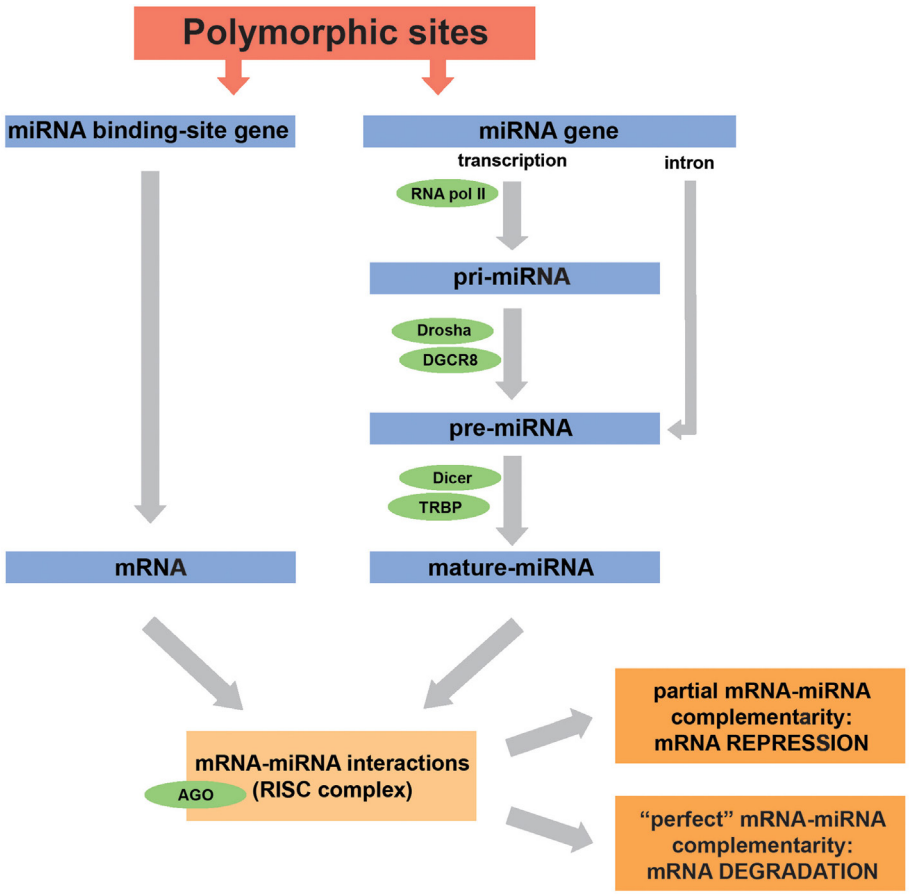

FIGURE 1 | Biogenesis and action of microRNAs (miRNAs) are affected by genetic variations. miRNA biogenesis is mediated by a number of enzymes and co-factors. Some of the most important enzymes for each miRNA processing step are indicated in green. Depending on the degree of miRNA-mRNA complementarity, mRNA expression will be repressed or the molecule will be directed to degradation. Genes encoding miRNAs or miRNA-binding sites can host polymorphisms that modify the action of these molecules. For example, polymorphisms in miRNA genes can downregulate miRNA expression, and polymorphisms in miRNA-binding sites can disrupt the miRNA–mRNA interaction. Furthermore, the effects of genetic variations in miRNA-machinery genes also have an important impact on the biogenesis of miRNAs. This figure was mainly based on the following references: Drury et al. (10), Lorio et al. (21), Ryan et al. (22), Ryan et al. (23), and Rupaimoole and Slack (24).

in addition to the potential clinical use of miRNAs as molecular biomarkers and therapeutic targets, we will present a review of the existing literature on these topics.

VIRAL INFECTIONS AND POLYMORPHIC VARIANTS THAT AFFECT miRNAs

HBV Infection, HBV-Associated Diseases, and miRNAs

It was estimated that around 30% of the world's population is a HBV carrier or has been infected with the virus in the past (34). HBV infection is classically responsible by triggering several types of liver damage, including cirrhosis and hepatocellular carcinoma (HCC) (34). Africa and Asia concentrate the largest number of countries with high prevalence of chronic HBV infection (34), and particularly in China, HBV infection is an endemic problem (35). Although several advances in the fight against HBV have been made, a large part of the Chinese population still suffers from HBV-associated diseases (36). Therefore, it was not a surprise, when the literature regarding the influence of miRNA-related polymorphisms on HBV-associated diseases was reviewed, that a large number of studies were performed with individuals from China.

In order to give a comprehensive idea of the studies approaching miRNA polymorphisms, minimizing the potential problems of comparing ethnically distinct populations, we will initially focus on studies performed in China; all other studies being gathered in the next section. Nevertheless, even considering only those studies, and centering in human populations with a relatively homogeneous ethnic origin, conflicting data arouses. In a research performed by Xu et al. (37), the GG genotype of miR-146a G/C SNP (rs2910164) was associated with increased risk of HCC in males. Their study compared 479 HCC patients with 504 controls. Of note, 88.9% of the HCC patients were infected with HBV. Moreover, through *in vitro* assays, the same authors

described how miR-146a rs2910164 would be linked to HCC. Briefly, the G allele increases miR-146a maturation, potentially contributing to HCC-related cell proliferation (37). A number of authors reported no impact of miR-146a rs2910164 on HBV-associated HCC (38–43). On the other hand, according to Cong et al. (44), the GG genotype and G allele of miR-146a rs2910164 increase the risk of HCC among HBV-infected individuals. In a recent meta-analysis including eleven studies performed in Chinese populations, the miR-146a rs2910164 was linked to an increased risk of HBV-associated HCC (45). Besides HCC development, other HBV-associated diseases are potentially influenced by this polymorphism. For example, Jiang et al. (46) investigated the miR-146a rs2910164 in patients with acute-on-chronic hepatitis B liver failure and in individuals with chronic HBV infection. Individuals carrying the GG genotype had reduced susceptibility to the disease, lower levels of TNF-α, and higher survival rate (46).

Xiang et al. (38) genotyped the miR-499a C/T SNP (rs3746444) in chronic HBV-infected individuals, HCC patients (HBV-infected and non-infected), and controls. They identified the CC genotype as a risk factor for the development of HBV-associated HCC (38). Posteriorly, and in conflict with the data from the previously cited work, a small case–control study found, in a dominant model, that AG + GG genotypes of miR-499a rs3746444 were associated with a reduced risk of HCC when HBV-infected patients were analyzed (47). In addition, another study with a small sample size reported an increased risk of HBV-associated HCC linked to the A allele of miR-499a rs3746444 (42). Ma et al. (48) investigated the miR-499 rs3746444 and the miR-423 A/C/T (rs6505162) SNP in 984 HCC patients and compared the genotype frequencies with a similar number of controls. Of note, among the HCC group, 760 individuals were infected with HBV. MiR-423 rs6505162 had no effect on HCC risk, independently of the HBV infection status. However, miR-499a TC + CC (in a dominant model) increased the risk of HBV-associated HCC, when compared to the TT genotype (48). Finally, a meta-analysis including case–control studies reinforced the involvement of miR-499a rs3746444 in the susceptibility to HCC among HBV-infected individuals (49). Nevertheless, it is important to consider that several authors did not found a statistically significant link between miR-499a rs3746444 and HBV-associated HCC (39, 41, 43, 50, 51). This fact evidences the need for new investigations aiming to establish with more robustness the impact of this SNP on HBV-associated HCC and reinforces the fact that, in multifactorial diseases, multiple variants of susceptibility can be identified, each of them with a small contribution.

In a study evaluating the miR-196a2 C/T SNP (rs11614913), Qi et al. (52) genotyped 199 chronic HBV-infected individuals without HCC, 361 chronic HBV-infected individuals with HCC, and 391 healthy controls. An increased risk of HBV-associated HCC was found in males carrying the C allele and the CC genotype. Regarding HCC progression, no statistically significant influence of miR-196a2 rs11614913 on tumor number, size, growth phase, stage, and lymph node metastasis was found. However, when stratified by sex, in male patients with lymphatic metastasis, a higher frequency of the T allele was observed (52).

The potential role of miR-196a2 rs11614913 on the risk of HBV-associated HCC was investigated by a number of authors. In a study performed by Hao et al. (39), CT and TT genotypes of miR-196a2 rs11614913 were considered risk factors for HCC development in HBV-infected individuals. In addition, the influence of miR-196a2 rs11614913 on HCC risk was investigated by Li et al. (43) in a small case–control sample (266 individuals in each group). 110 individuals from the HCC group and 32 individuals from the control group were HBV infected. Looking at these individuals, it comes out that CT + TT genotypes increase the risk of HCC development (43), a finding in line with the study performed by Hao et al. (39). However, conflicting results were also published. Kou et al. (41) evaluated this same miRNA variant site in 532 controls and 271 HCC patients. Approximately, 58% of the patients were HBV infected, and CT and TT genotypes presented a reduced risk of HCC (41). Furthermore, Zhang et al. (40) evaluated the miR-196a2 rs11614913 in a relatively large sample of the Chinese population. Their study included a control group (~1,000 individuals) and a group of HCC patients (~1,000, including 771 HBV-associated HCC patients). In brief, CT + TT genotypes and the T allele were linked to a lower chance of HBV-associated HCC development (40). Supporting this result, a small case–control study described CT and TT genotypes as well as the T allele of miR-196a2 rs11614913 as markers of reduced risk of HBV-associated HCC (53). Recently, the miR-196a2 rs11614913 was associated with a decreased risk of HBV-associated HCC in a meta-analysis including eleven studies carried out with Chinese populations (45). A previous meta-analysis (51), approaching a total of 2,693 HCC cases and 3,594 controls, had already associated the T allele and the TT genotype with reduced risk of HCC. Interestingly, this finding had been observed only considering the total pool of individuals, but not when stratifying the populations according to ethnicity (51). Actually, there are studies in Chinese populations where no statistically significant association between the miR-196a2 rs11614913 and risk of HBV-associated HCC were observed [see Yan et al. (54), for example], although these results seem to have been "diluted" with the inclusion of new studies in the more recent meta-analysis.

Another important point to be discussed refers to the interactions between viruses and host genetic factors. To highlight this point let's take the study from Han et al. (30), which, using quantitative PCR, explored the interaction of miR-196a2 rs11614913 and miR-34b/c T/C SNP (rs4938723) with HBV mutations in a sample of 3,325 individuals (1,021 of them with HBV-associated HCC). Among several results, the most interesting finding was that the effects caused by miRNA SNPs on HBV-associated HCC susceptibility can be strongly influenced by HBV mutations (30). Thus, host genetic polymorphisms may be relevant in the presence of an infection associated to a specific HBV genotype, but less important in the presence of HBVs showing different genetic features. In this sense, conflicting findings in studies evaluating the same particular host polymorphism in the context of HBV-associated diseases may be due not only to differences in the ethnic background of the studied population, but can also result from the HBV genetic variants circulating in this given population.

Some SNPs were studied in a particular context or population and few (or no) further studies were performed to confirm or refute these initial results. Wang et al. (55) investigated the

influence of miR-608 C/G SNP (rs4919510) and miR-149 C/T SNP (rs2292832) on the risk of HCC development. No link between miR-608 rs4919510 and HBV-associated HCC was reported. On the other hand, in men, the TT genotype of miR-149 rs2292832 was associated with an increased chance of HBV-associated HCC development when compared to the wild-type genotype (55). Differently, but also evaluating the miR-149 rs2292832, Liu et al. (56) found that the TC + CC genotypes, when compared with TT genotype, increased the risk of HCC in HBV-infected individuals. No link between miR-149 rs2292832 and HBV-associated HCC was reported in other studies (43, 50).

Wang et al. (57) investigated the miR-646 G/T SNP (rs6513497) in HCC patients and controls. Among the 771 HCC patients enrolled in the study, 81.1% were infected with HBV. Among males, the GT genotype and G allele were considered as protective factors against HBV-associated HCC (57). In this same direction, miR-378a C/T SNP (rs1076064) was also described as a protective factor of HBV-associated HCC. Specifically, AG + GG genotypes were associated with a decreased risk of HCC and higher HCC survival rate (58). Of note, these results were attributed, at least partially, to the effects that miR-378a rs1076064 exerts on miR-378 transcription (58). Although the results regarding miR-646 rs6513497 and miR-378a rs1076064 are quite interesting, the lack of confirmatory cohorts hinders further conclusions.

As the miR-122 expression is reduced in tissue samples of HBV-associated HCC (59), Liu et al. (60) evaluated the role of miR-122 A/C SNP (rs4309483) and miR-122 C/T SNP (rs4503880) on the risk of HCC. Their study included 1,300 HBV-infected patients with HCC, 1,344 HBV-infected patients without HCC, and 1,344 patients showing HBV clearance. The expression of pri-miR-122 and mature-miR-122 was measured in 29 HCC patients, comparing the levels in tumoral liver tissue and in adjacent tumor-free regions. In short, based on genotypes and gene expression, the authors concluded that miR-122 rs4309483 increases the risk of HBV-associated HCC (60). On the other hand, the same authors reported this SNP also acts as a protective factor against chronic HBV infection (60). This can be interpreted as follows: miR-122 rs4309483 hampers chronic HBV infection, but if the infection is established, this same SNP facilitates carcinogenesis.

Liu et al. (61) focused their investigation on the MCM7 C/T SNP (rs999885). Importantly, *MCM7* gene is the location of the miR-106b, miR-93 and miR-25 cluster (miR-106b-25) (61). They evaluated the influence of MCM7 rs999885 on the clinical outcome of HBV infection. In addition, the expression of miR-106b-25 was measured both in the HCC tissue and in adjacent tumor-free liver regions of 25 HBV-infected patients. AG/GG genotypes were associated to a higher expression of miR-106b-25 and a higher risk of HBV-associated HCC. Interestingly, these same genotypes were linked to lower risk of chronic HBV infection (61). The impact of MCM7 rs999885 of miR-106b-25 cluster on the outcome of HBV-associated HCC was also studied by Qi et al. (62). In summary, these authors observed that the AG/GG genotypes and G allele of MCM7 rs999885 were linked to a better HCC prognostic (62).

Zhou et al. (63) studied the GAGA ins/del polymorphism (rs17875871) of the 3′UTR of *IFNAR1* gene in a sample of HCC individuals and controls ($n = 420$ in each group). This polymorphism potentially affects the miR-1231-binding site. The deletion allele was associated with an increased risk of HCC, especially in the presence of HBV (63). The influence of polymorphisms in genes that affect the biogenesis/binding of miRNAs was also subject of study of Liu et al. (64). Specifically, polymorphisms in *DICER1*, *RAN*, *PIWIL1* genes (C/T rs1057035, A/C/G rs3803012, and C/T rs10773771, respectively) were genotyped in HBV-infected individuals with different clinical outcomes. Of note, DICER1 rs1057035 affects the miR-574-3p binding, RAN rs3803012 impacts the miR-199a-3p binding, and PIWIL1 rs10773771 influences the miR-1264 binding. The impact of the SNPs on the binding of these specific miRNAs was also tested *in vitro*. In brief, the authors found evidence that CT/CC genotypes of both DICER1 rs1057035 and PIWIL1 rs10773771 decreased the risk of HBV-associated HCC. Differently, RAN rs3803012 AG/GG genotypes were a risk factor of HBV persistent infection (64).

Xiong et al. (65) studied the KRAS G/T SNP (rs712), a genetic variation with implications to the binding of miR-let-7 and miR-181. According to these authors, the TT genotype increases the risk of HBV-associated HCC. This influence occurs possibly by a modified expression of *KRAS* due to the rs712-induced changes in the miR-let-7-binding site (65). Li et al. (66) explored the effect of five SNPs in miRNA-binding sites (located at *RAD52* gene) on the risk of HBV-associated HCC. The SNPs analyzed were: RAD52 A/G (rs1051669), RAD52 A/T (rs10774474), RAD52 A/T (rs11571378), RAD52 G/T (rs7963551), and RAD52 C/T (rs6489769). The C allele of RAD52 rs7963551 reduced the risk of HCC development. Of note, this SNP may affect the binding of miR-let-7. The authors also showed that CC or AC genotypes of RAD52 rs7963551 were associated with an increased RAD52 expression. Due to the role of RAD52 in DNA repair, changes in its expression or regulation caused by polymorphisms affecting the miRNA-binding sites may have a significant impact on the risk of HBV-associated HCC (66).

Zhang et al. (67) investigated the impact of PD1 A/G SNP (rs10204525) on the binding of miRNAs in the context of susceptibility to HBV-associated diseases. In summary, their results suggest that the *PD-1* regulation by miR-4717 is modified in response to PD1 rs10204525 genotypes. For example, *in vitro* experiments showed miR-4717 decreased *PD-1* expression in lymphocytes isolated from patients showing chronic HBV infection and GG genotype of PD1 rs10204525. In addition, this phenomenon was found in association with increased levels of TNF-α and IFN-γ. Together, these events may have an important impact on the HBV infection clinical course (67).

The influence of variations in genes of the miRNA machinery on chronic HBV infection was investigated by Shang et al. (68). Such study specifically addressed the following SNPs: DGCR8 A/G (rs3757), AGO1 A/G (rs636832), and GEMIN4 C/T (rs7813). The A allele of AGO1 rs636832 decreased the risk of chronic HBV infection. Moreover, compared to the AA genotype, AG + GG increased the risk of chronic HBV infection, suggesting the AA genotype as a protective factor to the disease. No statistically significant associations were reported in relation to the other analyzed SNPs (68).

In summary, it is evident that polymorphisms can interfere with the maturation and/or in the action of miRNAs, modifying

the risk of HBV-associated diseases. Therefore, it is important not only focus on genes that actually encode miRNAs or their binding sites, but also on those miRNA maturation/action modifier genes.

The interaction of a miR-122-binding site TTCA ins/del polymorphism (rs3783553, located at the *IL-1A* gene) and HBV mutations was investigated, in the context of HBV-associated HCC, by Du et al. (69). Interestingly, the TTCA insertion allele was linked to an increased frequency of the HBV C7A mutation. In general, rs3783553 did not modify the risk of HBV-associated HCC, but its interaction with HBV preS deletion reduced the risk of HCC development (69). According to the authors, host genetic polymorphisms influence the risk of HCC more subtly than the influence exerted by the genetic features of HBV. However, there is a strong interaction between viral and host genetic factors defining the course of HBV infection (69). Similar to Du et al. (69), Han et al. (70) evaluated the risk of HBV-associated HCC diseases taking into consideration virus–host interactions, meaning miR-218-2 A/G SNP (rs11134527) and HBV mutations. Briefly, miR-218-2 rs11134527 modified the risk of HCC, cirrhosis development, inflammation, and HBV clearance. Moreover, and again similar to the findings of Du et al. (69), the host genetic variation was associated with HBV preS deletion in men (70). However, in the study performed by Han et al. (70), the interaction of miR-218-2 rs11134527 with HBV preS deletion was linked to an increased risk of HCC. Finally, the T1674C/G HBV mutation reduced the increased risk of HCC linked to miR-218-2 rs11134527 (70). The results of these two studies exemplify the complex relationships between viral and host genetic factors. Besides, it is necessary to study the influence of gene–gene and gene–environment interactions to better understand the effect of miRNA SNPs on HBV-associated HCC (51).

Considering all articles mentioned above, we note that only a few miRNA SNPs have been studied in depth. This is the case of miR-146a G/C SNP (rs2910164) and miR-196a2 C/T SNP (rs11614913). The influence of these genetic variants on HBV-associated diseases is relatively well studied, at least in Chinese populations. However, even in these cases, conflicting results arouse. In order to synthesize the information described in this topic, the main interactions between miRNA SNPs and HVB-associated diseases were compiled in **Table 1**. In addition to data from studies performed with populations from China, **Table 1** also shows information obtained from studies performed in other populations. These studies will be discussed in the next topic.

HBV: More Studies With Diverse Human Populations

The potential role of miR-196a2 C/T SNP (rs11614913) on HBV-associated diseases was addressed by different authors in distinct human populations comprising ethnic backgrounds other than Chinese (focus of the previous topic). Data from a small case–control study performed by Akkiz et al. (73) in a Turkish population, pointed the C allele and the CC genotype as potential markers to identify individuals at high risk for developing HBV-associated HCC who could benefit from more frequent HCC preventive examinations. However, conflicting results regarding the effects of such variant were published later. Kim et al. (74) studied in a Korean population the impact of miR-196a2 rs11614913 and miR-196a2 A/C SNP (rs12304647) on the clinical outcome of HBV infection. In addition to 404 patients with HBV spontaneous recovery, the study included 313 HBV-infected patients with chronic hepatitis, 305 HBV-infected patients with liver cirrhosis, and 417 HBV-patients with HCC, in a total of 1,035 HBV-infected individuals. Briefly, among HBV-infected patients with chronic hepatitis or cirrhosis, the CC genotype of miR-196a2 rs12304647 was linked to a reduced risk of HCC, although no statistically significant influence of the miR-196a2 rs11614913 on HCC development was observed (74).

In a case–control study, Riazalhosseini et al. (75) genotyped three polymorphisms in three Malaysian ethnical groups (Malays, Chinese, and Indians): miR-196a2 C/T SNP (rs11614913), miR-196a2 A/C SNP (rs12304647), and miR-146a C/G SNP (rs2910164). The authors evaluated the influence of these SNPs on the development of HBV-associated cirrhosis and HCC, comparing 103 chronic HBV-infected patients with liver cirrhosis or with cirrhosis and HCC to 423 chronic HBV-infected patients without such conditions. No statistically significant influence of miR-196a2 rs11614913 and miR-146a rs2910164 on the HBV-associated diseases was observed. However, when compared to CC genotype, AA + AC genotype of miR-196a2 rs12304647 was linked to a reduced risk of cirrhosis/HCC (75).

Kim et al. (71) investigated in a case–control study with a Korean population the role of miR-196a2 C/T SNP (rs11614913), miR-149 C/T SNP (rs2292832), miR-146a C/G SNP (rs2910164), and miR-499a C/T SNP (rs3746444) on the risk of HCC development. Among 159 HCC patients, 127 were HBV infected. In relation to miR-149 rs2292832, CT genotype and CT + CC in a dominant model reduced the risk of HCC in HBV-infected and non-infected individuals. Considering miR-499a rs3746444, an AG + GG model also reduced the risk of HBV-associated HCC. No influence on HBV-associated HCC was observed for miR-146a rs2910164 and miR-196a2 rs11614913 in this study (71), although a meta-analysis (72) suggested that the miR-146a rs2910164 C allele decreases the risk of HCC in populations with an Asian ethnic background and also in Caucasians. No effect of miR-499a rs3746444 was observed in this same meta-analysis (72).

The influence of miR-149 C/T SNP (rs2292832) and miR-101-1 C/G/T SNP (rs7536540) on the risk of HCC in Thai population was evaluated by Pratedrat et al. (81), in a study including 95 healthy controls, 90 chronic HBV-infected individuals, and 104 HCC patients. However, no statistically significant association was found (81). In addition to miR-101-1 rs7536540, the influence of the following variants on clinical outcome of HBV infection was investigated in Korean individuals (77): miR-101-2 C/T SNP (rs17803780), miR-101-2 C/T SNP (rs12375841), and miR-338 C/T SNP (rs62073058). In brief, miR-101-1 rs7536540 had an impact on the risk of liver cirrhosis and HCC, and miR-101-2 rs12375841 and the haplotype ht2 (T-C) of miR-101-2 influenced the HBV clearance (77).

The role of three variants of the miRs-371-372-373 cluster (C/T SNP rs28461391, A/C rs3859501, and C/T rs12983273) on the risk of HCC and HBV clearance was investigated by Kwak et al. (76) in a sample of 1,439 Korean individuals. The miRs-371-373 rs3859501 and the ht2 (C-A-C) haplotype were linked to a reduced

TABLE 1 | Main microRNA (miRNA)-related polymorphisms showing statistically significant influence on hepatitis B virus (HBV) infection and HBV-related diseases.

miRNA or miRNA-binding site[a]	Polymorphism[b]	Influence on	Population	Reference
miR-146a	C/G rs2910164	Susceptibility to HBV infection	Chinese	Cong et al. (44)
		HBV-associated hepatocellular carcinoma (HCC)	Chinese	Zhou et al. (29); Cong et al. (44)
			Meta-analysis	Tian et al. (45)
		Acute-on-chronic hepatitis B liver failure	Chinese	Jiang et al. (46)
		Susceptibility to HBV infection; HBV clearance	Saudi Arabian	Al-Qahtani et al. (33)
miR-149	C/T rs2292832	HBV-associated HCC	Chinese	Wang et al. (55); Liu et al. (56)
			Korean	Kim et al. (71)
		Susceptibility to HBV infection; HBV clearance; HBV persistence; HBV-associated cirrhosis/HCC	Saudi Arabian	Al-Qahtani et al. (33)
miR-499	C/T rs3746444	HBV-associated HCC	Korean	Kim et al. (71)
			Chinese	Xiang et al. (38); Li et al. (42); Zou and Zhao (47); Ma et al. (48)
			Meta-analysis	Yu et al. (49)
miR-196a2	C/T rs11614913	HBV-associated HCC	Chinese	Hao et al. (39); Zhang et al. (40); Kou et al. (41); Li et al. (43); Qi et al. (52); Zhou et al. (53)
			Meta-analysis	Tian et al. (45); Zhu et al. (51); Xu et al. (72)
			Turkish	Akkiz et al. (73)
		Gene-gene interaction; HCC-related HBV mutations	Chinese	Han et al. (30)
		Susceptibility to HBV infection; HBV clearance; HBV-associated cirrhosis/HCC	Saudi Arabian	Al-Qahtani et al. (33)
miR-196a2	A/C rs12304647	HBV-associated HCC	Korean	Kim et al. (74)
		HBV-associated cirrhosis/HCC	Malaysian	Riazalhosseini et al. (75)
miR-34b/c	C/T rs4938723	Gene-gene interaction; HCC-related HBV mutations	Chinese	Han et al. (30)
miR-423	A/C/T rs6505162	HBV clearance; HBV-associated cirrhosis/HCC	Saudi Arabian	Al-Qahtani et al. (33)
miR-26a1	C/T rs7372209	HBV-associated cirrhosis/HCC	Saudi Arabian	Al-Qahtani et al. (33)
miR-608	C/G rs4919510	HBV-associated cirrhosis/HCC	Saudi Arabian	Al-Qahtani et al. (33)
miR-492	C/G rs2289030	HBV clearance	Saudi Arabian	Al-Qahtani et al. (33)
miR-30a	A/G rs1358379	Susceptibility to HBV infection; HBV clearance; HBV persistence; HBV-associated cirrhosis/HCC	Saudi Arabian	Al-Qahtani et al. (33)
miR-122	A/C rs4309483	Chronic HBV infection; HBV-associated HCC	Chinese	Liu et al. (60)
miR-122-binding site	ins/del rs3783553	HCC-related HBV mutations	Chinese	Du et al. (69)
miR-371-372-373 cluster	A/C rs3859501	HBV-associated HCC	Korean	Kwak et al. (76)
miR-106b-25 cluster	C/T rs999885	HBV-associated HCC	Chinese	Liu et al. (61); Qi et al. (62)
		Chronic HBV infection	Chinese	Liu et al. (61)
miR-101-1	C/G/T rs7536540	HBV-associated cirrhosis/HCC	Korean	Bae et al. (77)
miR-101-2	C/T rs12375841	HBV clearance	Korean	Bae et al. (77)
miR-1231-binding site	ins/del rs17875871	HBV-associated HCC	Chinese	Zhou et al. (63)
miR-219-1	A/G rs107822	HBV clearance	Korean	Cheong et al. (78)
miR-219-1	C/T rs421446	HBV clearance	Korean	Cheong et al. (78)
miR-219-1	C/T rs213210	HBV clearance	Korean	Cheong et al. (78)
miR-574-3p-binding site	C/T rs1057035	HBV-associated HCC	Chinese	Liu et al. (64)
miR-1264-binding site	C/T rs10773771	HBV-associated HCC	Chinese	Liu et al. (64)
miR-199a-3p-binding site	A/C/G rs3803012	HBV-associated HCC; HBV persistance	Chinese	Liu et al. (64)
miR-378	C/T rs1076064	HBV-associated HCC	Chinese	An et al. (58)
miR-604	C/T rs2368392	HBV-associated HCC; HBV persistance	Korean	Cheong et al. (79)
miR-218	A/G rs11134527	Gene-gene interaction; HCC-related HBV mutations; HBV-associated cirrhosis/HCC; HBV clearance	Chinese	Han et al. (70)
miR-646	G/T rs6513497	HBV-associated HCC	Chinese	Wang et al. (57)
miR-let-7-binding site	G/T rs7963551	HBV-associated HCC	Chinese	Li et al. (66)
miR-let-7-binding site	G/T rs712	HBV-associated HCC	Chinese	Xiong et al. (65)
miR-4717-binding site	A/G rs10204525	Chronic HBV infection	Chinese	Zhang et al. (67)
miR-323b	A/C/T rs56103835	HBV persistence	Korean	Yu et al. (80)

[a]*Seed or regulatory region.*

[b]*Polymorphism quotations were standardized according to the Single Nucleotide Polymorphism Database (dbSNP) of NCBI (https://www.ncbi.nlm.nih.gov/snp/), based on the reference SNP cluster (rs#) of each polymorphism.*

risk of HBV-associated HCC. However, no statistically significant influence of those SNPs was observed concerning HBV clearance (76). Another study from Korea evaluated the impact of three distinct variants of miR-219a1 (C/T rs421446, A/G rs107822, and C/T rs213210) on HBV clinical outcome (78). In brief, all SNPs evaluated and the ht1 (C-A-C) and ht2 (T-G-T) haplotypes showed some influence on HBV clearance. Conversely, no statistically significant influence of those SNPs on HBV-associated HCC was reported. These results indicate that miR-219a1 has an important influence specifically on HBV clearance. However, the mechanisms by which miR-219a1 acts on HBV infection and how its SNPs can affect those mechanisms are still unclear and may be subject to functional studies (78). Posteriorly, the T allele of miR-604 C/T SNP (rs2368392) was linked to HBV chronic infection in Korean patients (79), although, unexpectedly, in patients chronically infected with HBV this allele reduced the risk of HCC occurrence (79). In other words, this SNP seems to play a role in the maintenance of the infection, but it does not necessarily contribute to the mechanisms of hepatocarcinogenesis.

Still considering Korean patients, Yu et al. (80) evaluated the miR-323b A/C/T SNP (rs56103835) on HBV replication and clinical course of infection. In that study, miR-323b rs56103835 was associated with persistent infection and was hypothesized as a factor which facilitates chronic HBV infection. In line with this interpretation, this SNP promoted HBV replication *in vitro* (80). In association, these findings support an important role for miR-323b rs56103835 in HBV chronic infection, once miR-323b can be considered an HBV suppressor (80). Of note, some points in the study of Yu et al. (80) (statistical analysis and interpretation of results) were target of criticism (82) which should be taken into account when interpreting the results mentioned above.

Recently, Al-Qahtani et al. (33) investigated the role of a number of miRNA SNPs on HBV-associated liver diseases in Saudi Arabia, including 1,352 HBV-infected patients and 600 healthy HBV uninfected controls. The genotyped variants were: miR-499a C/T SNP (rs3746444), miR-423 A/C/T SNP (rs6505162), miR-26a1 SNP C/T (rs7372209), miR-608 C/G SNP (rs4919510), miR-604 C/T SNP (rs2368392), miR-492 C/G SNP (rs2289030), miR-149 C/T SNP (rs2292832), miR-146a C/G SNP (rs2910164), miR-196a2 C/T SNP (rs11614913), and miR-30a A/G SNP (rs1358379). Briefly, the authors evidenced that the polymorphisms of miR-149 rs2292832, miR-146a rs2910164, miR-196a2 rs11614913, and miR-30a rs1358379 were significantly more frequent in patients than in the control group (33). As a remark, Cong et al. (44) have already described that miRNA-146a rs2910164 may be involved in immune regulation during HBV infection in a Chinese population. Moreover, in this same study miR-30a rs1358379, miR-149 rs2292832, miR-146a rs2910164, miR-423 rs6505162, miR-492 rs2289030, and miR-196a2 rs11614913 were associated to HBV clearance (33). HBV persistence was impacted by miR-149 rs2292832 and miR-30a rs1358379. Finally, miR-196a2 rs11614913, miR-30a rs1358379, miR-26a1 rs7372209, miR-608 rs4919510, miR-149 rs2292832, and miR-423 rs6505162 impact the development of HBV-associated cirrhosis, or HBV-associated HCC. No statistically significant associations were reported concerning miR-604 rs2368392 or miR-499a rs3746444 on HBV-associated diseases (33). Of particular interest, the finding regarding miR-499a rs3746444 corroborates the previously mentioned study of Xu et al. (72).

Behelgardi et al. (83) studied the influence of IL-16 T/C (rs1131445), an SNP located in a miRNA-binding site in 3′UTR of the *IL-16* gene, and the risk of HBV chronic infection in an Iranian population. After adjustment for covariates, including age and gender, the TC genotype was associated with an increased risk of HBV chronic infection. IL-16 is a pro-inflammatory cytokine that activates T cells, monocytes, dendritic cells, and macrophages, as well as stimulates other pro-inflammatory cytokines, such as IL-1β, IL-6, and IL-15. Thus, polymorphisms that modulate the production of IL-16 could be important regulators of susceptibility to viral infections (83).

Hepatitis C Virus

The susceptibility to HCV infection as well as the progression of HCV-related diseases result from the interaction of host and viral genetic characteristics, and are mediated by environmental and different physio-metabolic factors (84). Focusing on the host genetics, the importance of miR-146a G/C SNP (rs2910164) (44, 53) and miR-196a2 C/T SNP (rs11614913) (39, 41, 53, 54) on HCV-associated disease was investigated in Chinese individuals. Despite these efforts, no statistically significant association was found between the SNPs and HCV-associated diseases (39, 41, 44, 53, 54). Furthermore, no statistically significant association between miR-196a2 rs11614913 and HCV-related HCC was reported in an investigation encompassing the Turkish population (73). Although disappointing at a first glance, these data are quite relevant. Knowing which SNPs (and genes) have little or no clinical importance on a particular disease helps to refine our choices and redirect new studies into variants and pathways relevant to the field.

MiR-122 is abundantly expressed in hepatic cells (85–87) and markedly influences the clinical course of HCV infection (86, 88). Some attempts to explain this influence have focused on genetic variants that affect miR-122 expression. For instance, Urban et al. (89) evaluated the relationships between the IFNL4/IL28B C/T SNP (rs12979860) and miR-122 expression in liver samples of HCV-infected patients from the United States presenting distinct ancestry (Asian, African American, Caucasian, and Hispanic). They observed a reduced miR-122 expression in samples of patients showing poor response to the treatment. However, this finding was independent of the IFNL4/IL28B rs12979860 genotype. On the other hand, this SNP may also influence the course of HCV infection independently, once carriers of CT or TT genotypes showed higher levels of interferon-stimulated genes compared to those levels linked to CC genotype (89). Evaluating the same SNP in a small sample of HCV-infected patients, Estrabaud et al. (86) observed an increased miR-122 expression in the liver of CC genotype carriers. In this same context, Spaniel et al. (59) reported a reduced miR-122 expression in non-tumor liver samples of HCV-infected individuals, a finding also linked to another SNP of *IFNL4/IL28B* gene: G/T rs8099917. In this study, the TG genotype was associated with a lower expression of miR-122 in non-tumor liver samples of HCV-infected Japanese individuals (59). Moreover, evaluation HCV-infected patients,

Su et al. (90) found an association between TT genotype of IFNL4/IL28B rs8099917 and high levels of serum miR-122. In agreement with results from population studies, an *in vitro* assay suggested that both IFNL4/IL28B rs12979860 and IFNL4/IL28B rs8099917 modulate the course of HCV infection, although how exactly this modulation happens is still not understood (87). However, as often is the case, there are conflicting data from studies that do not corroborate these associations (88, 91, 92).

The IFNL3/IL28B A/C SNP (rs4803217) affects the binding of HCV-induced miRNAs (miR-208b and miR-499a-5p) with the *IFNL3* mRNA (93, 94). According to McFarland et al. (93), this phenomenon has important implications for the HCV pathogenesis. Specifically, the G allele of IFNL3/IL28B rs4803217 impairs the activity of both these miRNAs, promoting a high expression of *IFLN3*. As a consequence, the G allele contributes to HCV clearance while the T allele favors (or is neutral) the infection process (93, 94). Based on the study of McFarland et al. (93), Tiang (94) highlighted that miR-208b and miR-499a-5p are potential targets for therapy against HCV infection. Posteriorly, the functional effects of IFNL3/IL28B rs4803217 on miR-208b and miR-499a-5p were challenged by other investigations (95, 96), since it was suggested that the influence of IFNL3/IL28B rs4803217 on HCV infection is promoted by miRNA-independent mechanisms (96). Thus, more studies regarding the role of IFNL3/IL28B rs4803217 polymorphism and miR-208b and miR-499a-5p on HCV infection are welcome.

Hepatitis C virus uses several strategies to evade the immune system, including miRNAs engagement (84). The disruption of miRNAs that promote HCV infection (for example, those that help HCV to evade the immune system) is a potential therapy for HCV-associated diseases (93). The understanding of how polymorphisms affect this phenomenon can help us in the development of new drugs based on this mechanism (**Figure 2**). Following these ideas, and based on a study investigating miRNA-101-1 and miRNA-221 expression and their respective SNPs (miR-101-1 C/G/T rs7536540 and miR-221 A/G rs17084733) in an Egyptian population, Shaker et al. (97) proposed the use of miR-101-1 and miR-221 as biomarkers of HCV-associated HCC. However, before applied to the clinical practice, these findings must be validated in different populations in studies with large sample sizes.

Finally, there is some evidence showing that TGFBR1 A/G SNP (rs868) (located at miR-let-7 and miR-98-binding sites) could have an impact on clinical parameters of HCV infection, especially on HCV RNA loads and hepatic inflammation (98). However, in the current scenario, the interaction between this TGFBR1 SNP (rs868) and HCV infection is poorly understood.

HIV Infection

According to Corbeau (99), different human miRNAs have a close relationship with HIV, both by interacting with HIV RNA as well as with mRNAs of cellular proteins essential for HIV replication. These interactions impact HIV replication, latency, pathogenesis, and also affect the host antiviral immune response. Therefore, the manipulation of these miRNAs expression can be approached as a potential therapeutic tool to mitigate the impact of HIV infection (99). Hariharan et al. (100) suggested that polymorphisms in miRNAs targeting HIV genes may influence the infection progression. However, although the relationship between HIV and miRNAs has already been studied and debated, the effects of miRNA SNPs on HIV-miRNAs interaction have been poorly explored.

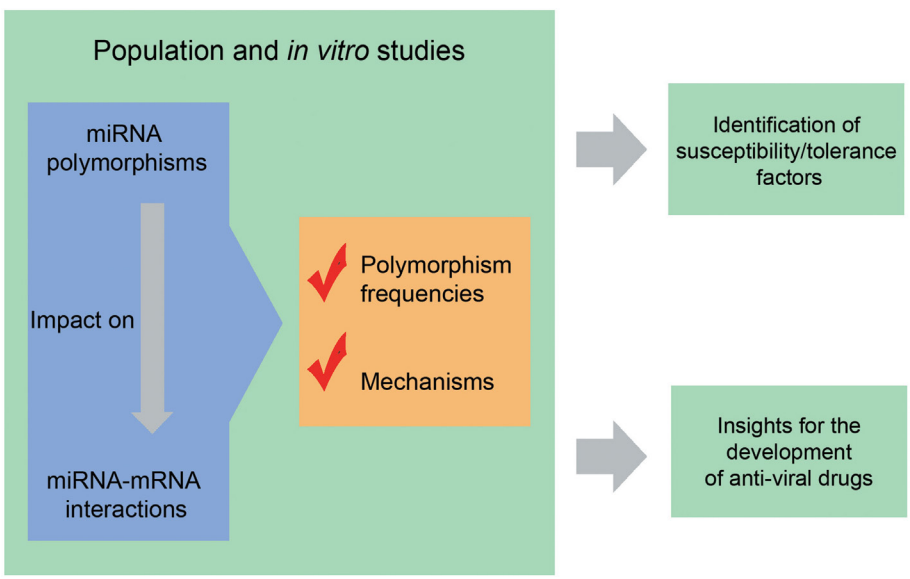

FIGURE 2 | Why to study polymorphisms in the context of viral diseases? Population or *in vitro* studies help to understand which and how polymorphisms impact on microRNA (miRNA)–mRNA interactions. Knowing the population distribution of polymorphisms and relationships of susceptibility/tolerance in the context of viral diseases, make it possible to identify individuals and populations with increased or decreased susceptibility to viral infections, allowing the development of strategies for infection control. In addition, the comprehension of the mechanisms by which miRNA-related polymorphisms influence the outcomes of viral diseases provides insights for the development of new antivirals. See text for references.

One of the few genes evaluated in the context of miRNA and HIV is the *Human Leukocyte Antigen-C* gene (*HLA-C*). The HLA-C ins/del variant (rs67384697) already evaluated in Europeans (101) and in Chinese populations (102), disrupts the binding site of miR-148, impacting the control of HIV infection. The deletion allele was associated to an HIV controller phenotype (low viral loads and high CD4 T^+ cell counts) and the insertion alleles were associated to an HIV noncontroller phenotype (high viral loads and low CD4 T^+ cell counts) (101, 102). The HIV controller phenotype was also associated to the CC genotype of HLA-C C/T SNP (rs9264942) (102), although it is worth to note that HLA-C rs67384697 and rs9264942 are in linkage disequilibrium (101, 102). Finally, miR-148a A/G SNP (rs735316) seems to influence the progression of HIV infection by interfering with the expression of HLA-C on the cell surface (103).

Peckham-Gregory et al. (104) evaluated 25 miRNA SNPs in patients with AIDS-associated non-Hodgkin lymphoma (AIDS-NHL) and HIV-infected controls. The authors included in their analyses SNPs located at miRNA coding regions, at miRNA biogenesis genes, and near/within miRNA-binding sites. Among the different results of this study, it worth to highlight: (I) The DDX20 C/T SNP (rs197412) affected miRNA biogenesis and this SNP C allele was associated with an increased risk of AIDS-NHL; (II) The T allele of miR-196a2 C/T SNP (rs11614913) (located at miR-196a2 coding region) was linked to a decreased risk of central nervous system (CNS) AIDS-NHL; (III) The T allele of HIF1A-AS2 C/T SNP (rs2057482) was associated with an increased risk of systemic AIDS-NHL, and (IV) the same allele decreased the risk of CNS AIDS-NHL (104). Of particular interest, HIF1A-AS2 rs2057482 is a variant that creates a binding-site to miR-196a2 (104).

Several CYP2B6 SNPs were evaluated concerning their potential influence in the metabolization of the anti-HIV drug Efavirenz in different contexts (105–109). Among the main findings, the CC genotype of CYP2B6 C/T SNP (rs1042389) was associated to low Efavirenz plasma concentration in Black HIV + individuals from South Africa (110). It is believed that this SNP modifies the expression of *CYP2B6* mRNA since it affects the binding-site of different miRNAs (110, 111). However, the association found in this study was quite weak and the clinical significance of this variant is controversial. Also in South Africa, Maharaj et al. (112) genotyped the miR-27a A/C/G/T SNP (rs895819) in HIV-negative and HIV-positive pregnant women subdivided according to a normotensive or a preeclamptic status. Although the TC/CC genotype of miR-27a rs895819 was associated to increased body mass index (BMI) in the group of HIV-positive women with preeclampsia, it was not associated with preeclampsia susceptibility (112). As miR-27a is an inhibitor of adipogenesis (113, 114) it is believed that miR-27a rs895819 can disrupt this miR-27a action, and then contribute to an increased BMI (112). The association between miR-27a rs895819 and BMI described by Maharaj et al. (112) is quite interesting and deserves to be replicated in other populations with different genetic backgrounds. Posteriorly, the same group described a potential impact of miR-146a C/G SNP (rs2910164) on HIV-positive South African women with preeclampsia (115). Specifically, GC/CC genotypes were associated with a reduced susceptibility to severe preeclampsia in HIV-positive pregnant women on HAART (Highly Active Antiretroviral Therapy). In addition, the miR-146a rs2910164 seems to have an influence on IL-2 levels of pregnant women (115). These results suggest an influence on the progression of HIV-related diseases. However, the patients studied by Maharaj et al. (112, 115) represent a very particular group of women, and before assuming that miR-27a rs895819 or miR-146a rs2910164 have an important influence on the clinical status of HIV-infected individuals from different genetic backgrounds, these SNPs must be studied in distinct populations (infected and non-infected by HIV) in studies recruiting men and women with different health status.

Finally, the A allele of TREX1 A/G SNP (rs3135945), a variant from a gene which encodes a restriction factor against HIV-1, was associated with higher susceptibility to HIV infection in a Caucasian cohort evaluated by Pontillo et al. (116). Since this SNP does not induce aminoacid sequence change, the authors hypothesized that a miRNA-mediated mechanism could explain how TREX1 rs3135945 impacts on HIV infection (116).

Epstein–Barr Virus

Epstein–Barr virus belongs to the herpesvirus family and is one of the most common viruses in humans, infecting more than 90% of the people worldwide. EBV is well known to cause the infectious mononucleosis (117). However, this virus is also associated with the development of several human tumors (118). EBV infection is a relevant susceptibility factor to nasopharyngeal carcinoma (NPC), and the few data available about the role of polymorphisms in miRNAs and binding target-sites in EBV infection came from studies focused in this type of cancer (32, 119, 120). Actually, the interest in NPC-associated EBV miRNAs emerged from the identification of EBV-encoded viral miRNA in lymphoid malignancies. Given that only a few viral latent proteins are expressed in NPC, researchers have hypothesized that EBV may contribute to cancer development through the viral miRNAs (120). The role of EBV miRNAs is still little known, but studies are pointing to important roles in both viral and cellular gene expression modulation (10, 121, 122).

An interesting case–control association study related to the current topic showed the influence of SNPs within mature-miRNA sequences in NPC susceptibility, assessing a southern China population (32). Further, these preliminary results were validated in a sample from eastern China. Eight SNPs were evaluated in the referred study, including miR-499 rs3746444 C/T, miR-608 rs4919510 C/G, miR-3152 rs13299349 A/G, miR-4293 rs12220909 C/G, miR-4513 rs2168518 C/T, miR-4520a rs8078913 C/T, miR-5579 rs11237828 C/T, and miR-5689 rs9295535 C/T. Among them, only the miR-608 rs4919510 SNP was associated with NPC risk. The presence of the G allele was reported as a susceptibility factor in both Chinese samples, in the two merged populations, and especially in individuals with EBV infection, where the risk effect was more prominent in comparison with individuals not infected. Aiming to evaluate the effects of the miR-608 rs4919510 SNP on NPC tumorigenesis, CNE-1 and CNE-2 cells (both NPC cell lines) were transfected with constructs containing G or C alleles and a soft-agar colony formation assay was performed. In agreement with population-based results,

functional analyses indicated that G allele of miR-608 rs4919510 SNP induced more colonies compared to C allele in CNE-2 cells (32). Another investigation also based on population-derived data and functional experiments had previously linked the miR-608 rs4910510 G allele with NPC locoregional recurrence (123). Based on this body of evidence, it is possible to assume that the G allele of miR-608 rs4919510 SNP significantly interacts with EBV, resulting in an increased NPC susceptibility (32). Some of the miR-608 target genes (immune system related genes, or genes associated to DNA repair, metastasis-related, cell death-related, among many others), can have their expression rates altered by the miR-608 rs4919510 SNP (32, 123). Furthermore, EBV can influence host gene transcription (32, 124, 125). In line with this view, Qiu et al. (32) suggested that miR-608 target genes could be directly activated by EBV and the influence of miR-608 rs4919510 SNP on gene transcription could be modified by EBV infection. These complex interaction networks would result in an increased NPC risk, as previously mentioned. Although the impact of miR-608 rs4919510 SNP on host gene expression and the interactions between EVB and host genes are plausible and supported by different data (32, 123–125), it must be characterized in more detail. Finally, the same authors also proposed the use of miR-608 rs4919510 SNP as a marker of NPC risk in a Chinese population (32). Although the above-mentioned data support this suggestion, it is important to replicate these findings in different populations before miR-608 rs4919510 SNP be used as a marker of NPC risk. Further functional studies fully characterizing the effects of this variant on gene regulation are also welcome to help us understand the role of this SNP (32).

Host–EBV interactions have also been investigated revealing that EBV gene regulation can be influenced by host transcriptional regulators. In addition, it was shown that EBV-encoded miRNAs can induce cell transformation in the host (126–128). In fact, EBV-encoded miRNAs have been involved in the regulation of both EBV and human gene expression in NPC. In a study from Lung et al. (120), two nucleotide variations in the primary transcript of miR-BART22 were identified as responsible for its increased biogenesis *in vitro*. This miRNA is coded by EBV and is highly expressed in NPC. Moreover, miR-BART22 modulates the EBV-encoded LMP2A protein expression, which is an oncoprotein recognized by cytotoxic T cells in the host (120). MiR-BART22-induced LMP2A down-modulation may promote EBV-infected cells evasion of the immune system (120). Based on these findings, it is possible to assume that miR-BART22 contributes to EBV pathogenesis. Thus, it makes sense the suggestion that polymorphisms in the miR-BART22 transcript could affect its maturation in NPC, contributing to a higher miR-BART22 expression, which in turn would induce a decreased LMP2A expression facilitating cancer development through the evasion of host immune response (120). Although this is not a case–control association study, it highlights and reinforces the importance of studies about polymorphisms in miRNAs and their binding target-sites in the context of the EBV infection. However, we consider that the most interesting in the study performed by Lung et al. (120) is that it supports the expression control of oncogenic and immunogenic viral proteins by EBV-derived miRNAs. Based on this information, polymorphisms affecting this control potentially play a pivotal role in the NPC development. In this sense, these polymorphisms may be used as models for the study of NPC, once understanding the mechanisms by which polymorphisms in EBV miRNAs interfere with the expression of proteins that modulate tumor biology may provide important insights for the development of NPC therapies. However, to achieve this goal, it is essential to perform functional studies focused on the understanding of the effect of EVB miRNAs and their polymorphisms in pathological and physiological contexts. Since EBV miRNAs modulate the expression of cancer-related proteins (120, 121, 128), they potentially also influence basic cellular physiological mechanisms, such as cell growth, differentiation, and signaling.

A recent characterization of the mRNA and miRNA transcriptome in NPC models (in cell lines that actually harbor EBV), provides a general view about miRNA–mRNA regulation and polymorphisms that can interfere in such regulation (127). This approach represents an interesting starting point for planning new studies about polymorphisms within miRNAs or miRNA target-sites potentially related to EBV infection.

Finally, we call attention to the need for conducting studies involving the characterization of EBV miRNAs, once information on this subject is still scarce. From the characterization of these miRNAs, it will be possible to deepen the investigations of polymorphisms found in sequences of EBV miRNAs. Although NPC is a disease of great relevance, it is also essential do not neglect other EBV-related diseases.

Human Papillomavirus

Studies encompassing miRNAs and HPV infection are incipient. Actually, a search in article databases returned only 12 articles focusing on genetic polymorphisms related to miRNAs and HPV, being the vast majority on HPV-related cancer development, progression, and prognosis. In this sense, miRNA-related polymorphisms that could modulate immune response and viral restriction, as well as cell cycle, proliferation and death, related to HPV infection often will be evaluated considering tumoral clinical outcomes.

Analyzing an Italian cohort of patients with penile squamous cell carcinomas (PSCCs), Peta et al. (129) investigated the association of a common functional miR-146a C/G SNP (rs2910164) with risk to cancer development. The frequencies of miR-146a rs2910164 genotypes in PSCCs patients, as well as its expression levels, were not different from the distribution observed in the general population, although miR-146a targets various genes that control of immune response, inflammation, cell proliferation, differentiation, and metastasis formation. Considering their potential effects, miRNA expression might act as a two-edged sword, being its upregulation related to immunosuppressive effects and its downregulation associated to cell proliferation and metastases development (129, 130). The authors found an inverse correlation regarding the expression levels of miR-146a and the expression of epidermal growth factor receptor (EGFR), a well-established target of miR-146a. The activation of EGFR pathways is known to increase keratinocytes proliferation and migration, and to be related to HPV-mediated cell immortalization and transformation (131). In fact, miR-146a expression levels were lower in high-risk HPV-positive than in HPV-negative patients,

although that difference was not statistically significant. This lower expression was also observed in HPV-positive carcinoma cell lines when compared to cultures from healthy cells. Thus, the authors suggested that HPV-16 E6 downregulates miR-146a expression, leading to an overexpression of EGFR, increasing, this way, the risk of cancer development (129).

Revathidevi et al. (132) studied the effect of a deletion in the *APOBEC3* gene cluster in an attempt to associate HPV infection and cancer development in a South Indian population. This polymorphism corresponds to a deletion of a 29.5-kb fragment, removing sequences from the fifth exon of *APOBEC3A* to the eighth exon of *APOBEC3B*. The polymorphic transcript encompasses the coding sequence of APOBEC3A and the 3′UTR *APOBEC3B*. The APOBEC3A/3B deletion polymorphism has been associated to poor HPV restriction and carcinogenesis promotion (133). Since APOBEC3A/3B deletion involves 3′UTR alterations, Revathidevi et al. (132) hypothesized that miRNA-mediated posttranscriptional regulation could be important to the *APOBEC3A/3B* overexpression. Nevertheless, no association between APOBEC3A/3B deletion polymorphism and the cancer development was observed, independently of the cancer type (they evaluated breast, cervical, and oral cancer samples), contrasting with previous studies that found associations of this polymorphism with cancer development (134, 135). Interestingly, the expression of an *APOBEC3B* miRNA that could regulate *APOBEC3A/3B* fusion transcript (miR-34b-3p) was downregulated in cervical tumor samples, suggesting that this miRNA may lead to a loss of miRNA repression and a consequently increased expression level of the APOBEC3A/3B protein.

In a Chinese population, Wu and Zhang (136) studied the association of miR-124 C/G (rs531564) with susceptibility to HPV infection and cervical cancer. The authors found that the miR-124 rs531564 G allele and the CG genotype were associated to a reduced risk of HPV infection, compared to the C allele and the CC genotype. Additionally, the miR-124 rs531564 G allele was described as associated to a reduced susceptibility to cervical cancer, corroborating other studies (137, 138). The authors hypothesize that the miR-124 rs531564 G allele promotes the expression of a mature form of this miRNA, leading to a lower risk of HPV infection and a subsequent reduced risk to cervical cancer.

Zhou et al. (139), studying another Chinese population, described the influence of miR-218 on HPV-related cervical cancer. Two polymorphisms were investigated: one at the primary-miR-218 (pri-miR-218) A/G SNP (rs11134527), and the second located at the 3′UTR of *LAMB3* (laminin-5 β3) gene (rs2566, C/T), a known target of miR-218, which is suppressed by the HPV-16 E6 protein. In fact, the expression of LAMB3 is augmented in the presence of the HPV-16 E6 oncoprotein and this effect is regulated through miR-218 (140). Laminin-5 plays an important role on the development of cervical lesions, and has been indicated as a marker of invasiveness (141). The authors evidenced an association of the pri-miR-218 rs11134527 variant homozygote GG genotype with a decreased susceptibility of cervical cancer development, as compared to the AA genotype. Regarding the LAMB3 rs2566 polymorphism, Zhou et al. (139) showed that the presence of the T allele, in a dominant model, was significantly associated to a higher risk of cervical cancer. Moreover, when these susceptibility variants were present together, the risk of cervical cancer was significantly higher, in a dose-dependent manner (139).

Several authors have studied the effect of miRNA-related polymorphisms on the development of oral squamous cell carcinoma (OSCC) and its variations [oropharynx (SCCOP) and oral cavity (SCCOC)] (142–147). These cancer types respond by the majority of head and neck malignant tumors worldwide and are highly associated to HPV infection. Song et al. (143) described the effect of four miRNA SNPs [miR-146 G/C (rs2910164), miR-149 C/T (rs2292832), miR-196 C/T (rs11614913), and miR-499 C/T (rs3746444)] in HPV-16 seropositivity and OSCC in a population from the United States. No statistically significant associations of these polymorphisms were observed. In fact, also an absence of association was observed between miR-146 rs2910164 and miR-196 rs11614913 with OSCC overall survival rates in a European cohort (144). However, Song et al. (143) described that, according to HPV-16 seropositivity, miRNA SNPs profiles could play a role in OSCC. Compared with individuals both miR-146 rs2910164 GG genotype and HPV-16 negative, those both GG genotype and HPV-16 positive presented an augmented risk of OSCC, and the susceptibility was even higher when the C allele was present. Similar results were obtained for the associations between miR-149 rs2292832 (CC genotype), miR-196 rs11614913 (C allele presence), and miR-499 rs3746444 (C allele presence) SNPs and risk of HPV-16-associated OSCC (143). Specifically to SCCOP, Guan et al. (142) described in the same population that compared to miR-146 rs2910164 CG/CC and miR-196 rs11614913 CC genotypes, individuals carrying both miR-146 rs2910164 GG and miR-196 rs11614913 CT/TT genotypes were significantly associated to a better overall, disease-specific, and disease-free survival in HPV-positive tumors (142). In line with these data, Song et al. (143) found that individuals with the combined miR-146 rs2910164 CG and CC genotypes had a higher risk of SCCOP than individuals with the GG genotype, and individuals with the miR-499 rs3746444 combined CT and CC genotypes had a higher risk of SCCOP than individuals with the TT genotype (143).

The same research group also studied the effect of polymorphisms located in putative miRNA-binding sites in the 3′UTR of genes related to DNA repair pathways in SCCOP recurrence in HPV-16-positive tumors (147). The authors found that only BRCA1 C/T (rs12516) and RAD51 A/G (rs7180135) SNPs were associated with SCCOP incidence. Patients with the variant genotypes of BRCA1 rs12516 (CT/TT) and RAD51 rs7180135 (AG/GG) SNPs presented a significantly lower susceptibility of disease recurrence as compared to patients with the corresponding common homozygous genotypes. Moreover, BRCA1 rs12516 CC genotype had a significantly higher BRCA1 protein expression, compared to CT/TT variant genotypes and RAD51 rs7180135 AA genotype had a borderline significant association to a higher expression of RAD51 protein, compared to RAD51 rs7180135 AG/GG variant genotypes. BRCA1 rs12516 SNP has been described as a potential binding site of several miRNAs, two of them (miR-118 and miR-639) have already been associated to cancer risk, while RAD51 rs7180135 SNP was described as a potential binding site of miR-197. Other miRNAs were

also described to target *RAD51* gene (miRNA-182, miR-155, miR-103, and miR-107), showing the importance of further studies to determine how this gene expression could be regulated.

Yuan et al. (146) and Zhang et al. (145) described in the same population that ins/del polymorphisms in 3′UTR of *E2F1* and *IL-1*α genes, respectively, are associated to OSCC HPV related (145, 146). Both genes have been described as important on the control of cell death and proliferation and variations on 3′UTR are supposed to impact on miRNA targeting. The authors showed that the E2F1 rs3213180 ins/del and ins/ins and the IL-1α 3′UTR (rs3783553) del/del genotypes, jointly to HPV seropositivity, are associated to a higher susceptibility to HPV-related OSCC. The E2F1 rs3213180 is the only miRNA-binding site described at E2F1 3′UTR that may affect E2F1 expression levels (148), while IL-1α rs3783553 interfere on miR-122-binding site, regulating IL-1α expression levels (149). In fact, Zhang et al. (145) described a significantly increased expression of IL-1α in patients with del/del genotype as compared to ins/ins and ins/del genotypes (145).

Another crucial regulatory gene is the *Cyclin-dependent kinase 6* (*CDK6*), which is associated with cell cycle and tumorigenesis. Various miRNAs are reported to be involved in CDK6-mediated tumorigenesis, such as miR-145, miR-320, and miR-29. In a Chinese population, Ye et al. (150) studied for the first time the effect of five genetic variations in the 3′UTR of *CDK6* gene (rs8179 G/A, rs4272 A/G, rs42033 A/T, rs42035 T/C, and rs42377 G/A) on susceptibility to precancerous cervical lesions. The authors found that the rs8179 A and rs42033 T alleles were associated to a lower risk to develop precancerous cervical lesions and had an antagonistic interaction with the HPV infection. This lower susceptibility to cervical lesions was also observed to rs8179 GA, compared to AA genotype and rs42033 AT, compared to AA genotype, after adjustments for HPV infection and others clinical and demographic characteristics. Strong linkage disequilibrium values were observed between rs8179, rs4272, rs42033 and rs42377 and the haplotype AGTA was significantly associated to a reduced risk to precancerous cervical lesions when compared to GAAG haplotype (150).

To our knowledge, only one study described polymorphisms in HPV related to miRNA-binding sites. Mandal et al. (151) showed that polymorphisms located at a short non-coding region (NCR2), commonly present between HPV E5 and L2 open reading frames, could lead to a loss of human miRNA sites. Through *in silico* analysis, the authors identified binding sites at the NCR2

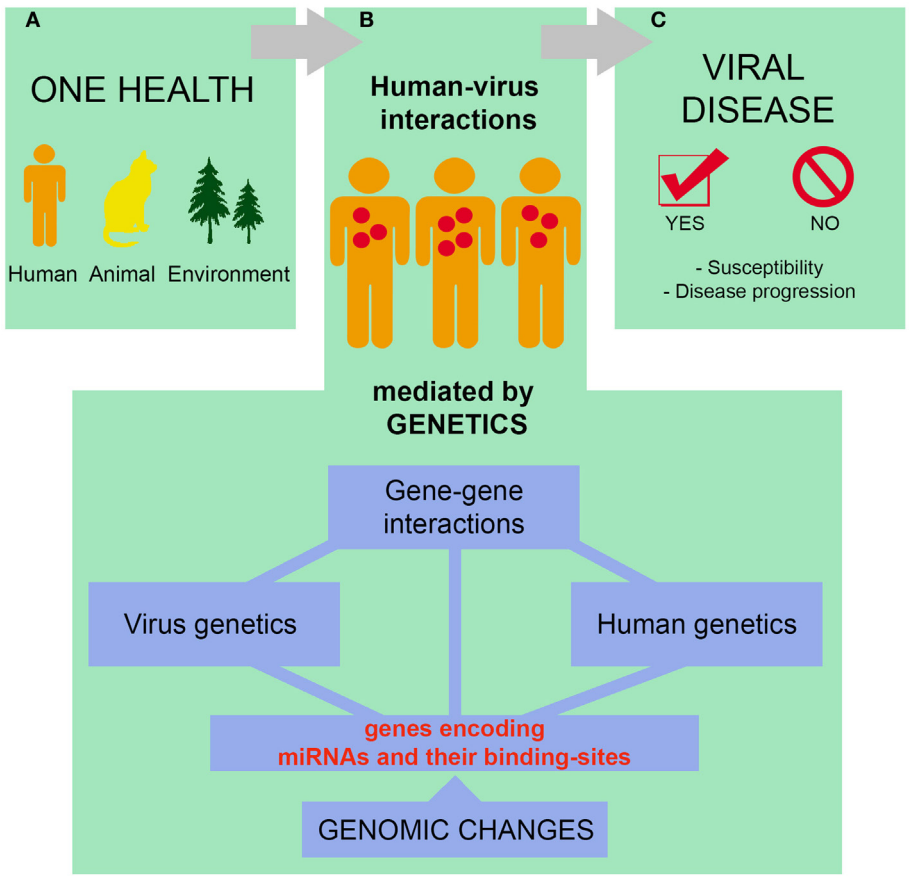

FIGURE 3 | Susceptibility to infections and progression of viral diseases are complex processes that must be thought within the context of One Health. Human, non-human, and environmental factors define whether a given individual will come into contact with a particular virus and the consequences of such interaction **(A)**. Of note, human–virus interactions are mediated by both host and viral genetic factors, including microRNAs (miRNA) and miRNA-related polymorphisms **(B)**. The complex interactions mentioned in **(A,B)** influence the susceptibility to infections as well as the progression of viral diseases **(C)**. See text for references.

region in HPV-16 corresponding to 14 human miRNAs (miR-3148, miR-3174, miR-3613-3p, miR-3916, miR-495, miR-548a-5p, miR-548b-5p, miR-548c-5p, miR-548d-5p, miR-548h-5p, miR-548i-5p, miR-548j-5p, miR-548w-5p, and miR-548y-5p). Moreover, the authors revealed the occurrence of an SNP (T4228C) in the NCR2 of a variant isolate, which could lead to loss of 9 miRNA-binding sites in the corresponding transcripts (151).

EMERGING TOPICS

Currently, the prevention and control of infectious diseases should be approached as a global strategy, in which human, animal, and environmental factors are integrated (152–154), allied to the evaluation of the genomic characteristics of the pathogens (155, 156). Among the human factors that should be taken into consideration, the investigation of genetic features that alter the susceptibility to infection and progression of viral diseases is essential for the prevention and clinical management of infectious diseases in specific human populations (157–159) (**Figure 3**).

SNPs are the most common form of genetic variation in the human genome (160). For many years human SNPs have been highly studied worldwide. However, it is necessary to focus those studies also on viral genetic variants and try to understand how these variants affect the action of miRNAs, for example. For instance, some evidence indicates that SNPs have a relevant impact on the biogenesis and action of Kaposi's sarcoma-associated herpesvirus (KSHV) miRNAs (161). KSHV infection is highly linked to Kaposi's sarcoma development (162). According to a set of *in vitro* analyzes based on clinical observations, Han et al. (161) have shown that different SNPs in KSHV miRNAs alter the expression level of these miRNAs, as well as modify their processing and silencing activities. These changes may alter KSHV pathogenesis, potentially impacting Kaposi's sarcoma development (161). Of note, a number of polymorphisms (SNPs, deletions, and insertions) in KSHV miRNAs have already been observed in pri-miRNAs, pre-miRNAs, and mature miRNAs (163–165). Looking at KSHV-related diseases, these polymorphisms affect miRNAs maturation and some of them may also affect the risk of Kaposi's sarcoma development in patients with AIDS (164), although as a whole the effects of these polymorphisms on KSHV pathogenesis are poorly understood. Furthermore, it has been shown that polymorphisms in KSHV miRNAs influence the risk for the development and the pathogenesis of multicentric Castleman disease and KSHV-associated inflammatory cytokine syndrome, diseases also linked to KSHV infection (165).

The number of KSHV miRNAs described in the literature is increasing (166–168). Similarly, the effects of them on immune response, KSHV pathogenesis and Kaposi's sarcoma development have already been described (166–172). The implications of KSHV miRNA SNPs on the miRNA processing are also being characterized (164, 173). However, there is much to be explored about the SNPs located in KSHV miRNAs. Once these SNPs are well characterized, we will better understand their effects on Kaposi's sarcoma development and other KSHV-related diseases.

Another interesting example of viral miRNA variant involves the human T cell leukemia virus-type 1 (HTLV-1); Host miR-28-3p is an inhibitor of HTLV-1 replication and infection (31). The Thr-to-Cys (AAT-to-AAC) polymorphism in ATK-1 HTLV strain (subtype 1A) disrupts the miR-28-3p target site. This disruption affects the anti-HTLV action of miRNA-28-3p. However,

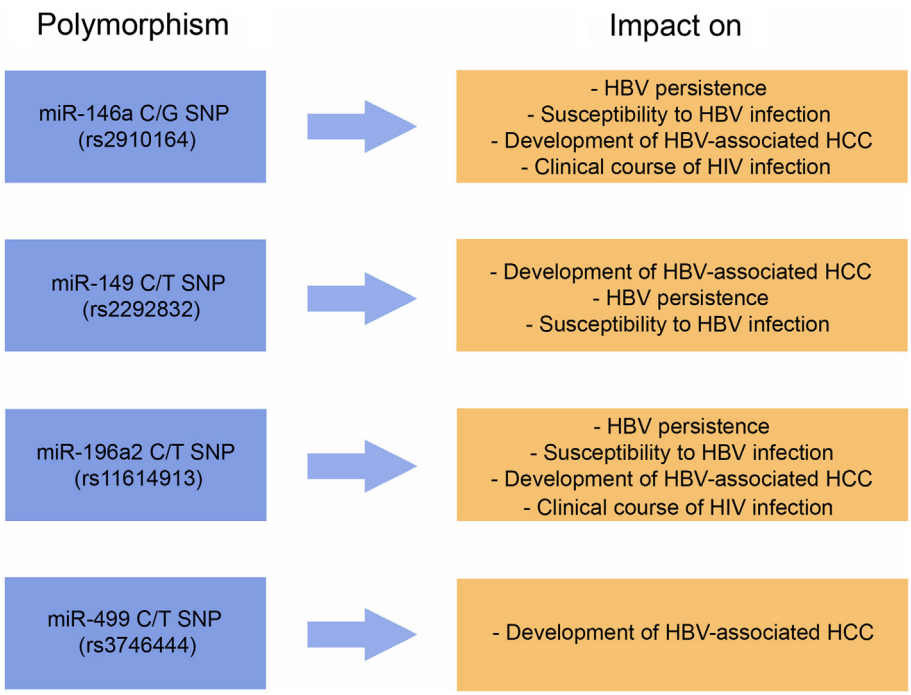

FIGURE 4 | Summary of the main miRNA-related polymorphisms discussed in this review and their impacts on viral diseases. See text and Tables for references.

miR-28-3p target site is highly conserved in the HTLV-1 subtypes B and C, and therefore, this miRNA has a therapeutic potential in strategies to control HTLV-1 infection (31).

The role of miRNA SNPs in viral infections other than those previously approached in the present review has been poorly explored, although some studies can be cited. For example, Misra et al. (174) evaluated the influence of the following host miRNAs SNPs on human cytomegalovirus (HCMV) infection: miR-146a C/G (rs2910164), miR-196a2 C/T (rs11614913), miR-499a C/T (rs3746444), and miR-149 C/T (rs2292832). In brief, with exception of miR-149 rs2292832, mutant genotypes of the other three SNPs were linked to increased risk of symptomatic HCMV infection. Multifactor Dimensionality Reduction analysis (applied to access SNP–SNP interactions) indicated an association between increased risk of symptomatic HCMV infection with the four interaction models tested (174). This result indicates that miRNA SNPs play a relevant role in the pathogenesis of HCMV. However, to the best of our knowledge, no other study focusing on the role of miRNA SNPs in HCMV infection was performed, making this a blank spot to further studies.

Finally, we should highlight the triad (I) exosomes, (II) miRNAs, and (III) viral infections. Exosomes are extracellular nanovesicles originated from multivesicular bodies. These vesicles have drawn attention from the scientific community due to their ability to transport protein, lipid, and genetic components between different cells in a highly regulated manner (175). Moreover, a large body of evidence showed that host and viral miRNAs are one of the major types of components transported by exosomes (176, 177). Exosomes have a relevant immunomodulatory action (176, 178) and have been shown to strongly interact with different viruses, such as HIV (179, 180), Ebola (181), HBV (182), and others (183). Taking into account the therapeutic potentials of the exosomes-mediated miRNA delivery pathway supported by recent findings (184, 185), in the near future, it will be possible to modulate the exosomes-mediated miRNA trafficking aiming to mitigate viral infections. In addition, a therapeutic control of exosomes-mediated miRNA delivery could be used to induce, or avoid, similar effects to those triggered by miRNA SNPs. However, in order to these therapeutics become a reality, one must to disclosure the influences of the miRNAs transported

TABLE 2 | Main microRNAs (miRNA)-related polymorphisms showing statistically significant influence on Epstein–Barr virus (EBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and Human Papillomavirus (HPV) infections.

Virus	miRNA or miRNA-binding site[a]	Polymorphism[b]	Influence on	Population	Reference
EBV	miR-608	C/G rs4919510	EBV-related nasopharyngeal carcinoma (NPC); NPC risk	Chinese	Qiu et al. (32)
HCV	miR-208b- and miR-499a-5p-binding sites	A/C rs4803217	HCV clearance	*In vitro* experiment	McFarland et al. (93)
	miR-let-7- and miR-98-binding sites	A/G rs868	HCV loads; hepatic inflammation	Polish	Sajjad et al. (98)
HIV	miR-148-binding site	ins/del rs67384697	HIV loads	European descendant	Kulkarni et al. (101)
			HIV loads; CD4 T^+ cell counts	Chinese	Blais et al. (102)
	miR-148-binding site	C/T rs9264942	HIV loads; CD4 T^+ cell counts	Chinese	Blais et al. (102)
	miR-148a	A/G rs735316	Progression of HIV infection	European descendant	Kulkarni et al. (103)
	miRNA biogenesis	C/T rs197412	AIDS-associated non-Hodgkin lymphoma risk	American	Peckham-Gregory et al. (104)
	miR-196a2	C/T rs11614913	Central nervous system (CNS) AIDS-associated non-Hodgkin lymphoma risk	American	Peckham-Gregory et al. (104)
	miR-196a2-binding site	C/T rs2057482	CNS and systemic AIDS-associated non-Hodgkin lymphoma risk	American	Peckham-Gregory et al. (104)
	miR-27a	A/C/G/T rs895819	HIV/AIDS-associated nutritional status	African descendant	Maharaj et al. (112)
	miR-146a	C/G rs2910164	HIV-related diseases (particularly preeclampsia)	African descendant	Maharaj et al. (115)
HPV	miR-146a	C/G rs2910164	HPV-related cancer	Chinese	Guan et al. (142); Song et al. (143)
	miR-149	C/T rs2292832	HPV-related cancer	Chinese	Song et al. (143)
	miR-196a	C/T rs11614913	HPV-related cancer	Chinese	Guan et al. (142); Song et al. (143)
	miR-499	C/T rs3746444	HPV-related cancer	Chinese	Song et al. (143)
	miRNA-binding sites	C/T rs12516 and A/G rs7180135	HPV-related cancer	Chinese	Zhu et al. (147)
	miRNA-binding sites	ins/del rs3213180	HPV-related cancer	Chinese	Yuan et al. (146)
	miR-122-binding site	ins/del rs3783553	HPV-related cancer	Chinese	Zhang et al. (145)
	miR-218	A/G rs11134527	HPV-related cancer	Chinese	Zhou et al. (139)
	miR-218-binding site	C/T rs2566	HPV-related cancer	Chinese	Zhou et al. (139)
	miRNA-binding sites	A/G rs8179 and A/T rs42033	HPV-related cancer	Chinese	Ye et al. (150)
	miR-124	C/G rs531564	HPV infection and cervical cancer	Chinese	Wu and Zhang (136)

[a]*Seed or regulatory region.*

[b]*Polymorphism quotations were standardized according to the Single Nucleotide Polymorphism Database (dbSNP) of NCBI (https://www.ncbi.nlm.nih.gov/snp/), based on the reference SNP cluster (rs#) of each polymorphism.*

by the exosomes in viral diseases, and to decipher how miRNA SNPs modulate the pathogenesis of different viruses.

CONCLUSION AND PERSPECTIVES

Based on the articles discussed in this review, we present in **Figure 4** the miRNA SNPs that were studied with greater robustness in the context of the viral infections, as well as the influences of these selected SNPs on viral diseases. In summary, development of HBV-associated HCC is influenced by the following polymorphisms: miR-146a G/C SNP (rs2910164), miR-149 C/T SNP (rs2292832), miR-196a2 C/T SNP (rs11614913), and miR-499 C/T SNP (rs3746444). In addition, in **Table 2** the main findings of the studies addressing HCV, HIV, EBV, and HPV infections are presented. The study of genetic variants located in miRNA genes or in genes of miRNA-binding sites is incipient. As research continues, new SNPs will be described and new influences of miRNA SNPs on viral diseases will be reported. It is also possible that future investigations will redirect the discussions about the biological or clinical significance of the variants presented in this review. On the other hand, part of these results can be strengthened with the completion of new studies. Of note, the majority of the studies mentioned in this review are punctual and specific of particular populations. Studies investigating miRNA SNPs in Asian populations highly outnumber the studies performed with non-Asian populations. Thus, it is essential to investigate the frequencies of miRNA SNPs in worldwide populations in order to gather better data about susceptibility and progression of viral diseases in different ethnic/genetic backgrounds. The identification of SNPs that influence characteristics of susceptibility or clinical outcome in different populations will be essential for the understanding of the biological significance of such genetic factors. Thus, new meta-analyses will be essential to establish with robustness the effects of those SNPs on infectious diseases.

We also draw attention to the fact that most of the studies within the scope of this review investigated human miRNA SNPs. It is necessary to explore the importance of viral miRNAs and their variants on the clinical course of infectious diseases. Also of great importance, the prevalence of viruses of different genotypes is variable around the world, which may or may not complicate what this review is dealing with. Thus, the evaluation of viral genetic characteristics is significant in population-based studies focused on miRNA SNPs. In this sense, when virus genotype is available, this information must be considered during the interpretation of the studies here mentioned.

Finally, directing further investigations to the SNPs discussed here may provide important insights for the development of new therapies against infectious diseases based on inhibitors or stimulators of the action of miRNAs. As discussed earlier, knowing how SNPs alter biogenesis, processing or the action of miRNAs may also be useful for the development of antiviral therapies or for the treatment of complications caused by viral infections. Technologies focused on the delivery of miRNAs in an accurate manner, as engineered exosomes, will also contribute to the success of these therapies. We believe that we are close to experiencing a boom of the miRNA-based therapies.

AUTHOR CONTRIBUTIONS

JE, FZ, and RG reviewed the studies and wrote the manuscript. JC wrote and reviewed the manuscript.

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CAPÍTULO X

CCR5 gene editing - Revisiting pros and cons of CCR5 absence

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CCR5 gene editing – Revisiting pros and cons of CCR5 absence

CCR5 gene editing in human embryos was recently announced, leading to significant debate in the scientific community regarding the ethical aspects and biological implications of this procedure (Cyranoski and Ledford, 2018). The announced genetic intervention was a supposedly CRISPR-mediated *CCR5* gene deletion. Discussing the ethical aspects of *CCR5* editing is not the focus of this article. Importantly, ethical concerns are being raised out by other authors elsewhere (Wang et al., 2018; Zhang et al., 2018). We further stress that different articles bring valuable information on the ethical aspects of genetic interventions in human embryos (Cartier-Lacave et al., 2016; Hildt, 2016; Krishan et al., 2016; Rossant, 2018). Actually, the precise consequences of this gene edition are unknown. However, previous studies that evaluated the absence or low expression of CCR5 through different approaches may bring us valuable information about the potential effects of *CCR5* gene editing.

CCR5 is a chemokine receptor with seven transmembrane domains (Parmentier, 2015), encoded by *CCR5* gene, and expressed primarily on the surface of leukocytes (Raport et al., 1996; Rottman et al., 1997; Wu et al., 1997; Lederman et al., 2006). CCR5 and its ligands (MIP-1α/CCL3, MIP-1β/CCL4, CCL5/RANTES, among others) mediate the migration of leukocytes to inflamed tissues and specific inflammatory sites (Lederman et al., 2006; Jones et al., 2011). More recently, the participation of CCR5 in different cellular processes and pathological conditions has been evidenced (Brelot and Chakrabarti, 2018; Scurci et al., 2018). Besides, CCR5 is a co-receptor necessary for HIV-1 infection (Parmentier, 2015; Brelot and Chakrabarti, 2018).

CCR5 gene has various polymorphisms that affect CCR5 expression, and these variants are found in different human populations (Ansari-Lari et al., 1997; Mummidi et al., 1997; Zhang et al., 2003; Barmania et al., 2013; Parmentier, 2015). Among *CCR5* polymorphisms, CCR5Δ32 is the most studied due to its association with protection against HIV infection. CCR5Δ32 is a 32-base pair deletion in *CCR5* coding region, found mainly in Caucasoid individuals and genetically admixed populations (Martinson et al., 1997; Solloch et al., 2017; Ellwanger et al., 2018). In homozygosis, CCR5Δ32 promotes the formation of a truncated protein, showing only four transmembrane domains. This truncated protein is not expressed on the cell membrane. Therefore, individuals with a homozygous genotype for CCR5Δ32 have a strong (but not complete) protection against HIV-1 infection, once they lack CCR5 expression. This lack of expression avoids HIV-CCR5 interaction and consequently the virus-cell fusion. On the other hand, the heterozygous genotype for CCR5Δ32 causes a reduced CCR5 expression on the cell surface, a condition associated with a slower progression of HIV infection/AIDS (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996; Balotta et al., 1997; Wu et al., 1997; Venkatesan et al., 2002; Brelot and Chakrabarti, 2018).

Considering that the absence of CCR5 expression was associated with a relative resistance against HIV infection, different approaches aiming the blockade or deletion of this molecule were performed. A study published in 2009 described the first and so far single case of long-term control of HIV infection as a result of non-pharmacological medical intervention. This result was obtained after a leukemic HIV-infected patient received an allogeneic hematopoietic stem cell transplant derived from a CCR5Δ32 homozygous donor (Hütter et al., 2009). The patient submitted to this procedure is popularly known as “the Berlin patient” (Brown, 2015). Currently, CCR5 blockers are used for HIV therapy and are already being tested for the treatment of other diseases, including cancer (Brelot and Chakrabarti, 2018; Vangelista and Vento, 2018).

Nevertheless, sometimes much attention is given to one single aspect of a given molecule, and the complex network of interactions behind it is somehow neglected. For example, although the relationship between CCR5 and HIV infection has been extensively studied, many biological aspects of CCR5 are still unknown. Considering this, here we highlight some potential pros and cons of CCR5 manipulation (Table 1), based on studies addressing the CCR5Δ32 allele, pharmacological CCR5 blockade, animal models, *in vitro* tests, and *CCR5* gene editing. In 2009, a similar discussion was held by our group by approaching the association of several pathological conditions and the potential effects of a null *CCR5* allele (Vargas et al., 2009). However, much was uncovered in recent years regarding the interactions of CCR5 in both healthiness and disease, and therefore, this issue should be revisited in light of the emerging gene editing technologies.

The pros and cons listed in Table 1 certainly do not exhaust the potential effects linked to CCR5 absence, reduced CCR5 expression, or those associated with the functional blockade of this molecule. The literature is full of examples showing different effects of both CCR5 absence and low expression in various pathological situations. These findings must be taken into consideration in future discussions regarding *CCR5* gene editing. The few examples listed here cover a broad spectrum of distinct conditions, ranging from infections, cancer, and autoimmune diseases to even pregnancy disorders, highlighting the complexity and extension regarding the effects of CCR5 absence. Moreover, those effects will depend on population-associated features, such as the ethnic/genetic background of a specific human population as well as to the presence of pathogens and environmental-related disease triggers. Of note, two major points must be highlighted. First, the absence of CCR5 due to gene-editing techniques may lead to different physiological consequences as compared to those observed in individuals homozygous for CCR5Δ32. The truncated form of CCR5 will not be present in such gene-edited individuals. Evolutionary forces may have influenced the selection of distinct genetic variants which eventually “compensate” the lack of CCR5 expression due to Δ32 allele and, within this same reasoning, a role of truncated CCR5 protein itself - even in the resistance against the HIV infection - has not been ruled out (Barmania and Pepper, 2013). Second, chemokine-ligand systems are classically considered as redundant, and although the absence of CCR5 molecule should be compensated by other chemokine receptors

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Table 1
Potential pros and cons of *CCR5* gene editing based on different lines of evidence.

Pros and cons	Approach	Some findings and methodological aspects	References
Pros			
Protection against HIV infection	*CCR5* gene editing	*CCR5* edition using CRISPR/Cas9 system promoted protection against HIV infection *in vitro* and *in vivo* (study using human cell lines and mice)	Kang et al. (2015); Xu et al., 2017
Protection against enteroviral cardiomyopathy	*CCR5* polymorphism (CCR5Δ32 cohort data)	CCR5Δ32 was associated with spontaneous myocardial enterovirus clearance and better outcomes (study in humans)	Lassner et al. (2018)
Reduced risk for autoimmune diseases	*CCR5* polymorphism (CCR5Δ32 cohort data)	CCR5Δ32 was associated with reduced risk for rheumatoid arthritis and multiple sclerosis (studies in humans)	Toson et al. (2017); Troncoso et al. (2018)
Better prognosis in colorectal cancer	Pharmacological CCR5 blockade	CCR5 blockade promoted better clinical responses in colorectal cancer patients (study in humans and *in vitro* tests)	Halama et al. (2016)
Low risk for preeclampsia development	*CCR5* polymorphism (CCR5Δ32 cohort data)	CCR5Δ32 was associated with protection against preeclampsia (studies in humans)	Gurdol et al. (2012); Telini et al. (2014)
Cons			
Worst outcome in West Nile virus infection	*CCR5* polymorphism (CCR5Δ32 cohort data)	CCR5Δ32 was associated with severe and fatal outcomes of symptomatic West Nile virus infection (study in humans)	Glass et al. (2006)
Increased heart dysfunction in Chagas disease	Low CCR5 expression	Low CCR5 expression was correlated with severe chronic chagasic cardiomyopathy (study using human cells)	Talvani et al. (2004)
Increased risk for worst outcome in colorectal cancer	Low CCR5 expression	Low CCR5 expression was associated with lymphatic dissemination of colorectal cancer (study using tissue samples from human patients and *in vitro* tests)	Zimmermann et al. (2010)
Impaired brain function	Low CCR5 expression and KO mice	CCR5 absence/low expression (using CCR5 knockout mice and *in vitro* tests) induced astrocyte activation, astrogliosis, amyloid-β deposit, and memory dysfunction	Lee et al. (2009); Hwang et al. (2016)

(Mantovani, 1999), it is possible that CCR5 performs unique functions not yet clarified (Chen et al., 2018).

CCR5 gene editing seems to be a double-edged sword: there are advantages and disadvantages, and we should be aware of such behavior. It is too early to conclude the actual effects of a procedure that abolishes the expression of a component of the immune system seemingly involved in so many different situations. The conclusions could only appear from the follow-up of individuals submitted to *CCR5* editing.

Conflicts of interest

The authors declare no conflicts of interest.

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CAPÍTULO XI

Host immunogenetics in Tick-borne encephalitis virus infection – The CCR5 crossroad

Este capítulo apresenta o seguinte artigo de revisão publicado no periódico *Ticks and Tick-borne Diseases*:

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Review Article

Host immunogenetics in tick-borne encephalitis virus infection—The CCR5 crossroad

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Inflammation

ABSTRACT

The human *Tick-borne encephalitis virus* (TBEV) infection is a complex event encompassing factors derived from the virus itself, the vectors, the final host, and the environment as well. Classically, genetic traits stand out among the human factors that modify the susceptibility and progression of infectious diseases. However, and although this is a changing scenario, studies evaluating the genetic factors that affect the susceptibility specifically to TBEV infection and TBEV-related diseases are still scarce. There are already some interesting pieces of evidence showing that some genes and polymorphisms have a real impact on TBEV infection. Also, the inflammatory processes involving tick-human interactions began to be understood in greater detail. This review focuses on the immunogenetic and inflammatory aspects concerning tick-host interactions, TBEV infections, and tick-borne encephalitis. Of note, it has been described that polymorphisms in *CD209*, *GSTM1*, *IL-10*, *IL-28B*, *MMP9*, *OAS2*, *OAS3,* and *TLR3* have a statistically significant impact on TBEV infection. Besides, CCR5, its ligands, and the CCR5Δ32 genetic variant seem to have a very important influence on the infection and its immune responses. Taking this information into consideration, a special discussion regarding the effects of CCR5 on TBEV infection and tick-borne encephalitis will be presented. Emerging topics (such as exosomes, evasins, and CCR5 blockers) involving immunological and inflammatory aspects of TBEV-human interactions will also be addressed. Lastly, the current picture of TBEV infection and the importance to address the TBEV-associated problems through the One Health perspective will be discussed.

1. Introduction – basic aspects of TBEV infection

Tick-borne encephalitis virus (TBEV) is an RNA virus that belongs to the *Flaviviridae* family, *Flavivirus* genus (Gritsun et al., 2003). Of note, modifications in the TBEV sub-type classification have already been proposed (Zlobin et al., 2001; Demina et al., 2012). This virus species is traditionally divided into three sub-types: Far Eastern, Siberian, and European viruses (Süss, 2011), although a new TBEV sub-type was proposed by Kovalev and Mukhacheva (2017), the Baikalian-TBEV. More recently, the Himalayan virus was described in China, potentially representing an additional TBEV sub-type (Dai et al., 2018). Moreover, the Siberian virus is the most genetically diverse TBEV sub-type, and the classification of their lineages are under frequent review (Kovalev and Mukhacheva, 2013, 2017). Tick-borne viral diseases represent critical veterinary and human medical problems in many countries. At least 17 tick-borne diseases affect humans (Dantas-Torres et al., 2012) and the TBEV has a prominent role among the etiological agents of such conditions.

TBEV is the causative agent of the tick-borne encephalitis (TBE), an infectious disease that affects people mostly in countries of Europe and Asia, being this virus considered a public health problem in such areas (Gritsun et al., 2003; Mansfield et al., 2009; Süss, 2011). TBEV has been known since 1937 when the Soviet virologist Lev Alexandrovich Zilber and a team of other scientists discovered the virus and characterized its link with TBE (Zlobin et al., 2017). Between 1990 and 2009, ˜170,000 clinical cases of TBE were reported in Russia and some European countries (Süss, 2011). In addition to TBEV, *Louping ill virus*, *Langat virus*, and *Powassan virus* can also cause TBE (Gritsun et al., 2003), but this review will mainly highlight studies focused on TBEV-related TBE.

In general, humans are infected by TBEV through the bite of a TBEV-infected tick (Gritsun et al., 2003; Süss, 2011). Less commonly the infection can be caused by human contact with aerosol or through ingestion of food products contaminated with the pathogen (Dörrbecker et al., 2010). *Ixodes ricinus* and *I. persulcatus* are the most important tick vectors of TBEV (Gritsun et al., 2003; Süss, 2011; Kazimírová et al., 2017). Wild rodents play a pivotal role in the ticks' life cycle and the

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TBEV maintenance in wild animals (Mansfield et al., 2009), probably being the main reservoirs of tick-borne pathogens (Vayssier-Taussat et al., 2015).

Due to the inexistence of curative treatment for the disease, the currently TBE therapy available is only supportive (Mansfield et al., 2009). Nevertheless, vaccination against TBEV infection is effective and recommended for people living on, or visiting, regions where the pathogen circulates (Süss, 2011). Although vaccination is highly recommended for populations in countries at high risk for TBEV infection, each country in endemic TBEV regions applies vaccination strategies based on their epidemiological situation and vaccination policies (Zavadska et al., 2013). There are different TBEV vaccines, including FSME-Immun (Baxter Vaccines, Austria), Encepur (Novartis Vaccines and Diagnostics, Germany), EnceVir (Virion Corporation, Russia), and TBE-Moscow (Chumakov Institute for Poliomyelitis and Viral Encephalitides, Russia) (Lehrer and Holbrook, 2011). In general, TBEV vaccines are well tolerated. Systemic and local adverse effects may occur in some individuals following vaccination, especially in children. The adverse effects are mild and transient, such as fever and pain at the site of vaccination. However, neurological manifestations may occur in a small portion of the vaccinated individuals. When they happen, neuritis and headache are the main manifestations. However, there is no strong association of TBEV vaccines with neurological adverse effects, and the vaccines are generally safe (Grzeszczuk et al., 1998; Kunz, 2003; Lindquist and Vapalahti, 2008; Rendi-Wagner, 2008; Demicheli et al., 2009; Šmit and Postma, 2015; Bogovic and Strle, 2015; Galgani et al., 2017). Ecotourism, camping, and adventure tourism facilitate the transmission of tick-borne diseases once such activities put humans in close contact with nature and wildlife. Thus, as mentioned above, tourists visiting endemic TBEV regions may receive TBEV vaccine (Jensenius et al., 2006).

The clinical manifestations and outcome of TBE can be quite particular. The following forms of TBE are known: febrile, meningeal (the most common), meningoencephalitic, poliomyelitic, polyradiculoneuritic, and chronic (Gritsun et al., 2003). In brief, fever, nausea, vomiting, muscular pain, and meningeal disorders are classic TBE symptoms (Gritsun et al., 2003). These non-specific symptoms make TBE diagnosis a clinical challenge (Zavadska et al., 2013). Such infection can initially cause only a febrile syndrome, which may or may not progress and then affect the CNS and promote neurological disorders (Mansfield et al., 2009). In general, clinical TBE is characterized by a biphasic disease, in which the patient shows flu-like symptoms in the first stage of the illness and CNS/neurologic symptoms in the second stage. These stages are intercalated by an asymptomatic period (Süss et al., 2010; Süss, 2011). In general, ˜20% of TBE patients have some sequel, being age and protein concentration in CSF important risk factors for sequels development (Czupryna et al., 2018). The TBE progression pattern may vary according to the particular TBEV sub-type which is infecting the host (Mansfield et al., 2009). Other viral factors, such as virus tropism, and specific host characteristics are determining factors of TBEV infection outcome (Süss, 2011). Among host factors, age, immune status, and some genetic features have a meaningful impact on the susceptibility and progression of TBEV infection (Süss, 2011). In this context, recent results obtained by Vora et al. (2017) suggested that host immunological background influences the tick-associated fibrinogenolytic activity. Together, the information mentioned above demonstrates that human-tick interactions are quite complex, being affected by factors derived from human and tick.

The cysteine-cysteine chemokine receptor 5 (CCR5) modulates the immune response against different human infectious diseases. In some infections, CCR5 can act as a “protective molecule,” helping the host mitigate the infection. On other occasions, nevertheless, CCR5 can mediate post-infection inflammatory responses which can be harmful to the host (Klein, 2008). Considering the scenario mentioned above, in this review we will address the genetic factors involved in the susceptibility to TBEV infection and disease progression, focusing on studies evaluating the CCR5 and its ligands. Before that, we will briefly describe the fundamental aspects of the host immune response in the context of the human-tick interaction. Lastly, we will bring emerging topics regarding human-tick interactions in the context of the One Health concept.

2. Immune responses during human-tick interactions and TBEV infection

As blood-feeding arthropods, ticks need to circumvent the host immune defenses to obtain their bloodmeals adequately. The interaction between tick and host skin can last for days, and this will correspond to the blood feeding period. Host inflammatory responses at the tick biting site would hamper the feeding process. Thus, ticks developed the ability to disrupt the host chemokine-mediated immune response. Such disruption enables these parasites to feed on host blood “free” of a local inflammatory response at the site of interaction between the tick mouthparts and the host damaged skin (Ribeiro et al., 1985; Hajnická et al., 2005; Vančová et al., 2007; Hovius, 2009; Vančová et al., 2010; Wikel, 2013; Mason et al., 2014; Kotál et al., 2015; Chmelař et al., 2016; Bonnet et al., 2018). For example, different studies have shown that tick saliva suppresses the activity of a number of cytokines and chemokines, including IL-2, IL-4, IL-6, CXCL8/IL-8, IL-12, TNF-α, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL11/eotaxin, and CCL5/RANTES (Ribeiro et al., 1985; Gillespie et al., 2001; Hajnická et al., 2001, 2005; Frauenschuh et al., 2007; Vančová et al., 2007; Déruaz et al., 2008; Oliveira et al., 2008; Peterková et al., 2008; Hovius, 2009; Vančová et al., 2010; Marchal et al., 2011; Poole et al., 2013; Mason et al., 2014; Hayward et al., 2017). It is possible that such a simultaneous disruption of the activity of multiple chemokines results from the complex interactions and robustness of the chemokine-receptor system network (Mantovani, 1999). In other words, ticks need to disrupt the entire cytokine/chemokine network at a local level to obtain blood from the host (Hajnická et al., 2005). Besides, tick saliva could also modulate the host immune system at a systemic level (Kazimírová et al., 2017). Importantly, tick species, developmental stage, the amount of saliva used by the tick, and the duration of the feeding process (number of days) are influencing factors of the tick-derived anti-chemokine activity (Vančová et al., 2010). Prostaglandin E_2 (Poole et al., 2013) and the chemokine-binding proteins known as evasins (Bonvin et al., 2016; Hayward et al., 2017) are some molecules that regulate the chemokine-mediated local immune response triggered by ticks saliva. However, a wide variety of molecules present in the tick saliva account for the immune regulation (Beaufays et al., 2008; Oliveira et al., 2011; Hidano et al., 2014; Kazimírová et al., 2017; Šimo et al., 2017). On the other hand, some reports point to a tick saliva-induced increase in the production of different chemokines (Langhansová et al., 2015). Nevertheless, it appears that tick saliva contains immune-regulatory molecules and that it induces the secretion of such molecules as well (Poole et al., 2013).

It was shown recently in an animal model that, in the very early stage of the tick feeding process, the CCR5 is up-regulated, potentially due to the initial inflammatory response observed when the tick damages the skin (Thangamani et al., 2017). In general, in the first 3 h of the tick feeding process, the bite site can be considered an inflammatory environment (Thangamani et al., 2017). Interestingly, TBEV-infected ticks trigger higher recruitment of inflammatory cells to the bite site in comparison to non-infected ticks (Thangamani et al., 2017). In this context, tick-mediated TBEV infection in early stages of tick bite occurs in an “inflammatory” environment, which in turn, could favor the host infection (Thangamani et al., 2017). In humans, the early stages of ticks-induced skin lesions activate the innate immune system (Glatz et al., 2017). In the presence of pathogens, such immune response will depend on the pathogens transmitted by the tick (Pulendran et al., 2001; Akira et al., 2006; de la Fuente et al., 2017). After a tick bite, macrophages, neutrophils, and dendritic cells are recruited to the skin

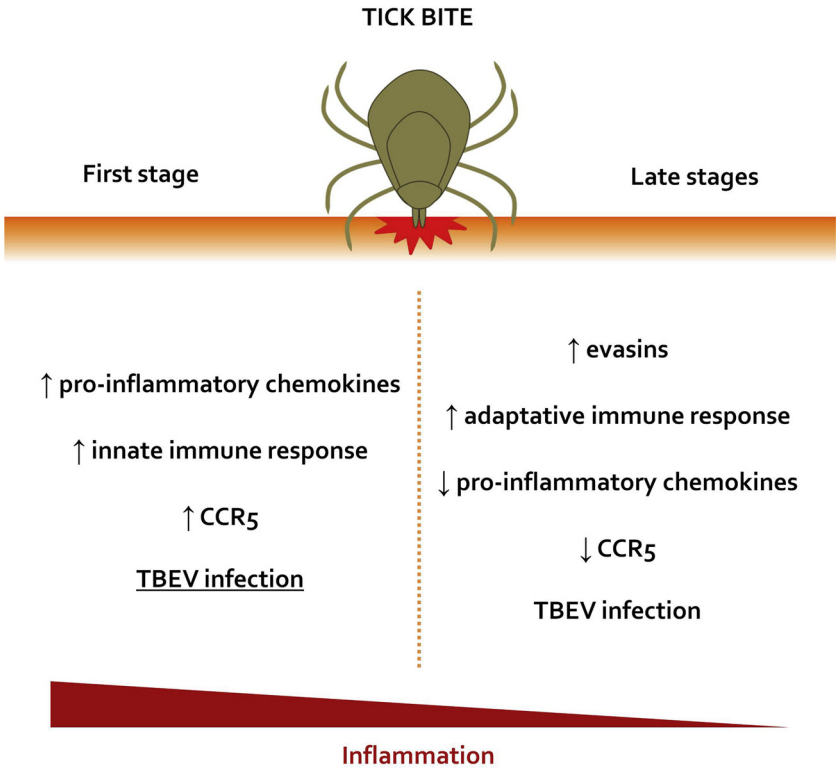

Fig. 1. Inflammatory events that probably occur in the human skin in the first and late stages of a tick bite. In the first stage of the tick bite, there is an increase of the local host inflammatory response, with activation of the innate immune system associated with an increase of pro-inflammatory chemokines and expression of inflammatory receptors such as CCR5. TBEV infection is likely to happen more easily in this early stage of the tick bite, although it may also occur in other stages. During the late stages of the tick bite occurs the development of an adaptive immune response. Concomitantly, an increased release of tick saliva-derived immune-suppressive molecules (mainly evasins) associated with a decreased pro-inflammatory chemokines production and expression of inflammatory receptors (including CCR5) by the host cells is observed. See text for references.

lesion (Glatz et al., 2017). After the first 24 h of tick-skin interaction/bite, the inflammatory context is reduced due to the anti-inflammatory action of the tick saliva; this event is accompanied by the development of an adaptive immune response (Glatz et al., 2017). Fig. 1 summarizes the potential inflammatory events that occur during the first and late stages of a tick bite. The dual effect of tick saliva on the host inflammatory response demonstrates that ticks have sophisticated mechanisms to control the action of inflammatory cells. Finally, it is also important to mention that the dual inflammatory/anti-inflammatory effect of tick saliva on host cells also depends on the cell type exposed to tick saliva (Scholl et al., 2016).

The immune response of the mammalian host against the TBEV infection is quite complex. In the first place, it will depend on the route of infection by the pathogen: tick bite, ingestion (TBEV-contaminated milk products), or inhalation (aerosol containing TBEV) (Dörrbecker et al., 2010). Depending on the transmission route of the pathogen to the host, different tissues and organs will participate in the immune response (skin, stomach, and olfactory tract, among others). How and how easily the TBEV will overcome the blood-brain barrier (BBB) will also depend on the entry route of the virus into CNS. For example, the TBEV entry by the olfactory tract probably facilitates its passage through the BBB, which eventually allows the virus to reach the brain directly (Dörrbecker et al., 2010).

Furthermore, TBEV-infected cells can mediate the pathogen passage through the BBB (Dörrbecker et al., 2010). TBEV-infected human brain microvascular endothelial cells promote brain infection without causing substantial disruptions to BBB (Palus et al., 2017). Of note, Zhou et al. (2018) reported that exosomes support TBEV transmission through the BBB. These processes occur in a microenvironment where the host attempts to regulate BBB integrity to avoid infection or mitigate it through the action of inflammatory cells and orchestration of chemokine release (Miner and Diamond, 2016). Lastly, the impact of the virus on the CNS as well as the infection-related tissue-specific response will depend on the pattern and types of inflammatory cells, humoral response, as well as the cellular components and chemokines/cytokines engaged by the host (Dörrbecker et al., 2010).

In general, infection by tick-borne flaviviruses has a significant impact on the release of many cytokines and chemokines. Also, which cytokines or chemokines will be released and in which tissue this release occurs will depend on the viral species by which the host was infected (Tigabu et al., 2010). We suggest that this differential pattern of immune response can also occur in infections with different TBEV sub-types. As described in the introduction of this review, such different modes of immune response will vary according to the characteristics of each patient, including age, immune status, and genetic features (Süss, 2011). In the next section, we will address the influence of human genetic factors on the immune response during TBEV infection and TBE pathogenesis.

3. Host immunogenetics in the TBEV infection context

Different genetic factors from both the pathogen and the host influence the susceptibility to an infectious disease as well as the disease outcome (Powell et al., 2000; Toan et al., 2006; Janssen et al., 2007; Jayadev and Garden, 2009; Chapman and Hill, 2012; Moore et al., 2013; da Silva et al., 2014; Rajoriya et al., 2017; Valverde-Villegas et al., 2017). For example, and as recently reviewed, microRNAs-related polymorphisms have significant impacts on the susceptibility and progression of several human viral infections (Ellwanger et al., 2018a). Looking specifically at TBEV-host interactions, Barkhash et al. (2016b) argued that in the Central Asian Mongoloid populations the susceptibility to TBEV infection is modulated by various genes and polymorphisms. Moreover, a study using mice models with different genetic patterns of susceptibility to TBEV infection indicated that the genetic background has a meaningful impact on the clinical course of TBE (Palus et al., 2013). Such data points to the existence of genes that control TBEV infection susceptibility.

Recently, a system biology analysis performed by Ignatieva et al. (2017) have pointed to *IL-10*, *IFNL3/IL-28B*, *ARID1B*, *IFNAR1*, and *CCR5* as important genes of the immune response against TBEV. Based on the number of genes/proteins interactions, *IFNAR1* and *CCR5* were assumed as the most relevant amongst all candidate genes. These

Table 1
Studies that evaluated the impact of gene variants on TBEV infection/TBE.

Study	Gene	Variants evaluated	Population	Sample size (*n*)[a]		Main findings
				Cases	Controls	
Barkhash et al. (2010)	*OAS1*	rs1131454, **rs10774671**, rs1131476, rs1051042, rs2660, rs6489865	Russian	142	302	No evident influence of the polymorphisms on TBE/TBEV infection.
	OAS2	rs2384075, rs2072138, rs1293762, rs2240185, rs929291, rs2240184, rs15895, rs1732778				rs1293762, rs15895, and rs1732778 have an impact on TBE outcome.
	OAS3	rs7967461, rs1156361, rs2285932, rs2072136, rs2240187, rs1557866, rs2010549				rs2285932 and rs2072136 have an impact on TBE outcome.
	OASL	rs3213545, rs12819210				No evident influence of both gene variants on TBE/TBEV infection.
Kindberg et al. (2011)	*OAS1*	**rs10774671**	Lithuanian	128	135	No impact of this SNP on TBE/TBEV infection.
	TLR3	rs5743305, **rs3775291**				rs3775291 (wild-type genotype/allele) was a risk-factor for TBE/TBEV infection; No impacto of rs5743305 on TBE/TBEV infection.
Barkhash et al. (2012)	*CD209*	**rs4804803**, **rs2287886**	Russian	136	263	rs2287886 (A allele and AA genotype) was linked to a higher predisposition to severe forms of TBE; No statistically significant differences regarding rs4804803 genotype/allele frequencies between TBE and control groups.
Barkhash et al. (2013)	*TLR3*	**rs3775291**	Russian	137	269	GG genotype and G allele (wild-type genotype/allele) were linked to higher predisposition to TBE.
Mickienė et al. (2014)	*TLR3*	**rs3775291**	Lithuanian	348	212	Wild-type allele was higher in TBE patients than in controls; The SNP influenced the TBE severity in adults; Dual effect of *TLR3* on TBEV infection.
Grygorczuk et al. (2015)	*IFNL4/IL-28B*	**rs12979860**	Polish	15	[b]	rs12979860 influenced the IFN λ3 concentrations in cerebrospinal fluid.
	IL-10	**rs1800872**, **rs1800896**				No effect of both gene variants on TBE was detected.
	CD209	**rs287886**, **rs4804803**				rs287886 influenced the IL-10 concentrations in cerebrospinal fluid. No effect of rs4804803 on TBE was detected.
Barkhash et al. (2016a)	*IL28B*	rs8103142, rs12980275	Russian	132	221	rs8103142 TT genotype and T allele were linked to higher TBE predisposition; rs12980275 AA genotype and A allele were linked to higher TBE predisposition.
	IL-10	**rs1800872**, rs3021094, rs3024498				rs1800872 AA genotype was linked to higher TBE predisposition. No influence of other two SNPs on TBE was observed.
Czupryna et al. (2017)	*CD209*	**rs2287886, rs4804803**	Polish	59	57	rs2287886 AG genotype was linked to higher susceptibility to TBE; No important impact of rs4804803 on severe forms of TBE.
	IL-10	**rs1800872, rs1800896**				No important impact of both SNPs on severe forms of TBE.
	IFNL4/IL-28B	**rs12979860**				No important impact of this SNP on severe forms of TBE.
Ilyinskikh and Ilyinskikh, (2017)	*GSTM1*	*GSTM1* deletion	Russian	120	124	The inactive form of both genes was linked to increased TBEV-associated cytogenetic aberrations in young patients.
	GSTT1	*GSTT1* deletion				
Barkhash et al. (2018)	*SCRIB*	rs6558394	Russian	150	228	No influence of this SNP on TBE at population level.
	MMP9	rs17576				rs17576 G allele was linked to severe TBE.

TBE: Tick-borne encephalitis; TBEV: Tick-borne encephalitis virus.

[a] See the original studies for the exact number of subjects genotyped for each polymorphisms.

[b] No controls were included in the genetic analysis (patients were stratified according to clinical criteria); Gene variants evaluated in more than one study are highlighted in bold; Gene variants that had a significant impact on TBEV infection/TBE are pointed in "main findings" column and summarized in Fig. 2.

findings are of great importance, but functional and population-based studies are needed to confirm the effects of such genes and proteins on TBEV infection. In an interesting initiative, Ignatieva et al. (2017) developed an online platform which provides a variety of information on the genes (140 so far) linked to the immune response against TBEV infection, the "TBEVHostDB" (http://icg.nsc.ru/TBEVHostDB/).

The genes and polymorphisms that may be involved in the susceptibility to TBEV infection and TBE development are summarized in Table 1. Studies involving the *CCR5* will be addressed separately in the next sections of this review.

Barkhash et al. (2010) evaluated 23 SNPs in genes of the 2′-5′-oligoadenylate synthetase (2′-5′-OAS) proteins family. Such proteins show antiviral activity and, in brief, five SNPs showed important influences on TBE outcome: rs1293762, rs15895, rs1732778 (located on *OAS2*), rs2285932, and rs2072136 (located on *OAS3*). These results suggest that the *OAS* family plays a prominent role in the clinical course of TBEV infection. However, functional studies associated with population-based studies are not yet available, being quite essential to confirm these findings.

Looking at *TLR3* rs3775291, Kindberg et al. (2011) found that the wild-type genotype/allele of this polymorphism was a risk-factor for TBE/TBEV infection. A disrupted TLR3-mediated immune response associated with gene variants could decrease the deleterious effects of the exacerbated inflammation/immune response during TBEV infection (Kindberg et al., 2011). In this case, the variant allele would act as a protective factor against TBEV-related diseases. In concordance, this association first observed in the Lithuanian population was corroborated in the Russian population. In the later study, the wild-type genotype/allele of *TLR3* rs3775291 was also a risk factor for TBE (Barkhash et al., 2013). On the other hand, partial results reported in a study performed by Mickienė et al. (2014) challenged the scenario mentioned above regarding the role of *TLR3* rs3775291 on TBE. The functional TLR3 can indeed promote the pathogenesis of TBE. However, TLR3 can also be necessary for the anti-TBEV response when the virus is in the brain. Moreover, the role of *TLR3* rs3775291 on TBEV infection can differ between adults and children (Mickienė et al., 2014), data that together with those previously mentioned, suggest a dual effect of TLR3 during TBEV infection.

Besides the genes mentioned in Table 1, other candidate genes have potential importance in the context of TBEV infection. For example, based on gene expression data obtained from an *in vitro* model of tick-borne flavivirus (TBFV) infection, relevant effects on the TBFV persistence were assigned to genes such as *CXCL10, INF-β1*, and *TNF-α* (Mlera et al., 2016). An interesting table including various other genes observed up- or down-regulated post-infection can be found in the original study (Mlera et al., 2016). Furthermore, recent studies in mice suggest that *CD33*, *KLK1B22*, *SIGLECE*, *KLK1B16*, *FUT2*, *GRWD1*, *ABCC6*, *OTOG*, and *MKRN3* (all located on chromosome 7) may also be important in the context of susceptibility and progression of the TBEV infection and should be better studied, also considering their ortholog human genes (Palus et al., 2018). According to Barkhash et al. (2012), studies focused on genetic variants that influence the predisposition to TBEV-related diseases may be useful to understand the pathogenesis of TBEV. Furthermore, such studies may provide insights into the development of therapies for the treatment of TBEV-related diseases (Barkhash et al., 2012). Once the influence of genes on human-TBEV interactions is well established, genetic markers could be used as predictors of the clinical progression of TBEV infection (Ignatieva et al., 2017). Finally, Fig. 2 shows the genes and polymorphisms that have already been statistically linked to some effect on TBEV infection and disease progression. The proportion of genetic factors involved in "protection" and "susceptibility", gene-gene interactions (Phillips, 2008), gene penetrance (Alcaïs et al., 2009), and gene-environment interactions (Hunter, 2005) may affect the influence of genes and polymorphisms on TBEV infections and therefore all such situations are mentioned in Fig. 2. Furthermore, it is important to highlight that a statistically significant association sometimes does not represent a critical biological effect and therefore must be interpreted with prudence (Cordell, 2009; EFSA Scientific Committee, 2011; Lovell, 2011).

4. The CCR5, CCR5Δ32, and CCR5 ligands in TBEV infection and TBE outcome

The pattern of the immune response against TBEV has a pivotal role in the course of infection in humans since sometimes the immune response *per se* is responsible for the damage associated to the infection (Dörrbecker et al., 2010). Similarly, individual genetic factors play a remarkable role in the susceptibility to TBEV infection and clinical course of TBE, as demonstrated by studies focusing on single nucleotide polymorphisms (Table 1; as discussed in the previous section). In this context, we would like to draw attention to the potential role of the CCR5 and the CCR5Δ32 genetic variant in TBEV infection.

CCR5 is a cell-surface receptor (Raport et al., 1996; Signoret et al., 2000) expressed in macrophages (Raport et al., 1996; Rottman et al., 1997), lymphocytes (Raport et al., 1996; Rottman et al., 1997; Wu et al., 1997), and monocytes (Rottman et al., 1997; Wu et al., 1997), being observed also in non-blood cells (Rottman et al., 1997). CCR5 regulates a variety of immune functions, mainly leukocyte migration to sites of inflammation (Lederman et al., 2006). The main CCR5 ligands are CCL3 (Samson et al., 1996a; Mueller et al., 2006), CCL4 (Samson et al., 1996a; Bondue et al., 2002), and CCL5 (Samson et al., 1996a; Mbemba et al., 2001; Lin et al., 2008). Fig. 3 shows a schematic representation of CCR5.

The interaction of type 1 human immunodeficiency virus (HIV-1) with surface CCR5 in $CD4^+$ T cells allows the virus entry into the cell (Proudfoot, 2002; Brelot and Chakrabarti, 2018). The cellular susceptibility to HIV infection correlates with the levels of CCR5 expression (Wu et al., 1997; Fear et al., 1998; Zella et al., 1998). CCR5 is expressed by the gene of the same name, located on chromosome 3 (Raport et al., 1996). CCR5Δ32 (rs333) is a 32-base pair deletion in *CCR5*. In general, CCR5Δ32 homozygous individuals are not infected by HIV-1, due to the absence of CCR5 on the cell surface, although heterozygous individuals for CCR5Δ32 present a slower progression to AIDS mainly due to reduced expression of functional CCR5 (Dean et al., 1996; Liu et al., 1996; Wu et al., 1997; Proudfoot, 2002; Venkatesan et al., 2002; Brelot and Chakrabarti, 2018). The CCR5Δ32 allelic frequency can reach ~16% in some Euro-derived populations (Solloch et al., 2017), being rare in Afro-derived individuals (Martinson et al., 1997; Solloch et al., 2017). In admixed populations, such as the Brazilian, the allelic frequency is 3–6% (Silva-Carvalho et al., 2016; Ellwanger et al., 2018b). The protection against HIV infection attributed by CCR5Δ32 was described in 1996 (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996b). Since then such discovery has led to significant advances in HIV therapy once CCR5Δ32 implications have provided support for the development and use of CCR5 blockers. Currently, Aplaviroc, Cenicriviroc, Maraviroc, and Vicriviroc are examples of CCR5 antagonists under study in humans (Vangelista and Vento, 2018). Among them, Maraviroc is considered effective and safe, already being used in the clinical practice (CONITEC, 2012; Brites et al., 2015). Besides HIV infection, CCR5 and CCR5Δ32 also play a relevant role in some inflammatory-associated diseases (Kohem et al., 2007; Martin-Blondel et al., 2016; Schauren et al., 2013; Scheibel et al., 2008) and different viral infections (Glass et al., 2006; Marques et al., 2015; Rustemoglu et al., 2017).

4.1. CCR5 and CCR5Δ32

The studies approaching the role of the CCR5 and the CCR5Δ32 polymorphism in TBVE infection are summarized in Table 2, suggesting altogether that CCR5 plays a prominent role in TBE infection. This view is mainly supported by data from Kindberg et al. (2008), Mickienė et al. (2014), Michlmayr et al. (2016), Ignatieva et al. (2017), and

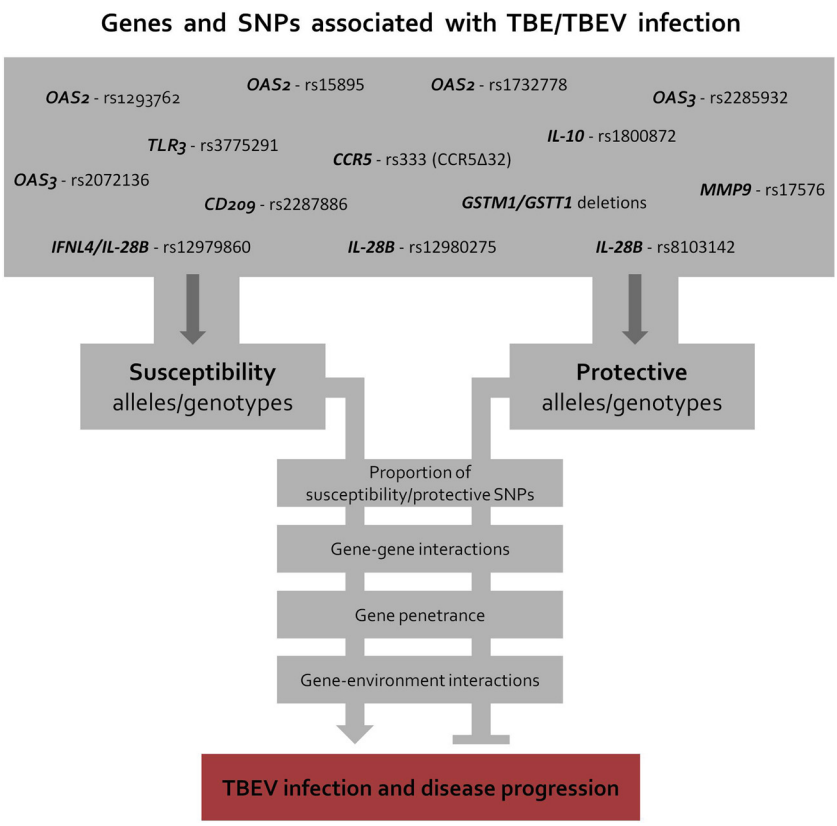

Fig. 2. Genes and polymorphisms associated with TBEV infection and TBEV-related disease progression. The proportion of "protective" and "susceptibility" genetic factors, gene-gene interactions, and gene penetrance may affect the impact of genes and polymorphisms on TBEV infection. Of note, non-human factors and gene-environment interactions should also be taken into account when interpreting the effect of a particular gene or polymorphism on TBEV infection. The effect of each polymorphism shown in this figure is detailed in Table 1. See text and Table 1 for references.

Thangamani et al. (2017). Specifically, functional CCR5$^+$ cells seem to be important in the immune response against the TBEV infection and its effects in the brain. CCR5Δ32 disrupts CCR5 and potentially affects the CCR5$^+$ cell function, thus favoring TBEV pathogenesis and TBE progression (Kindberg et al., 2008; Mickienė et al., 2014), although part of the data presented by Grygorczuk et al. (2016) challenge this view. Interesting, using a mouse model, Michlmayr et al. (2016) suggested that CCR5 deficiency is a contributing factor to symptomatic TBEV infection. In line with a potential role of the CCR5 in the TBEV pathogenesis, it has been demonstrated that CCR5Δ32 is an important risk factor for symptomatic West Nile virus infection (Glass et al., 2006), a condition that also affects the central nervous system. Nevertheless,

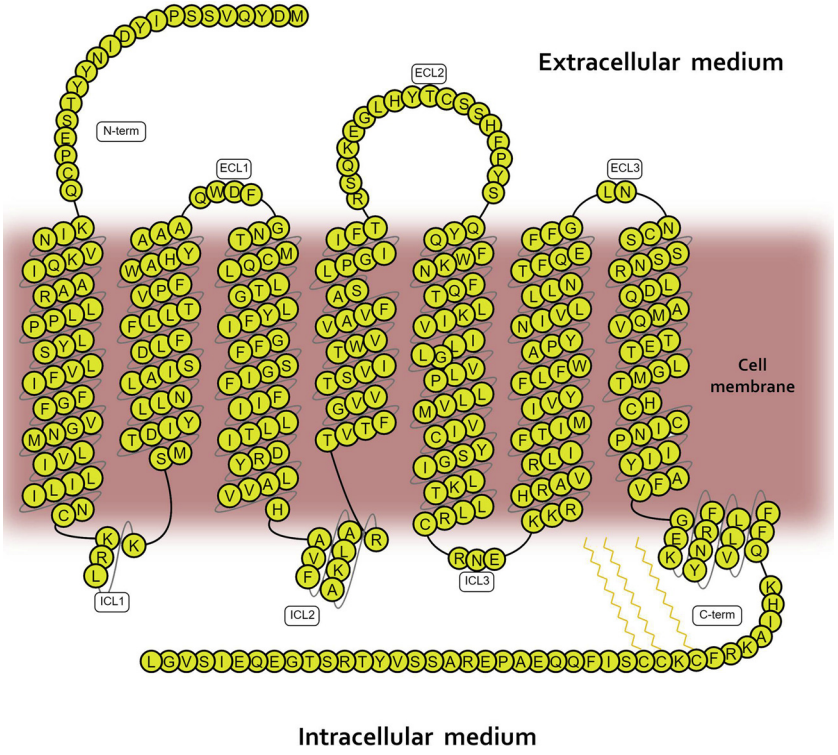

Fig. 3. CCR5 structure. Schematic representation (2D, linear) of CCR5 structure. The figure shows seven transmembrane domains, extracellular loops (ECL1, ECL2, ECL3), intracellular loops (ICL1, ICL2, ICL3), N-terminus (N-term), and C-terminus (C-term). Three palmitoylated cysteines (that interact with the cell membrane) are indicated in the figure. Capital letters indicate amino acids. Basic CCR5 structure was created using the GPCR database: http://gpcrdb.org/ (Pándy-Szekeres et al., 2018) and then edited based on information obtained in previous studies (Oppermann, 2004; Lederman et al., 2006; Parmentier, 2015; Brelot and Chakrabarti, 2018).

Table 2
Impacts of CCR5 and CCR5Δ32 on TBEV infection/TBE.

Reference	Population evaluated or study model	Sample size (*n*)		Main findings
		Cases	Controls	
Kindberg et al. (2008)	Lithuanian	$n = 129$	$n = 134$	CCR5Δ32 homozygous frequency was higher ($p < 0.05$) in TBE patients than in controls; CCR5Δ32 allele frequency was higher in TBE patients than in controls and in TBE patients with moderate/severe disease than in patients with mild symptoms (but not significantly).
Engman et al. (2012)	Swedish	$n = 8$	$n = 15$	No influence of CCR5Δ32 on TBE susceptibility.
Barkhash et al. (2013)	Russian	$n = 137$	$n = 268$	No influence of CCR5Δ32 on TBE susceptibility or clinical manifestations.
Mickienė et al. (2014)	Lithuanian	$n = 349$	$n = 213$	CCR5Δ32 homozygous frequency was higher ($p < 0.05$) in TBE patients than in controls; Δ32 allele was a risk factor for clinical TBEV infection ($p < 0.05$, using subgroups data); No influence of CCR5Δ32 on TBE severity.
Grygorczuk et al. (2015)	Polish	$n = 15$	[a]	No influence of CCR5Δ32 on cytokine concentrations of TBE patients.
Grygorczuk et al. (2016)	Polish	$n = 79$	$n = 18$	CCR5 plays a role in the pathogenesis of TBEV infection (neurologic phase); CCR5 may act in the early response to TBEV infection; No influence of low CCR5 expression in peripheral blood lymphocytes on TBE susceptibility; No significant impact of CCR5Δ32 on TBE clinical manifestations.
Michlmayr et al. (2016)	Mouse model			CCR5 plays a pivotal role in the immune response against TBEV infection in the central nervous system; CCR5 deficiency may promote symptomatic TBEV infection.
Czupryna et al. (2017)	Polish	$n = 59$	$n = 57$	No influence of CCR5Δ32 on TBE susceptibility or clinical manifestations.
Ignatieva et al. (2017)	Systems biology			*CCR5* gene is suggested as having remarkable participation in the immune response to TBEV infection.
Thangamani et al. (2017)	Mice model			*CCR5* gene is up-regulated after TBEV-infected tick feeding.

TBE: Tick-borne encephalitis; TBEV: Tick-borne encephalitis virus.
[a] No controls were included (patients were stratified according to clinical criteria).

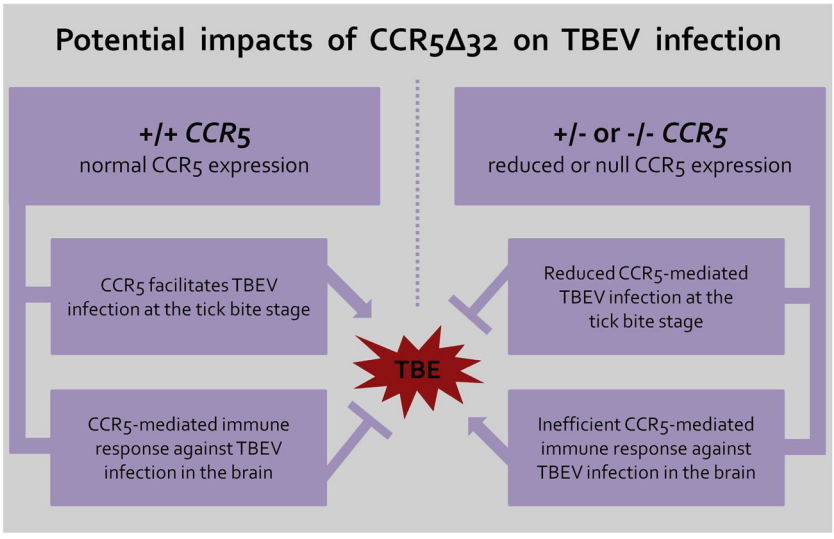

Fig. 4. Potential impacts of CCR5Δ32 on TBEV infection. Functional CCR5 is important in the immune response against TBEV infection and its effects in the brain, but CCR5 can facilitate the infection during the early stages of the tick bite. CCR5Δ32 disrupts CCR5 expression, potentially favoring TBEV pathogenesis and TBE progression. On the other hand, CCR5Δ32-derived low or null expression probably protects against TBEV-infection at the biting stage. See text for references.

some of the studies mentioned here evaluated CCR5Δ32 (Barkhash et al., 2013; Czupryna et al., 2017; Engman et al., 2012; Grygorczuk et al., 2015, 2016) and fail to describe a direct influence of this genetic variant on TBE susceptibility or clinical parameters. However, the studies of Czupryna et al. (2017), Engman et al. (2012), Grygorczuk et al. (2015), and Grygorczuk et al. (2016) were performed on small samples, which may have influenced their results. Considering the findings, investigations on the frequency of CCR5Δ32 in different non-vaccinated populations from geographical locations where the TBEV circulates are needed. When evaluated together, the data obtained from these studies will give us a better understanding of the possible influence of CCR5Δ32 on susceptibility to TBEV infection and disease progression. Based on the studies mentioned here, we have schematized in Fig. 4 the potential impacts of CCR5Δ32 on TBEV infection.

The analysis of gene interaction networks points to *CCR5* as a player in the TBEV-derived host immune response (Ignatieva et al., 2017). Further functional studies allied with bioinformatics analyses focusing on the role of CCR5 in the immune response against TBEV infection are of great relevance. This approach is fundamental, especially taking into account the increasing amount of studies assessing the potential clinical implications of CCR5 blockade in different contexts (Vangelista and Vento, 2018). Actually, a better comprehension of the CCR5 roles in TBEV infection may be quite relevant for the development of CCR5-based TBE therapies since we are facing a potential challenging crossroad: CCR5 agonists/modulators could be useful for treating TBE, but simultaneously we should be aware of the unknown impacts of CCR5 blockade on TBEV infection susceptibility. Such hypotheses should be evaluated.

4.2. *CCR5 ligands*

It is important to remember that CCL3, CCL4, and CCL5 are the main CCR5 agonists, regulating the action of different $CCR5^+$ leukocytes (Samson et al., 1996a; Jones et al., 2011). Oliveira et al. (2008) demonstrated that *in vitro* chemotaxis of immature dendritic cells (DCs) is inhibited by tick saliva. Specifically, such inhibition is mediated by down-regulation of CCR5 expression on the cell surface. In line with these findings, the study also has shown that tick saliva disrupts CCL3

chemotactic function (Oliveira et al., 2008). Evasin-1 is a candidate molecule in this pathway, since it binds to CCL3, besides binding to CCL4 and CCL18/PARC (Frauenschuh et al., 2007). A study by Carvalho-Costa et al. (2015) also showed that tick saliva decreased the CCR5 expression in DCs and reduced the migration of these cells, reinforcing and complementing the findings reported by Oliveira et al. (2008). Carvalho-Costa et al. (2015) also showed that tick saliva has a suppressive action on DCs differentiation. Prostaglandin E_2 must be responsible at least in part by the results found in the study (Carvalho-Costa et al., 2015).

Prostaglandin E_2 from tick saliva decreases the CCL5 levels released by macrophages (Poole et al., 2013). This tick saliva-induced CCL5 reduction potentially prevents a pro-inflammatory response at the site of the tick bite (Poole et al., 2013). Evasin-4 also binds to CCL5 (Déruaz et al., 2008; Bonvin et al., 2014), inhibiting its function (Déruaz et al., 2013). Nevertheless, the inhibitory activity of evasin-4 is not exclusive upon CCL5. Other chemokines are also inhibited by evasin-4 (Déruaz et al., 2013), and some data suggests that other evasins also bind to CCL5 (Singh et al., 2017).

Besides viruses, ticks are hosts of different microorganisms (Asman et al., 2015; Beltrame et al., 2018; Johnson et al., 2018; Karasartova et al., 2018). Therefore, the immunomodulatory effects of tick saliva can be influenced by the presence of these microorganisms. The incubation of THP-1 cells (a human monocytic cell line) with tick saliva in the presence of *Borrelia burgdorferi* inhibited the production of CCL3, CCL4, and CCL5, compared to THP-1 cells exposed only to *B. burgdorferi*, as evaluated by a cytokine bead array (Scholl et al., 2016). Although these results suggest that the observed immunomodulatory effect was derived from tick saliva components and not evoked by *B. burgdorferi*, such data draw attention to the need of considering that the immunomodulatory effects of tick saliva can occur concomitantly with the host inflammatory response against different tick-derived microorganisms, such as bacteria and protozoa.

Increased production of pro-inflammatory chemokines, including CCL5, CCL3, and CCL4, was observed in the brain of mice infected with TBEV (Palus et al., 2013). A similar phenomenon seems to occur in humans since Palus et al. (2015) have observed increased levels of some inflammatory markers in TBE compared to controls; however, the levels of the CCR5 agonists CCL3, CCL4, and CCL5 were not statistically different between the groups (Palus et al., 2015). No difference of CCL3 and CCL4 levels between TBE patients and controls were also reported in another study (Grygorczuk et al., 2016). On the other hand, using a Langat virus-induced TBE rat model, Maffioli et al. (2014) observed higher CCL5 levels in the cerebrospinal fluid of infected rats in comparison to controls, in accordance with data from TBEV-infected humans evaluated in the same study (Maffioli et al., 2014). Other studies also described higher levels of CCL3 (Grygorczuk et al., 2006) and CCL5 (Grygorczuk et al., 2016) in TBE patients compared to controls. In line with these findings, an *in vitro* study performed by Palus et al. (2014) showed that TBEV infection in human astrocytes induced an increased expression of CCL4 and other pro-inflammatory cytokines and chemokines. According to the same study, astrocytes are the potential source of the pro-inflammatory chemokines found in the brain during TBEV infection. Thus, such TBEV-infected astrocytes would mediate the inflammation-related neurodegenerative events found in TBEV infection (Palus et al., 2014).

In an overall perspective, CCL5 should be considered as an important mediator of the inflammatory response related to TBEV infection (Zhang et al., 2016). Up-regulation of *CCL5* expression was observed in an *in vitro* model of tick-borne flavivirus infection (Mlera et al., 2016). In line with this finding, Zhang et al. (2016) have shown that intracerebral TBEV infection induces CCL5 expression in brain tissue of BALB/c mice. Also in the context of TBEV infection using the BALB/c mice model, CCL5 inhibition was linked to extended survival and reduced brain inflammation (Zhang et al., 2016). Furthermore, the CCL5 up-regulation was confirmed using human brain-derived cell lines, and such up-regulation seems to be mediated by the interferon regulatory factor 3 (IRF-3) activation (Zhang et al., 2016). Finally, a recent study suggested the TBEV nonstructural protein NS5 as the viral activation factor of the IRF-3-associated CCL5 up-regulation in the context of TBEV infection (Zheng et al., 2018). Taking together, data from TBEV-infected humans, animal models, and *in vitro* studies reinforce the involvement of CCR5 agonists in the pathogenesis of TBEV infection.

5. Perspectives and emerging topics

5.1. Exosomes and TBEV infection

It is quite evident that tick-borne viruses exploit tick-saliva components and their immune regulation properties to evade the host immune system and to promote infection (Kazimírová et al., 2017). Recently, it has been suggested that TBEV infection from ticks to humans can be facilitated by tick-derived exosomes (Zhou et al., 2018). Exosomes are nanovesicles released in the extracellular medium by different cell types. Such vesicles enable the communication and transport of molecules between cells (Properzi et al., 2013; Ellwanger et al., 2017a; Samanta et al., 2018; de la Torre Gomez et al., 2018). Also, it is likely that host-derived exosomes may affect the TBEV infection of neuronal cells (Zhou et al., 2018). In concordance, it is already known that exosomes and other extracellular vesicles influence human-virus interactions and the course of different viral infections (Chahar et al., 2015; Ellwanger et al., 2018c; Liu et al., 2017; Anderson et al., 2018; Hackenberg and Kotsyfakis, 2018; Martelli et al., 2018; Vora et al., 2018; Wang et al., 2018; Wu et al., 2018; Yao et al., 2018). Interestingly, it is possible that some viruses explore the budding and transport machinery of exosomes to evade the immune system and promote infection (Gould et al., 2003). Taking these points into consideration, the role of exosomes in TBEV infection is an interesting topic that deserves to be explored in detail. Finally, we speculate that the exosomes participate, at least in part, in the regulation of the anti-inflammatory microenvironment induced by ticks during their bloodmeal at the bite site.

5.2. Modulating the immune response and TBEV infection: Evasins and CCR5 blockade

The therapeutic potential of inflammatory modulators based on evasins activity has been addressed in different studies (Déruaz et al., 2008; Bonvin et al., 2016). Indeed, several evasins may be used for therapeutic strategies (Hayward et al., 2017). Interestingly, it is possible that evasins and other tick saliva-derived anti-inflammatory molecules are not (or are scarcely) immunogenic (Déruaz et al., 2008), which makes the use of these molecules for the treatment of inflammatory diseases even more promising.

Also looking at the pharmacological modulation of the immune response, the use of CCR5 blockers stands out in the current literature. The effects of CCR5 blockers may be different on cells of the central nervous system as compared to cells of peripheral organs. Besides, the CCR5 blockers administered in the central nervous system may have different effects from those caused by the systemic CCR5 deficiency due to the CCR5Δ32 allele (Zhang et al., 2016). Currently, there is a growing use of CCR5 blockers to treat HIV infection and in strategies of Pre-exposure prophylaxis (PrEp) to prevent new cases of infection (Luz et al., 2018; Riddell et al., 2018). However, the impacts of population-scale use of CCR5 blockers (due to HIV treatment or prevention) on TBEV infection are unknown. Immunization against TBEV has been suggested before initiating the use of CCR5 blockers in HIV-infected patients (Klein, 2008).

5.3. Current status of TBEV infection: look at pathogen, ticks, human factors, and the environment in an integrated way

Currently, there are phylogenetic data pointing to the existence of at least five TBEV sub-types (Kovalev and Mukhacheva, 2017; Dai et al., 2018). However, the public health importance of the newly discovered Himalayan-TBEV (Dai et al., 2018) and Baikalian-TBEV (Kovalev and Mukhacheva, 2017) is unknown and therefore need to be investigated. In this sense, a better understanding of the biological and pathogenic particularities of each TBEV sub-type will allow the establishment of therapies and vaccines specific for each type of virus. In addition, mapping the circulation of each TBEV sub-type is essential to develop actions focused on pathogen control at a population level.

In essence, TBEV infection is an emerging zoonosis (Mansfield et al., 2009) that represents not only a medical problem but also an ecological challenge. Contradictorily, compared to other infectious diseases, tick-borne viral diseases are considered of little importance on a global epidemiological scale (Kazimírová et al., 2017). The lack of implementation of prevention and control measures is a contributing factor to the spread of tick-borne viral diseases (Kazimírová et al., 2017).

Climate change, alterations in ticks ecology, and social factors (urbanization, international travels, human migration, outdoor activities, among others) are involved in the increase/decrease of TBEV-related problems (Süss et al., 2010; Süss, 2011), but such factors are still poorly understood and subject of debate (Süss, 2011). However, there is no doubt that unbalances between human, animal, and environmental factors are involved in the emergence of infectious diseases (Mwangi et al., 2016; Ellwanger et al., 2017b; Destoumieux-Garzón et al., 2018), including tick-borne diseases (Vayssier-Taussat et al., 2015). Moreover, looking specifically at the human factor, individual genetic features have a pivotal role in the susceptibility and progression of viral diseases (Chapman and Hill, 2012; Ellwanger et al., 2018a). This role is evidenced in this review through different studies involving TBEV infection.

Considering the number of factors that influence different aspects of TBEV ecology and human-TBEV interactions, the risk of TBEV infection should be assessed both at the individual as well as at the population levels (Süss et al., 2010). Understanding the immunological processes that permeate the relationships between ticks, humans, and tick-borne viruses is essential for the development of new control strategies targeting tick-borne infections (Wikel, 2013; Kazimírová et al., 2017). The challenge is to identify what are these factors and how to control them to contain the pathogen and disease emergence. At a population level, tick-borne viral diseases may be faced within the One Health perspective, in which the characteristics of the pathogens are considered together with human, animal, and environmental factors in the application of broad-spectrum disease control strategies (Vayssier-Taussat et al., 2015; de la Fuente, 2018). For such approaches to be effectively implemented, professionals from different areas, such as microbiology, veterinary, epidemiology, human health, and sociology should be involved (Vayssier-Taussat et al., 2015).

The points discussed above in association with characteristics from ticks and humans may represent barriers or bridges that TBEV must overcome to infect humans. For example, viral genetic factors can serve as bridges or barriers, increasing or decreasing viral pathogenesis according to TBEV sub-types (Mansfield et al., 2009; Robertson et al., 2009). Similarly, it is possible that viral factors also influence the TBEV infectivity. In addition, protective or susceptibility human genetic factors (see some examples in Table 1 and Fig. 2) may represent barriers or bridges to TBEV infection. Finally, Fig. 5 puts together all the factors that should be taken into consideration when TBEV infection control is sought at the individual and population levels.

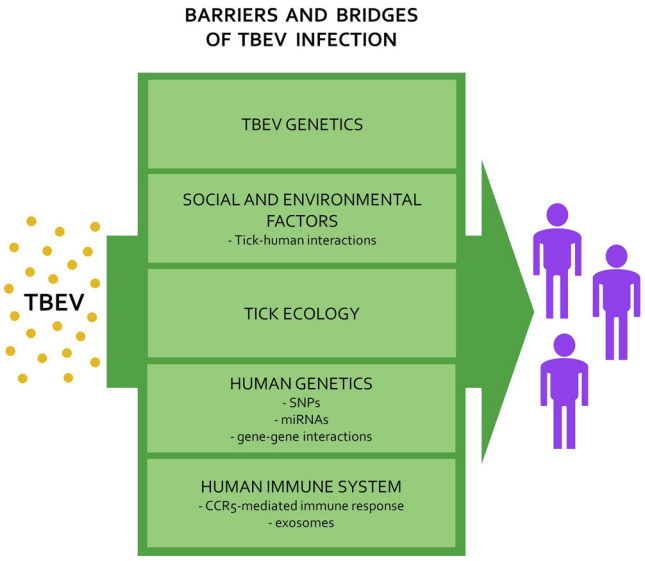

Fig. 5. Barriers and bridges of TBEV infection. Several factors separate TBEV from humans. Some of these factors can be compared to "bridges" that facilitate infection processes in humans (for example, viral genetic factors that increase virulence or outdoor activities that put humans in close contact with ticks). On the other hand, some factors can be considered as "barriers" that make TBEV infection difficult (for example, host protective immunogenetic factors). All these barriers and bridges should be taken into consideration in control and mitigation measures of TBEV infection. The approach that integrates animal, human, and environmental factors for the control and prevention of infectious disease is in line with the One Health perspective. See text for references.

6. Conclusions

TBEV infection is a complex zoonosis. Therefore, prevention of human TBEV infection should be performed through vaccination initiatives especially in populations in endemic areas. In association, actions focused on the One Health perspective can contribute to the control of the tick populations. Looking at human factors, it is clear that the host genetic polymorphisms (as highlighted in Fig. 2) have a critical impact on the susceptibility to TBEV infection and disease progression. In this context, *CCR5* plays a prominent role, both at the gene level as well as at the protein level. The CCR5 is important in the immune response against TBEV infection and its effects in the brain, although it may facilitate the infection in the early stages of the tick bite. Furthermore, it seems that the CCR5Δ32-associated disruption of the CCR5 function favors TBEV pathogenesis and TBE progression. On the other hand, CCR5 down-regulation due to CCR5Δ32 would protect against TBEV infection. Complementing these findings, different types of evidence have shown that host-derived CCR5 agonists CCL3, CCL4, and CCL5 have a significant influence on the clinical outcome of TBEV infection. Studies addressing tick-human interactions point to the potential pharmacological use of immunomodulatory proteins present in the tick saliva. Finally, the use of CCR5 blockers to treat HIV infection can interfere with TBEV infection in HIV/TBEV co-infected individuals.

Conflict of interest

No conflict of interest to declare.

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CAPÍTULO XII

CCR5Δ32 in HCV infection, HCV/HIV co-infection, and HCV-related diseases

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Short communication

CCR5Δ32 in HCV infection, HCV/HIV co-infection, and HCV-related diseases

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ABSTRACT

Although a potential involvement of the CCR5Δ32 variant has already been suggested in relation to susceptibility to hepatitis C virus (HCV) infection, data from the literature is still quite controversial. Thus, our study evaluated the influence of the CCR5Δ32 allele variant in HCV infection, HCV/HIV co-infection, and HCV-related diseases in individuals from southern Brazil. A total of 1352 individuals were included in this study, divided into the following groups: Control ($n = 274$); HCV+ ($n = 674$); HIV+ ($n = 300$); HCV+/HIV+ ($n = 104$). Individuals from the HCV+ group were further stratified according to clinical/histological criteria, as HCV+/control ($n = 124$); HCV+/fibrosis ($n = 268$); HCV+/cirrhosis ($n = 190$); HCV+/hepatocarcinoma ($n = 92$). Considering all individuals included in this study, the following genotype frequencies were observed: wild-type homozygous (wt/wt), 88.76%; heterozygous (wt/Δ32), 10.72%; variant homozygous (Δ32/Δ32), 0.52%. Genotypic frequencies were very similar between the groups. Of note, the frequency of the Δ32 homozygous was quite similar comparing control uninfected against the HCV+ individuals ($p > 0.999$). The overall Δ32 allele frequency was estimated at 5.88%. Considering the number of Δ32 allele carriers and non-carriers, no statistically significant differences ($p > 0.05$) between the groups were observed, suggesting that the CCR5Δ32 variant does not influence the susceptibility to HCV infection, HCV/HIV co-infection, or HCV-related diseases in individuals from southern Brazil.

1. Introduction

The C-C chemokine receptor type 5 (CCR5) molecule is a G-protein coupled receptor expressed in different cell types, including T cells and macrophages. Among other cellular and physiological functions, CCR5 modulates leukocyte trafficking and immune responses (Alkhatib, 2009). The CCR5Δ32 variant is characterized by a 32 base-pair (bp) deletion in the coding region of the *CCR5* gene, resulting in a truncated protein, which is not expressed on the cell surface of homozygous individuals (Alkhatib, 2009; Samson et al., 1996). Among Euro-descendant populations, the Δ32 allele frequency varies around 10% (Galvani and Novembre, 2005). In Brazil, it was estimated at 4% (Silva-Carvalho et al., 2016).

The infection by hepatitis C virus (HCV) is strongly influenced by host genetic factors (Ellwanger et al., 2017). Of note, it was suggested that changes in the CCR5 expression due to CCR5Δ32 could impact the susceptibility to HCV infection and progression of HCV-related diseases, once CCR5 signalling is important for the immune response against HCV (Ahlenstiel et al., 2004; Coenen and Nattermann, 2010). Nevertheless, data concerning the influence of CCR5 on hepatitis C virus (HCV) infection susceptibility is still controversial. In this context, our study evaluated the influence of CCR5Δ32 in HCV infection, HCV/HIV co-infection, and HCV-related diseases in a large sample of Brazilian individuals.

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2. Methods

We genotyped 1352 DNA samples of individuals from the Rio Grande do Sul State (the southernmost state of Brazil). In addition to uninfected controls, HCV+, HIV+, and HCV/HIV co-infected individuals were selected for this study (approved by the Ethics Committees of UFRGS, ULBRA, and Hospital de Clínicas de Porto Alegre, Brazil). All participants signed an informed consent according to the Declaration of Helsinki.

CCR5Δ32 (rs333) was genotyped according to Chies and Hutz (2003), with minor adaptations. For the amplifications, the following primers were used: CCR5a 5′-GGTCTTCATTACACCTGC-3′; CCR5b 5′-AGGATTCCCGAGTAGCAGATG-3′. The PCR mix reaction (total volume of 25 μl) was composed of: 1 μl of DNA (0.2–0.5 μg), 2.5 μl of 10× buffer with 30 mM of $MgCl_2$, 0.2 μl Taq DNA polymerase (5 U/μl), 10 mM dNTP, and 10 pmol of each primer. PCR reactions underwent one initial denaturation cycle at 94 °C for 7 min, followed by 40 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and then a final extension cycle at 72 °C for 7 min. Genotyping of PCR products was performed on a 3% agarose gel with ethidium bromide under UV light. The pattern of bands visualized is: wild-type homozygous (wt/wt) – a single 137 bp band; heterozygous (wt/Δ32) – two bands of 137 and 105 bp respectively; variant homozygous (Δ32/Δ32) – a single 105 bp band.

Due to the low number of CCR5Δ32 homozygous, individuals were classified into Δ32 allele carriers and Δ32 allele non-carriers, and the number of carriers and non-carriers were used for comparisons between groups. Initially, to evaluate the potential involvement of the Δ32 allele on the susceptibility to HCV infection or HCV/HIV co-infection, the number of Δ32 allele carriers and non-carriers was compared between groups as follows: HCV+ group *vs.* Control group; HIV+ group *vs.* Control group; HCV+ group *vs.* HIV+ group; HCV+/HIV+ group *vs.* Control group; HCV+/HIV+ group *vs.* HCV+ group; HCV+/HIV+ group *vs.* HIV+ group. Then, to evaluate the potential involvement of the Δ32 allele in HCV-related diseases, individuals from the HCV+ group were stratified according to clinical/histological criteria and compared as follows: HCV+/fibrosis group *vs.* Control group; HCV+/fibrosis group *vs.* HCV+/control group; HCV+/cirrhosis group *vs.* Control group; HCV+/cirrhosis group *vs.* HCV+/control group; HCV+/HCC group *vs.* Control group; HCV+/HCC group *vs.* HCV+/control group. For clarification, “Control group” refers to control/unfected individuals, “HCV+/control group” refers to HCV+ individuals without HCV-related diseases, and “HCC” refers to hepatocarcinoma.

The Hardy-Weinberg equilibrium was verified in all groups using the chi-square test. Using WINPEPI 11.65, the number of carriers and non-carriers of the Δ32 allele was compared between the groups through the Pearson's chi-square test with Yates's correction. Two-tailed Fisher's test was used when appropriate. p-Value < 0.05 was set as statistically significant.

3. Results

Table 1 shows sociodemographic data and the number of individuals included in each group. Table 2 shows HCV+ individuals data after stratification. The number/frequency of Δ32 allele carriers and non-carriers and the genotype frequencies observed in each group are shown in Table 3.

Considering all individuals included in this study (controls and infected individuals), 88.76% of them showed a wild-type homozygous genotype (wt/wt), 10.72% a heterozygous genotype (wt/Δ32), and 0.52% had a variant homozygous genotype (Δ32/Δ32). A very similar distribution of genotypes was found among all groups. According to our results, and considering the absence of significantly differences regarding the frequency of the variant homozygous genotype (Δ32/Δ32) between the HCV+ and the control groups ($p > 0.999$), this genotype is not related to susceptibility or protection against HCV infection.

Table 1
Demographic data of the individuals included in this study.

Demographic data	Control group $n = 274$	HCV+ group $n = 674$	HIV+ group $n = 300$	HCV+/HIV+ group $n = 104$
Age, mean ± SD	44.4 ± 8.3	55.8 ± 10.3	42.4 ± 9.6	44.7 ± 8.9
Sex, *n* (%)				
Female	86 (31.4)	344 (51.0)	142 (47.3)	38 (36.5)
Male	188 (68.6)	330 (49.0)	158 (52.7)	66 (63.5)
Ethnicity[a], *n* (%)				
Caucasians	223 (81.4)	472 (70.0)	191 (63.7)	43 (41.3)
Non-Caucausians	51 (18.6)	202 (30.0)	109 (36.3)	61 (58.7)

n, sample number. SD, standard deviation.
[a] Based on skin-color and self-declaration.

Table 2
Demographic and clinical data of the individuals from HCV+ group stratified according to clinical/histological criteria.

Demographic and clinical data	Groups resulting from the stratification of the HCV+ group, $n = 674$			
	HCV+/control group $n = 124$	HCV+/fibrosis group $n = 268$	HCV+/cirrhosis group $n = 190$	HCV+/HCC group $n = 92$
Age, mean ± SD	53.1 ± 11.1	53.1 ± 10.5	58.9 ± 8.5	61.2 ± 8.3
Sex, *n* (%)				
Female	52 (41.9)	153 (57.1)	101 (53.2)	38 (41.3)
Male	72 (58.1)	115 (42.9)	89 (46.8)	54 (58.7)
Ethnicity[a], *n* (%)				
Caucasians	84 (67.7)	181 (67.5)	138 (72.6)	69 (75.0)
Non-Caucausians	40 (32.3)	87 (32.5)	52 (27.4)	23 (25.0)
BMI, mean ± SD	26.2 ± 4.3	26.7 ± 4.9	27.8 ± 5.2	26.4 ± 4.2
Declared smoker[b], *n* (%)	11 (8.9)	47 (17.5)	88 (46.3)	62 (67.4)
Declared alcohol drinker[b], *n* (%)	13 (10.5)	14 (5.2)	19 (10.0)	23 (25.0)
HCV infection *via* blood transfusion, *n* (%)	26 (21.0)	56 (20.9)	69 (36.3)	33 (35.9)

n, sample number. SD, standard deviation. BMI, body mass index.
[a] Based on skin-color and self-declaration.
[b] Currently or in the past.

Importantly, all groups were in Hardy-Weinberg equilibrium ($p > 0.05$ in all groups).

Considering controls and infected individuals, we observed an overall Δ32 allele frequency of 5.88%. In relation to Δ32 allele carriers and non-carriers, the comparisons performed between the groups did not indicate an influence of CCR5Δ32 on the susceptibility to HCV infection, HCV/HIV co-infection, or HCV-related diseases (p-values > 0.05 resulted from all comparisons performed). These results remained unchanged when the individuals were stratified by ethnicity (data not shown). Of note, 184 individuals (~27%, Table 2) from the HCV+ group ($n = 674$) were infected by HCV through blood transfusions. Not even this important risk factor seems to have influenced our results.

4. Discussion

In a study performed in Germany, Woitas et al. (2002) reported a high number of CCR5Δ32 homozygous individuals in a group of HCV+ patients in comparison to controls, which was interpreted as indicating this variant as a susceptibility factor to HCV infection. In this same study, HCV/HIV co-infected individuals also showed a higher frequency of the Δ32 allele compared to HIV mono-infected patients (Woitas et al., 2002). Although it is true that this work drew attention to the potential roles of CCR5 and CCR5Δ32 on the susceptibility to HCV infection and

Table 3
Distribution of the CCR5Δ32 profiles.

CCR5Δ32 profile	Control group (n = 274)	HCV+ group (n = 674)	HIV+ group (n = 300)	HCV+/HIV+ group (n = 104)	Groups resulting from the stratification of the HCV+ group, n = 674			
					HCV+/control group (n = 124)	HCV+/fibrosis group (n = 268)	HCV+/cirrhosis group (n = 190)	HCV+/HCC group (n = 92)
wt/wt, n (%)	240 (87.6)	601 (89.2)	265 (88.3)	94 (90.4)	106 (85.5)	246 (91.8)	171 (90.0)	78 (84.8)
wt/Δ32, n (%)	32 (11.7)	68 (10.1)	35 (11.7)	10 (9.6)	16 (12.9)	21 (7.8)	18 (9.5)	13 (14.1)
Δ32/Δ32, n (%)	2 (0.7)	5 (0.7)	–	–	2 (1.6)	1 (0.4)	1 (0.5)	1 (1.1)
Δ32 carrier, n (%)	34 (12.4)	73 (10.8)	35 (11.7)	10 (9.6)	18 (14.5)	22 (8.2)	19 (10.0)	14 (15.2)
Δ32 non-carrier, n (%)	240 (87.6)	601 (89.2)	265 (88.3)	94 (90.4)	106 (85.5)	246 (91.8)	171 (90.0)	78 (84.8)
Δ32 allele frequency	0.066	0.058	0.058	0.048	0.081	0.043	0.053	0.082

n, sample number. wt/wt, wild-type homozygous genotype. wt/Δ32, heterozygous genotype. Δ32/Δ32, variant homozygous genotype. HCC, hepatocarcinoma.
Δ32 allele frequency = (2 × n individuals Δ32/Δ32) + (n individuals wt/Δ32) / (2 × n total individuals).

HCV-related diseases, due to potential confounding situations (*i.e.* high number of hemophiliac patients included, among others), it has generated a great debate (Klein, 2003; Mangia et al., 2003; Poljak et al., 2003; Zhang et al., 2003). In line with the criticisms made regarding this study conclusions, our results do not support an influence of the CCR5Δ32 allele on the susceptibility to HCV infection or HCV/HIV co-infection in the Brazilian population. Our findings are in agreement with several other studies evaluating different populations (Glas et al., 2003; Goyal et al., 2006; Promrat et al., 2003; Thoelen et al., 2005; Wald et al., 2004; Wasmuth et al., 2004). Moreover, previous studies of our group indicate that the ethnic background impacts the susceptibility to infectious and autoimmune diseases in the Brazilian population (Glesse et al., 2017; Schauren et al., 2013; Valverde-Villegas et al., 2017). However, in the present study, ethnic origin (based on skin-color and self-declaration) did not influence the results. The lack of a direct influence of CCR5Δ32 on the susceptibility to HCV infection is quite plausible since HCV does not use CCR5 as a cellular co-receptor to infect cells (Lindenbach and Rice, 2013; Woitas et al., 2002). Possible modifications in the susceptibility to HCV infection related to CCR5Δ32 would be associated with disturbance of the immune response induced by the low expression of the CCR5, instead of direct interactions of HCV and the CCR5 molecule.

Some data also suggested that CCR5Δ32 could influence the progression of HCV-related liver diseases (Goulding et al., 2005; Hellier et al., 2003; Wald et al., 2004). However, after stratifying HCV+ individuals according to clinical/histological criteria, no association of CCR5Δ32 with these HCV-related diseases was observed. Similarly, several studies evaluating distinct populations did not show evidence of a significant influence of CCR5Δ32 on the progression of HCV-related diseases (Goyal et al., 2006; Mangia et al., 2003; Morard et al., 2014; Ruiz-Ferrer et al., 2004; Wasmuth et al., 2004). In conclusion, in individuals from southern Brazil, CCR5Δ32 did not influence the susceptibility to HCV infection, HCV/HIV co-infection, or HCV-related liver disease.

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CAPÍTULO XIII

CCR5Δ32 in HBV infection and HIV/HBV coinfection

Este capítulo apresenta o seguinte artigo de dados a ser submetido ao periódico *Infection, Genetics and Evolution*:

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CCR5Δ32 in HBV infection and HBV/HIV co-infection

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Abstract

CCR5 is a chemokine receptor that mediates the action of inflammatory cells, besides acting as an HIV co-receptor. CCR5Δ32 is a 32 base pair deletion in the coding region of the *CCR5* gene. CCR5Δ32 in homozygosis results in the lack of CCR5expression on cell surface, promoting protection against HIV infection. Heterozygous individuals for CCR5Δ32 have a reduced expression of CCR5. Recent evidence demonstrates that CCR5 and CCR5Δ32 are involved in the pathogenesis of other viral infections besides HIV infection. In this context, the role of CCR5 and CCR5Δ32 in HBV infection is not clear and conflicting/contradictory results have been described. Thus, the objective of this study was to investigate the role of CCR5Δ32 in HBV infection and HBV/HIV co-infection in a population from Southern Brazil. We genotyped 783 individuals divided into: Control group (*n*=334), HBV+ group (*n*=335), and HBV+/HIV+ group (*n*=144). We also included in this study an HIV+ group to complement the analyzes (*n*=300, obtained from a previous study of our group). The Δ32 allelic frequencies found in the Control group, HBV+ group, and HBV+/HIV+ group were 7.5%, 9.0%, and 3.1%, respectively. The individuals were classified in Δ32 allele carriers and Δ32 allele non-carriers. Then, the groups were compared using binary logistic regression adjusted for ethnicity. We did not observe a significant effect of CCR5Δ32 on the susceptibility or protection against HBV infection in individuals from Southern Brazil ($p>0.05$). The impact of CCR5Δ32 on HBV/HIV co-infection was inconclusive. Finally, this study contributes to elucidate the role of CCR5 in HBV infection, bringing evidence for the lack of effect of CCR5Δ32 on susceptibility to infection. We encourage the development of studies evaluating the role of CCR5Δ32 in the pathogenesis of HBV infection. New studies addressing CCR5Δ32 on HBV/HIV co-infection should also be performed.

Keywords: CCR5; CCR5Δ32; co-infection; immunogenetics; genetic susceptibility; HBV.

1. Introduction

Hepatitis B virus (HBV) is an enveloped DNA virus of the *Hepadnaviridae* family (Liaw and Chu, 2009; Li, 2015). HBV has tropism for hepatocytes and uses the sodium taurocholate cotransporting polypeptide (NTCP) as a receptor for cell entry (Yan et al., 2012; Li, 2015). There are three basic forms of HBV infection: acute, chronic, and occult (Li, 2015). Most individuals infected with HBV eliminate the virus after acute infection, but the disease can become chronic in a small number of patients (~5%), leading to different health problems (Liaw and Chu, 2009; Moudi et al., 2016), including hepatic decompensation, cirrhosis, and hepatocellular carcinoma (Liaw and Chu, 2009). There is no virological cure for HBV infection, but the chronic disease can be treated, and the suppression of viral replication can be achieved (Li, 2015; Polaris Observatory Collaborators, 2018).

Around 290 million people are infected with HBV worldwide, which makes this viral infection a global public health problem (Polaris Observatory Collaborators, 2018). This scenario is worsened by HBV/HIV co-infection. Co-infected patients have more severe hepatic problems compared to HBV monoinfected individuals, once HIV can increase HBV pathogenesis. Also, co-infected patients have an increased risk of death (Kourtis et al., 2012; Singh et al., 2017).

Viral, environmental, and host factors determinate the susceptibility and outcome of viral diseases, including HBV infection. Looking at host genetics, several polymorphisms in microRNA genes have important effects on HBV infection (Ellwanger et al., 2018a). Moreover, polymorphisms of genes *IL-6*, *IL-10*, *IL-28B*, *TGF-β*, *INF-γ*, *TNF-α*, *MIF*, *HLA*, *VDR*, and *CCR5* also have some impact on HBV infection (Moudi et al., 2016). Of note, polymorphisms in genes of the immune system can modify the immune response against HBV infection, affecting the disease outcome (Moudi et al., 2016).

The *CCR5* gene encodes the CCR5 protein, which is a chemokine receptor that coordinates the action of different leucocytes during inflammation. CCR5 also participates in a variety of physiological and pathological processes, including HIV infection (Brelot and Chakrabarti, 2018). CCR5Δ32 (rs333) is a polymorphism that in homozygosis causes the lack of CCR5 expression on the cell membrane. In heterozygosis, CCR5 expression is found in reduced levels (Wu et al. 1997; Venkatesan et al. 2002). Once HIV uses CCR5 as co-receptor, CCR5Δ32 allele confers protection against HIV infection: almost total protection when found in homozygosis and a delay of ~2 years in HIV disease progression when present in heterozygosis (Brelot and Chakrabarti, 2018). Beyond HIV infection, it is now known that CCR5Δ32 also affects the susceptibility and outcome of other viral infections (Glass et al., 2006; Mickienė et al., 2014; Rustemoglu et al., 2017; Lassner et al., 2018) and non-viral diseases (Schauren et al., 2013; Toson et al., 2017; Troncoso et al., 2018).

It is accepted that CCR5 and its ligands are involved in the immune response against HBV infection (Sanchooli et al., 2014; Li et al., 2016) and influence the outcome of other liver diseases

(Ajuebor et al., 2006), although the molecular mechanisms involved in these phenomenon are poorly understood. Similarly, the impact of CCR5Δ32 on HBV infection is controversial (Sanchooli et al., 2014; Drozd-Dąbrowska et al., 2017). Thus, the objective of this study was to evaluate the role of CCR5Δ32 in HBV infection and HBV/HIV co-infection in a population from Southern Brazil.

2. Methods

DNA samples, groups, and ethical aspects

Genomic DNA samples of individuals from Southern Brazil (Rio Grande do Sul State) were obtained. This study genotyped 783 individuals, belonging to the following groups: Control group (*n*=334), HBV+ group (*n*=335), HBV+/HIV+ group (*n*=144). We also included in this study 300 HIV-infected individuals (HIV+ group) to complement the analyzes. This group was obtained from a previous study developed in our laboratory (Ellwanger et al., 2018b). In total, 1,113 subjects were analyzed in this study (Table 1).

The participants of this study were recruited at reference centers for the treatment of infectious diseases in three cities of Rio Grande do Sul State (Bento Gonçalves, Canoas, and Caxias do Sul). Demographic and clinical data of HBV-infected individuals included in this study were described previously (Pereira et al., 2017; de Paoli et al., 2018). Subjects were classified as "Caucasians" and "Non-Caucasians" based on skin-color and self-declaration (Table 1). The diagnosis of HBV infection and HBV/HIV co-infection was performed through serological tests. Individuals who composed the control group were recruited from blood banks or from convenience sampling. Individuals in the control group had negative serology for HBV and HIV. This study is part of a research project approved by the Ethics Committee of the Lutheran University of Brazil (ULBRA, Rio Grande do Sul, Brazil). All participants signed a consent form developed following Resolution No. 466 from Ministry of Health (Brasil, 2012).

CCR5Δ32 genotyping

CCR5Δ32 (rs333) was genotyped according to Chies and Hutz (2003), with minor adaptations described in Ellwanger et al. (2018b). In brief, the following primers were used for DNA amplification: CCR5a 5′-GGTCTTCATTACACCTGC-3′; CCR5b 5′-AGGATTCCCGAGTAGCAGATG-3′. PCR mix reaction was composed of: 1 μl of DNA (0.2–0.5 μg), 2.5 μl of 10× buffer with 30 mM of $MgCl_2$, 0.2 μl Taq DNA polymerase (5 U/μl), 10 mM dNTP, and 10 pmol of each primer (total volume: 25 μl each reaction). Reactions were performed as follows: one initial denaturation cycle at 94 °C for 7 min, followed by 40 cycles at 94 °C for 1 min, 55 °C for 1

min, 72 °C for 1 min, and then a final extension cycle (at 72 °C for 7 min). Finally, PCR products were genotyped using 3% agarose gel with ethidium bromide under UV light. The pattern of bands visualized is: a single 137 bp band (wild-type homozygous genotype, wt/wt); two bands, including one 137 bp band and a second 105 bp band (heterozygous genotype, wt/Δ32); a single 105 bp band (homozygous variant genotype, Δ32/Δ32).

Statistical analysis

We assessed the Hardy-Weinberg equilibrium in all groups using the chi-square test, with the aid of the web-tool available at OEGE - Online Encyclopedia for Genetic Epidemiology studies (http://www.oege.org/software/hwe-mr-calc.shtml) (Rodriguez et al., 2009). The individuals were classified into Δ32 allele carriers and Δ32 allele non-carriers due to the low number of CCR5Δ32 homozygous, as performed previously (Toson et al., 2017; Ellwanger et al., 2018b). The groups were compared using binary logistic regression adjusted for ethnicity using *Statistical Package for the Social Sciences* (SPSS, IBM, v.18). In multivariate analyses the odds ratio were adjusted by ethnicity because previous studies have shown that CCR5Δ32 is more frequent in Caucasians (Martinson et al., 1997; Novembre et al., 2005; Solloch et al., 2017). The *p*-value <0.05 was set as statistically significant.

3. Results

Table 2 shows the allelic and genotypic frequencies of CCR5Δ32 observed in the groups. The frequency of Δ32 allele found in the controls and HBV+ group were 7.5% and 9.0%, respectively. The allelic frequencies observed in HBV+/HIV+ group and HIV+ group were 3.1% and 5.8%, respectively. All groups showed genotypic and allelic frequencies according to the Hardy-Weinberg equilibrium ($p>0.05$ in all groups).

In the comparisons among groups (Table 3), the potential effect of CCR5Δ32 on susceptibility to HBV infection and HBV/HIV co-infection was adjusted for ethnicity in all logistic regression models. First, all HBV-infected individuals were grouped into one group (considering HBV-monoinfected plus HBV/HIV co-infected individuals) and compared to the Control group. Subsequently, only the HBV-monoinfected individuals (HBV+ group) were compared to the Control group. No statistically significant differences between the groups were observed ($p>0.05$, Table 3). This result indicates that CCR5Δ32 did not affect the susceptibility to HBV infection in the population analyzed in this study.

As mentioned above, the HBV/HIV co-infected group showed the lowest Δ32 allele frequency among the groups (3.1%). Then, we compared the HBV/HIV co-infected group to the Control group,

HBV+ group, and HIV+ group. All comparisons resulted in statistically significant differences ($p<0.05$) between the groups (Table 3). Our results suggest that the Δ32 allele is associated with protection against HBV/HIV co-infection. However, this effect is probably due to the low frequency of CCR5Δ32 allele observed in HIV+ monoinfected and HBV/HIV co-infected individuals (Table 2). CCR5Δ32 allele can provide partial protection against HIV infection (Hoffman et al., 1997; Marmor et al., 2001; Philpott et al., 2003; Trecarichi et al., 2006), which may result in a lower frequency of CCR5Δ32 among HIV-infected individuals or, in this case, in HBV+/HIV+ group.

4. Discussion

The frequency of the Δ32 allele observed in control individuals (7.5%) was high when compared to Δ32 allele frequency observed in the Brazilian population as a whole, estimated at 4.0% (Silva-Carvalho et al., 2016). However, the allelic frequency was similar to that found in other studies performed with the population of Southern Brazil (Boldt et al., 2009; Schauren et al., 2013). This is an expected result, once the Southern region of Brazil was colonized mainly by European populations, explaining the higher frequency of the Δ32 allele in this region (Pena et al., 2011; Silva-Carvalho et al., 2016).

The CCR5Δ32 heterozygous genotype was associated with higher susceptibility to HBV infection in a study performed in India (Suneetha et al., 2006). On the other hand, CCR5Δ32 was a protective factor against HBV infection in Iranian individuals (Abdolmohammadi et al., 2016). However, our results did not indicate a statistically significant effect of CCR5Δ32 on the susceptibility or protection to HBV infection monoinfection in individuals from Southern Brazil ($p>0.05$). Although our analyses indicated a potential protection of the Δ32 allele against HBV/HIV co-infection, this is probably a biased association due to the presence of HIV+ individuals in the HBV+/HIV+ group, as explained in the results section.

The lack of CCR5 expression due to CCR5Δ32 homozygosity does not prevent HBV infection (Nguyên et al., 1999). Considering that HBV does not use CCR5 as a receptor for cell entry (Yan et al., 2012; Li, 2015), potential modifications in susceptibility to HBV infection would be due to CCR5 absence or haploinsufficiency promoted by CCR5Δ32, which could disrupt the immune response against HBV infection (Khorramdelazad et al., 2013). However, our study does not point in this direction. In agreement with our results, no association between susceptibility to HBV infection and CCR5Δ32 was found in individuals from South-East Iran (Khorramdelazad et al., 2013). Moreover, no association between CCR5Δ32 and chronic HBV infection was found in Moroccan individuals (Rebbani et al., 2014).

A potential effect of CCR5Δ32 can be found on the pathogenesis and outcome of HBV infection (Thio et al., 2007), although the studies that evaluated these aspects showed mixed results.

When assessed alone, the CCR5Δ32 was not associated with markers of HBV disease severity or treatment outcome in the Indian population (Suneetha et al., 2006; Goel et al., 2013). However, in association, the genotypes Wt/Wt (CCR5Δ32), a/a (*VDR*, rs7975232), and T/T (*VDR*, rs731236) were linked to severe HBV-related liver disease in an Indian population (Suneetha et al., 2006). No influence of CCR5Δ32 on HBV infection recovery was found in a small sample of individuals from Iran (Safari et al., 2017). However, Thio et al. (2007) observed an association between CCR5Δ32 and HBV recovery in Caucasian individuals, suggesting that Δ32 allele is a protecting factor against persistent HBV infection. Using the same sample, the effect of CCR5Δ32 and *RANTES* (Regulated on Activation, Normal T cell Expressed and Secreted gene) polymorphisms on HBV recovery was evaluated in a later study (Thio et al., 2008), which described an association between the genotypic model "CCR5Δ32 + *RANTES* -403A" and HBV recovery (Thio et al., 2008).

Finally, some authors assessed the potential influence of CCR5Δ32 in the context of HBV infection in populations from Asia. However, the Δ32 was not detected (Ahn et al., 2006; Li et al., 2011) or no link between the polymorphism and HBV infection was found (Zhang et al., 2018). These are expected results since CCR5Δ32 frequency is generally low in Asian populations (Martinson et al., 1997; Solloch et al., 2017).

Conducting studies that evaluate the effect of a polymorphism on the susceptibility to infections is a challenge. The exposure of different individuals to pathogens is varied and difficult to assess. It is necessary to take into account that this limitation may have influenced the results of our study. Also, the human genetic background, viral characteristics, environmental aspects, and limitations in the methodological approaches are some factors that may explain the variable impacts of CCR5Δ32 on HBV infection observed in studies involving different populations (Sanchooli et al., 2014; Drozd-Dąbrowska et al., 2017).

5. Conclusions

Recently, we have discussed the pros and cons of CCR5 absence in different diseases (Ellwanger et al., 2019). It is clear that the effects of CCR5Δ32 is beyond protection against HIV infection and should be explored in different contexts. Currently, the benefits of CCR5 blockers for the treatment of various diseases are being evidenced (Halama et al., 2016; Moy et al., 2017; Puengel et al., 2017; Coppola et al., 2018). For this additional reason, studies involving CCR5Δ32 may shed light on the potential effects of the CCR5 blockade in different therapeutic strategies. In this study, we added a piece to this puzzle: our results did not indicate a significant effect of CCR5Δ32 on the susceptibility or protection against HBV infection in individuals from Southern Brazil. The impact of CCR5Δ32 on HBV/HIV co-infection was inconclusive and remains an open question to be explored in further studies.

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Table 1. Characteristics of the individuals included in this study.

Characteristic	Control group *n*=334	HBV+ group *n*=335	HBV+/HIV+ group *n*=144	HIV+ group* *n*=300
Age, mean ± SD	47.1 ± 11.9	47.3 ± 12.1	43.5 ± 10.2	42.4 ± 9.6
Sex, *n* (%)				
Female	153 (45.8)	154 (46)	50 (34.7)	142 (47.3)
Male	181 (54.2)	181 (54)	94 (65.3)	158 (52.7)
Ethnicity, *n* (%)**				
Caucasians	287 (86.4)	283 (85.5)	61 (42.7)	191 (63.7)
Non-Caucausians	45 (13.6)	48 (14.5)	82 (57.3)	109 (36.3)

n, sample number. SD, standard deviation. *Obtained from Ellwanger et al. (2017). **Valid percent (excluding missing data).

Table 2. Distribution of CCR5Δ32 allelic and genotypic frequencies among studied groups.

CCR5Δ32 profile	Control group (*n*=334)	HBV+ group (*n*=335)	HBV+/HIV+ group (*n*=144)	HIV+ group (*n*=300)*
wt/wt, *n* (%)	284 (85.0)	278 (83.0)	135 (93.8)	265 (88.3)
wt/Δ32, *n* (%)	50 (15.0)	54 (16.1)	9 (6.2)	35 (11.7)
Δ32/Δ32, *n* (%)	-	3 (0.90)	-	-
Δ32 allele carrier, *n* (%)	50 (15.0)	57 (17.0)	9 (6.2)	35 (11.7)
Δ32 allele non-carrier, *n* (%)	284 (85.0)	278 (83.0)	135 (93.8)	265 (88.3)
Δ32 allele frequency	0.075	0.090	0.031	0.058
wt allele frequency	0.925	0.910	0.969	0.942

n, sample number. wt/wt, wild-type homozygous genotype. wt/Δ32, heterozygous genotype. Δ32/Δ32, variant homozygous genotype. *Obtained from Ellwanger et al. (2017).

Table 3. **Effect of CCR5Δ32 on HBV infection and HBV/HIV co-infection.**

Comparison between groups*	O.R.	C.I. 95%	*p*-value
HBV+ individuals** *versus* Control group	0.96	0.64-1.43	0.828
HBV+ group *versus* Control group	1.15	0.76-1.75	0.500
HBV+/HIV+ group *versus* Control group	0.38	0.17-0.85	**0.018**
HBV+/HIV+ group *versus* HBV+ group	0.35	0.16-0.78	**0.010**
HBV+/HIV+ group *versus* HIV+ group	0.44	0.20-0.96	**0.039**

O.R., odds ratio. C.I., confidence interval. *****Binary logistic regression adjusted for ethnicity (reference categories: Allele = Δ32 non-carrier and Ethnicity = Non-Caucasians). **HBV+ group plus HBV+/HIV+ group. Statistically significant *p*-values are shown in bold.

CAPÍTULO XIV

Discussão, conclusões e perspectivas

DISCUSSÃO

Por muito tempo, acreditou-se que o desenvolvimento socioeconômico e o envelhecimento da população seriam acompanhados por uma importante diminuição nos casos de doenças infecciosas e um aumento nos casos das doenças crônico-degenerativas, a chamada "transição epidemiológica". Porém, esse processo não aconteceu da forma como se esperava. Apesar de alguns avanços terem sido conquistados através de medidas como a vacinação, as doenças infecciosas ainda representam um importante problema de saúde pública no mundo todo (Waldman, 2001; Paz e Bercini, 2009; Pedroso e Rocha, 2009; Grisotti, 2010). Além disso, atualmente é evidente que muitos patógenos são também responsáveis por doenças crônicas (Grisotti, 2010). Um bom exemplo é a infecção pelo HIV que, quando tratada, já é considerada uma doença crônica. Ou seja, as doenças crônico-degenerativas são de fato um importante problema deste século, juntamente com as doenças infecciosas agudas ou crônicas, e não no lugar delas.

A relação entre problemas de ordem planetária e a emergência das doenças infecciosas já está amplamente estabelecida. Em consequência disso, tais doenças serão controladas de forma duradoura e realística apenas quando a relação entre o homem e o ambiente natural for mais harmônica do que é atualmente. Por exemplo, o desenvolvimento de uma vacina pode ser efetivo para controlar uma epidemia causada por uma arbovirose. Porém, a redução dos impactos ambientais que estimulam as mudanças climáticas, o adequado manejo de lixo e a correta urbanização (entre outros fatores) também são necessários para que se possa controlar a proliferação de vetores e reduzir a emergência de novas epidemias, diminuindo a demanda por novas vacinas e medicamentos.

Para que os problemas de ordem planetária sejam resolvidos, deve haver comprometimento de diferentes líderes nacionais e internacionais. O envolvimento dos órgãos governamentais é decisivo para que ações que visam a saúde planetária sejam de fato implementadas, pois elas dependem da integração entre políticas sociais, econômicas e ambientais (Whitmee et al., 2015).

Em nível local, muito pode ser feito pelas comunidades e indivíduos para fortalecer a saúde planetária. Hancock et al. (2017) apresentaram princípios e ações práticas que

podem ser executadas em nível local e global, levando em consideração fatores socioculturais e ambientais e que impactam a saúde planetária. Especificamente, tais princípios e ações estão focados nos dez seguintes pilares: saúde e felicidade, equidade e fortalecimento da economia local, cultura e comunidade, solo e natureza, uso sustentável da água, alimentação local e sustentável, viagem e transporte, produtos e materiais, redução do consumo e desperdício e redução de fontes de energia baseadas em carbono (Hancock et al., 2017). Neste sentido, outro bom exemplo é a cartilha "Biodiversidade faz bem à saúde: um guia prático", lançada em 2017 pela FIOCRUZ trazendo recomendações práticas sobre como a população pode usufruir dos recursos naturais causando o mínimo de impactos ambientais, e assim prevenindo a emergência de doenças infecciosas. A cartilha apresenta recomendações para o manejo e cuidado dos animais, da água, dos alimentos, da casa e quintal, das pessoas e da biodiversidade. Além disso, divulga de forma bastante compreensível informações sobre vetores, zoonones, patógenos transmitidos pela água, solo e alimentos, além de outras recomendações práticas e efetivas na prevenção das doenças infecciosas e preservação da biodiversidade (FIOCRUZ, 2017). Iniciativas como essas transformam conceitos e discussões complexas em recomendações práticas, que podem ser facilmente assimiladas pela população. Esse tipo de iniciativa deve ser incentivado e replicado em diferentes países, comunidades, além de estar presente nos mais variados contextos sociais e ambientais.

As políticas de educação ambiental e preservação da biodiversidade devem ser aplicadas junto com o fornecimento à população de garantias no que se refere à saúde e educação. As estratégias de detecção, vigilância, prevenção e mitigação das doenças infecciosas emergentes e reemergentes dependem de sociedades com um adequado desenvolvimento econômico, que garanta a viabilidade de tais estratégias e supra os indivíduos com renda adequada para ser aplicada em educação de qualidade e cuidados com a saúde (Waldman, 2001; Pedroso e Rocha, 2009). Esse fato ajuda a entender porque em países de baixo desenvolvimento econômico os problemas causados pelas doenças infecciosas emergentes são maiores do que em países desenvolvidos.

O entendimento de que eventos de ordem planetária afetam a saúde humana é clássico e histórico. Porém, o termo Saúde planetária apenas recentemente começou a ser disseminado, principalmente entre a comunidade científica atuante na área das doenças infecciosas. Apenas em 2017 foi criada uma revista científica de grande visibilidade

dedicada a assuntos relacionados à Saúde planetária, a *The Lancet Planetary Health* (https://www.thelancet.com/journals/lanplh/home). Esse cenário demonstra que ainda há um longo caminho a ser percorrido para que diferentes ações voltadas à saúde do planeta sejam alinhadas e colocados em prática (Horton e Lo, 2015). Entretanto, os ecossistemas já foram submetidos a uma carga muito grande de agressões. As mudanças climáticas em decorrência do excesso de emissão de CO_2 pelas populações humanas constituem um importante exemplo. Dessa forma, é necessário que as discussões sobre saúde planetária sejam constantes para que então se traduzam em ações práticas, seja através da população, mediada pelos seus governos ou, como seria o ideal, através de ambos.

No que se refere às ações focadas na promoção da saúde global, deve-se levar em consideração que as grandes instituições comprometidas com tais medidas estão sediadas majoritariamente em países desenvolvidos, longe dos focos dos principais problemas que afetam a saúde globalmente, como é o caso da Organização Mundial da Saúde, sediada na Suíça, e do CDC, sediado nos EUA (Beaglehole e Bonita, 2010). Dessa forma, a comunidade científica e as instituições governamentais brasileiras devem assumir uma posição de vanguarda nos campos tanto da saúde planetária quanto da saúde global, visto que o Brasil é um dos países mais biodiversos do mundo e possui capacidade técnica e recursos para o enfrentamento das doenças infecciosas emergentes, reemergentes e negligenciadas. Através do fornecimento de estímulos e recursos por parte do setor público, os profissionais comprometidos com o enfrentamento de tais doenças poderão atuar de forma adequada. Tais estímulos devem ser concretizados em verbas para que os profissionais adquiram formação acadêmica e técnica na área das doenças infecciosas e vigilância epidemiológica, além do fornecimento de condições físicas adequadas para que estes profissionais trabalhem de forma digna em termos trabalhistas e segura em termos de biossegurança. Neste processo, a sociedade civil tem papel essencial, devendo exigir que esses recursos sejam adequadamente fornecidos aos órgãos de saúde pública, pesquisa e vigilância epidemiológica (Luna, 2002). Obviamente, há uma série de problemas envolvendo formação profissional, infraestrutura e destinação de recursos para saúde pública e pesquisa que precisam ser sanados.

No ano 2000, o Ministério da Saúde implementou no Brasil o Sistema de Vigilância Ambiental em Saúde (atualmente “Vigilância em Saúde Ambiental”), que se configura como um conjunto de ações focadas na detecção de modificações ambientais que possam

afetar a saúde da população (Pignatti, 2004), sendo coordenado pelo Ministério da Saúde do Brasil. Programas como esses precisam ser ampliados, fortalecidos e conectados com as universidades, os institutos de pesquisa e a indústria, para que os avanços nas estratégias de prevenção e controle das doenças estejam alinhados com os princípios das saúdes planetária e global. O desenvolvimento de vacinas ou medicamentos antivirais deve estar conectado com as políticas públicas de controle do desmatamento, por exemplo.

Conforme Piggnati (2004), a maioria dos estudos epidemiológicos realizados no Brasil está focada na identificação de fatores de risco das doenças. Porém, as questões sociais, ambientais e políticas que interferem em tais fatores geralmente não recebem a devida atenção, apesar de algumas inciativas interessantes terem sido implementadas no Brasil, como o já mencionado sistema de Vigilância em Saúde Ambiental. Ações que parecem desconectadas em um primeiro momento, quando colocadas em prática de forma conjunta, tornam-se estratégias robustas na prevenção e no controle das doenças infecciosas no Brasil e no mundo. Ou seja, os estudos epidemiológicos que investigam doenças emergentes e reemergentes devem ser associados com a investigação ambiental, social e cultural, fatores estes que permeiam as causas das doenças infecciosas, principalmente em países como o Brasil, onde as causas e emergência das doenças infecciosas estão muitas vezes associadas com questões sociais e ambientais. Essa estratégia facilitará a formulação de planos de resposta à emergência epidemiológica de forma realística e adequada ao cenário onde ela se desenvolve. Por exemplo, um importante surto de doença priônica (Doença de Creutzfeldt-Jakob) foi detectado entre nativos da Nova Guiné na década de 1950. Na época, não se conhecia o agente causador da doença, nem mesmos suas bases patológicas eram entendidas. Foi com o auxílio da investigação antropológica que a natureza contagiosa da doença priônica foi reconhecida (a transmissão se dava através do canibalismo), gerando importantes avanços no entendimento da doença (Rhodes, 1998). Outro exemplo bastante interessante é a relação entre práticas funerárias e a tranmissão do vírus Ebola. A realização de funerais de indivíduos mortos em decorrência de febre hemorrárica Ebola é um evento que propicia a transmissão do patógeno para outras pessoas, principalmente em decorrência dos rituais que envolvem a manipulação e preparo do cadáver para o funeral. Por outro lado, medidas que informam e orientam a população sobre os riscos de contágio durante os funerais e, por consequência, modificam e adaptam comportamentos culturais, são efetivas para o controle

da tranmissão do vírus (Victory et al., 2015; Curran et al., 2016). Esses casos relacionados à doença priônica e ao Ebola exemplificam a importância de considerar fatores culturais e sociais quando se trata da investigação e controle das doenças infecciosas.

Em suma, a preservação do equilíbrio ambiental, medidas que suportem a qualidade de vida da população, vacinação e um sistema adequado de vigilância epidemiológica formam uma estratégia básica e eficiente para o controle das doenças infecciosas em nível populacional (Boulus, 2001). A abordagem *One Health* deixa evidente a grande complexidade dos processos e fatores envolvidos na emergência das doenças infecciosas. Por esse motivo é necessário investigar novos fatores que influenciam a emergência de tais doenças, bem como investir no estudo das melhores alternativas para prevenção e controle das mesmas.

No que se refere aos estudos envolvendo *One Health*, é clássica a representação desta abordagem na forma de figuras contendo a "saúde humana", "ambiental" e "animal". Porém, quase sempre os patógenos não estão presentes em tais representações. É possível que isso seja em decorrência da dificuldade de alocar os patógenos em um ponto específico da figura, uma vez que o "ambiente" dos patógenos é bastante variado, podendo ser uma célula, o ambiente natural, a corrente sanguínea ou um animal selvagem. Dessa forma, propõem-se a apresentação dos patógenos conforme a Figura 1, representando a circulação dos mesmos de forma dinâmica entre os três componentes da *One Health*.

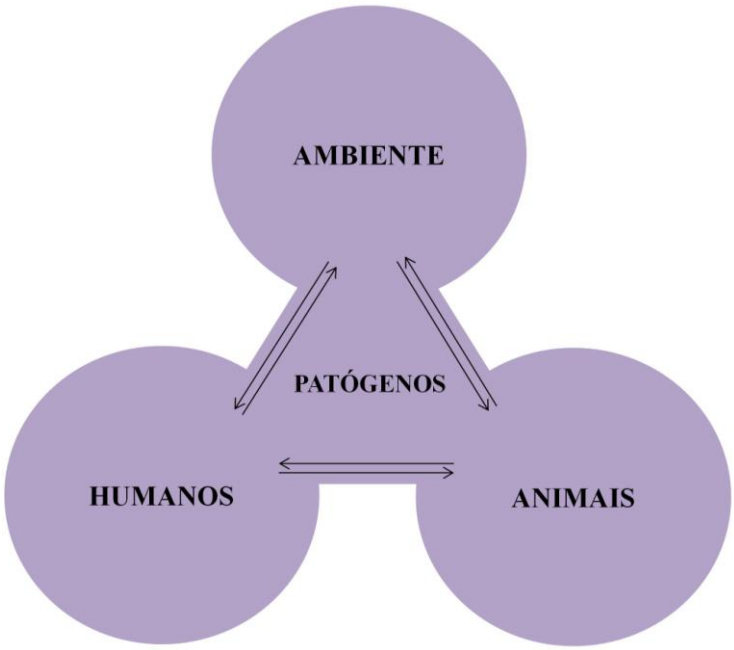

Figura 1. Proposta para a representação da *One Health* no contexto do estudo das doenças infecciosas. Fonte: figura do autor.

Os trabalhos apresentados no Capítulo II desta tese contribuem para a discussão sobre o cenário atual das doenças emergentes e reemergentes no Brasil, além de abordarem ações de prevenção dessas doenças. O primeiro trabalho trata da importância do estudo da ecologia viral como estratégia de prevenção de surtos e epidemias (Ellwanger e Chies, 2016). Este trabalho evidencia a importância de conhecer os fatores ecológicos envolvidos no ciclo de vida dos vírus. Em posse desse conhecimento, estratégias de controle dos patógenos podem ser tomadas de forma efetiva. Complementam essa discussão os outros dois manuscritos apresentados na sequência do Capítulo II.

De acordo com Lipkin e Anthony (2015), a ocorrência de qualquer doença infecciosa nunca foi prevista através de algoritmos, sendo que a melhor estratégia seria focar os esforços na construção de estruturas concentradas em vigilância e diagnóstico das doenças infecciosas, possibilitando uma adequada resposta quando elas emergirem (Lipkin e Anthony, 2015). Essa abordagem exige ferramentas para a detecção de patógenos de forma acurada e rápida. Nesse sentido, o segundo trabalho desta tese aborda estratégias focadas em métodos de detecção e vigilância das doenças infecciosas (Ellwanger et al., 2017a). Atualmente existe uma variedade de "biossensores" com capacidade de auxiliar a identificação de diferentes patógenos (Wang et al., 2002; Khan et al., 2016; Brindha et al., 2018). Nesse contexto, nosso trabalho defende o uso dessas ferramentas nas estratégias de detecção de surtos e epidemias de forma rápida e possibilitando a detecção de uma ampla variedade de patógenos utilizando-se uma única amostra biológica. No Brasil, é muito comum a circulação simultânea de diferentes arbovírus, por exemplo, o que muitas vezes torna problemático o diagnóstico acurado de uma determinada infecção. Além disso, a coinfecção de indivíduos por diferentes microrganismos patogênicos é considerada um problema negligenciado (Vogels et al., 2019). Nossa discussão refere-se especificamente ao uso dos "chips de DNA" (Khan et al., 2016) como forma de reduzir esses problemas, porém é evidente que as técnicas de metagenômica também se tornarão ferramentas cada vez mais comuns na vigilância epidemiológica das doenças infecciosas (Gardy e Loman, 2018).

O terceiro trabalho do Capítulo II faz um apelo sobre a necessidade de fortalecer as ações focadas na vigilância das doenças zoonóticas no Brasil (Ellwanger e Chies, 2018a) visto que as condições ecológicas do país parecem ser cada vez mais permissivas à emergência dessas doenças em razão da crescente degradação ambiental observada no

território nacional. Como exemplo, foi amplamente divulgado que o desmatamento da Amazônia voltou a crescer (Watanabe e Maisonnave, 2018). Além disso, tragédias ambientais de grandes proporções são frequentemente registradas no Brasil, exemplificadas pelo rompimento de duas barragens de rejeitos, uma no município de Mariana em 2015 e outra no município de Brumadinho, em 2019 (Campos-Silva e Peres, 2019; Zimmermann, 2019).

As zoonoses não afetam os humanos apenas de forma direta e em decorrência dos problemas médicos causados pela infecção. Mesmo quando os humanos não entram no ciclo de infecção das doenças de animais, perdas econômicas causadas por doenças infecciosas que afetam animais de criação, por exemplo, também causam prejuízos aos produtores e suas famílias que dependem da fonte de renda gerada por esses animais (Zanella, 2016). São clássicas e dramáticas as cenas de abates em grande escala de gado, suínos e aves em épocas de surtos e epidemias nas quais esses animais estão envolvidos no ciclo de transmissão ou amplificação de determinado patógeno. Este é mais um exemplo dos motivos pelos quais a vigilância de zoonoses deve ser ampliada no Brasil.

Os três primeiros trabalhos apresentados no Capítulo II relacionam-se com o fator "ambiental" da tríade *One Health* e abrem espaço para a apresentação do quarto artigo desta tese. Deve-se levar em consideração que o desenvolvimento de trabalhos focados no componente ambiental é essencial, visto que alguns autores criticam a abordagem *One Health* por muitas vezes dar maior ênfase à saúde humana e veterinária, em detrimento da saúde ambiental (Lerner e Berg, 2017).

Apesar dos *drivers* das doenças infecciosas serem geralmente desequilíbrios ambientais, fatores naturais também têm forte influência sobre a dinâmica dessas doenças. Em Ellwanger e Chies (2018b), estão destacadas as potenciais influências do vento sobre as doenças infecciosas, através da discussão dos resultados de um trabalho recente que avaliou como o vento pode impactar o comportamento de mosquitos do gênero *Anopheles* e, por consequência, os casos de malária (Endo e Eltahir, 2018b). Este manuscrito também se relaciona com o fator ambiental da *One Health*. Porém, é raro o vento ser reconhecido como importante componente da *One Health*. Dessa forma, o manuscrito apresentado também traz uma contribuição importante ao chamar a atenção para esta questão pouco explorada. O vento deve ser levado em conta não só em situações que avaliam a

transmissão de patógenos que formam aerossol, mas também como fator influenciador no comportamento de vetores.

Por último, o Capítulo II também traz um manuscrito descrevendo os principais alvos para investimentos de recursos e esforços focados na prevenção das doenças infecciosas (Ellwanger et al., 2019a). Este trabalho foi formulado visando contribuir com as discussões atuais apresentadas na literatura internacional que trazem visões conflitantes sobre quais alvos e estratégias de prevenção seriam mais adequados para serem implementados, levando em consideração limitações, custos e efetividade (Caroll et al., 2018; Holmes et al., 2018). Este trabalho organiza e define os alvos que muitas vezes aparecem pouco caracterizados na literatura. Além disso, também estão apresentados prós e contras de cada alvo, fornecendo aos pesquisadores subsídios teóricos para formular as ações e pesquisas voltadas à prevenção das doenças emergentes e reemergentes.

Os problemas causados pelas doenças infecciosas no Brasil mais estudados são geralmente aqueles que afetam um grande número de pessoas, como as infecções por HIV, HCV e as epidemias de gripe. Porém, o Brasil também enfrenta de forma recorrente a emergência de "novas" doenças. Recentemente o país sofreu graves prejuízos humanos e econômicos em decorrência da epidemia causada pelo ZIKV. Porém, outros vírus que já preocuparam a comunidade científica brasileira em determinados momentos históricos rapidamente deixam de ser lembrados após o término do surto ou da epidemia. Exemplos clássicos dessa situação são o SABV (Capítulo III) e o ROCV (Capítulo IV).

O artigo que reúne as informações sobre o SABV (Ellwanger e Chies, 2017) foi desenvolvido com o objetivo de descrever as informações históricas relacionadas ao patógeno, bem como reunir os dados biológicos disponíveis sobre o mesmo. Apesar deste patógeno não ter forte potencial para causar surtos de grandes proporções, o SABV é altamente patogênico. Levando em consideração a escassez de estudos sobre o SABV, o trabalho apresentado nesta tese representa uma importante fonte de informações que poderá ser útil para pesquisadores que venham a estudar o patógeno ou até mesmo para a tomada de decisões caso o SABV volte a causar infecções em humanos.

O mesmo cenário descrito em relação ao SABV pode ser aplicado ao ROCV (Ellwanger et al., 2017b), com uma diferença básica: apesar do SABV ser mais patogênico do que o ROCV, o número de infecções causadas pelo ROCV na população brasileira foi muito maior. Além disso, deve-se levar em consideração o fato do ROCV ser transmitido

por mosquitos. Considerando os sérios problemas que o Brasil enfrenta em relação ao controle de vetores, o ressurgimento do ROCV pode ser mais grave atualmente do que foi ao final de década de 1980. Além disso, a urbanização de um potencial surto pelo ROCV talvez causasse problemas com proporções similares à recente epidemia causada pelo ZIKV. Deve-se recordar que o ZIKV é conhecido desde a década de 1940, tendo sido negligenciado até a epidemia de 2015 emergir no Brasil (Baud et al., 2017). Talvez se estratégias robustas de vigilância epidemiológica estivessem ativas no Brasil, a circulação do ZIKV tivesse sido detectada antes de tomar proporções epidêmicas. A detecção precoce da circulação do ZIKA no país poderia ter estimulado as pesquisas sobre este vírus emergente. É possível que estudos com modelos animais pudessem ter demonstrado que o ZIKA causa problemas de desenvolvimento fetal antes que tais problemas fossem detectados em humanos, potencialmente estimulando de forma precoce as medidas de prevenção da infecção por ZIKA em gestantes. Além disso, caso estudos envolvendo a patogênese do ZIKV tivessem sido conduzidos em anos anteriores, a resposta ao vírus durante a epidemia poderia ter sido mais robusta e rápida, pois estaria baseada em evidências já disponíveis na literatura. Além dos cenários mencionados serem hipotéticos, abordar problemas relacionados ao ZIKV não é um dos objetivos desta tese. Entretanto, esses acontecimentos servem como um bom estudo de caso que reverbera sobre a atual situação em que se encontram as pesquisas sobre o SABV e o ROCV. A realização de estudos com objetivanto entender de forma detalhada a patogênese e os aspectos ecológicos do SABV e do ROCV potencialmente mostrarão em qual medida esses vírus são preocupantes em termos clínicos e epidemiológicos.

Levando em consideração o grande número de vírus existentes na natureza (Paez-Espino et al., 2016), talvez seja mais vantajoso investir na vigilância de patógenos já conhecidos como causadores de doenças em humanos (como o SABV e o ROCV) do que destinar recursos e esforços na descoberta de novos patógenos (Holmes et al., 2018). Conforme discutido no último manuscrito do Capítulo II, em Ellwanger et al. (2019a), a integração das duas estratégias seria o ideal. Entretanto, em situações nas quais há escassez de recursos, o fortalecimento da vigilância epidemiológica de patógenos selecionados parece ser a estratégia mais adequada a ser seguida.

Restam muitas questões em aberto a serem respondidas tanto sobre o SABV quanto sobre o ROCV. Os dois vírus surgiram no Brasil, causaram importantes problemas em

humanos e, de forma intrigante, desapareceram. O reservatório exato do SABV nunca foi encontrado. De forma similar, os fatores que estimularam a emergência do surto de encefalite pelo ROCV nunca foram completamente entendidos. Voltar a estudar estes patógenos é fundamental, pois essa ação apresenta implicações para a saúde pública brasileira.

Até o Capítulo IV, esta tese teve como foco principal o componente ambiental da abordagem *One Health*, os patógenos em si, e questões relacionadas aos animais, visto que muitas discussões envolveram vetores, animais reservatórios e doenças zoonóticas. A partir do Capítulo V as interações do tipo patógeno-hospedeiro começam a ser exploradas, fazendo com que o componente humano da *One Health* ganhe destaque.

As relações patógeno-hospedeiro foram exploradas nos Capítulo V e VI usando como modelo de estudo o impacto dos exossomos derivados do hospedeiro sobre a infecção pelo HIV. Essa interação foi escolhida porque o HIV representa uma importante doença infecciosa no Brasil (Murray et al., 2014) e a influência dos exossomos sobre as infecções é um tema emergente na literatura científica (Lawson et al., 2016).

O artigo apresentado no Capítulo V revisa os aspectos básicos das interações entre exossomos e o HIV (Ellwanger et al., 2017c). Este trabalho foi iniciado com o objetivo de reunir as informações sobre este tópico e explorar principalmente a teoria do "exossomo Troiano" (Gould et al., 2013). Entretanto, ao desenvolver o trabalho os autores se depararam com uma grande quantidade de questões em aberto e até mesmo contradições no que se refere ao papel dos exossomos na infecção pelo HIV ou em relação ao estudo dos exossomos *per se*. Por exemplo, ainda há um grande debate sobre os métodos mais adequados para isolar e caracterizar os exossomos. Ainda, é possível que os exossomos atuem tanto na proteção contra a infecção pelo HIV, bem como auxiliando a patogênese do vírus, dependendo do tecido, fluido biológico ou contexto fisiológico no qual essa interação é analisada. Ao término da revisão, fica evidente que a interação humano-HIV sofre diferentes tipos de influências dos exossomos, porém os detalhes de tais influências ainda não são compreendidos de forma completa. É possível que, em razão do crescente número de trabalhos envolvendo exossomos (Lawson et al., 2016), essas questões sejam em breve respondidas de forma detalhada.

Atualmente, diferentes trabalhos indicam que não só o HIV, mas também outros vírus podem explorar a maquinaria de brotamento e transporte de exossomos para formar

novas partículas virais ou evadir o sistema imune (Anderson et al., 2016; Pleet et al., 2016; Raab-Traub e Dittmer, 2017; Vora et al., 2018; Zhou et al., 2018). Apesar de aparentemente haver uma forte relação evolutiva entre os vírus e a maquinaria celular responsável pelos exossomos, ainda não se sabe se os vírus exploraram essa maquinaria pré-existente ou se tanto a maquinaria celular dos exossomos como o processo de formação de novas partículas virais foi direcionado por processos evolutivos independentes.

A relação HIV-exossomos continua a ser explorada no Capítulo VI através de um artigo de hipótese sobre a influência dos exossomos na terapia de células dendríticas contra o HIV (Ellwanger et al., 2016). O aprimoramento deste tipo de terapia é uma importante estratégia de potencial tratamento da infecção pelo HIV (García et al., 2013; Coelho et al., 2016). Entretanto, os fatores imunológicos que afetam a resposta do hospedeiro a esta terapia não são entendidos de forma completa. Este artigo de hipótese surgiu a partir de uma discussão entre membros do Laboratório de Imunobiologia e Imunogenética da UFRGS com pesquisadores da USP e da UFPE sobre a possível influência dos exossomos especificamente sobre resultados obtidos no estudo da terapia de células dendríticas contra o HIV desenvolvido na USP. Este trabalho não traz explicações ou afirma como os exossomos influenciam a resposta à terapia, mas sim descreve uma possível forma de como esta influência poderia acontecer, com base em dados clínicos dos pacientes estudados e em análises de expressão gênica.

Em suma, os artigos que compõem os Capítulos V e VI adicionam um fator entre os outros tantos que fazem parte do componente humano da *One Health*: os exossomos. As microvesículas celulares de forma geral são temas emergentes no estudo das doenças infecciosas. Apesar do papel das microvesículas sobre a patogênese das doenças infecciosas virais ser evidente, ainda pouco se sabe sobre as potenciais influências das mesmas sobre a suscetibilidade às doenças infecciosas. É importante que esta questão seja estudada porque poderá ajudar a explicar padrões diferenciados de suscetibilidade às infecções.

O Capítulo VII enquadra-se no estudo da resposta do hospedeiro frente à infecção pelo HIV. Especificamente, o manuscrito apresentado em tal capítulo demonstra que os níveis da citocina pró-inflamatória IL-8 encontram-se elevados em indivíduos portadores do HIV sob uso de ARV (Ellwanger et al., 2019b; submetido para publicação). Esse

trabalho reforça as crescentes evidências de que a inflamação persistente é um dos principais problemas enfrentados pelos portadores do HIV, além de demonstrar que o tratamento utilizado atualmente não é suficiente para controlar a inflamação. A inflamação crônica é um dos principais fatores causais dos problemas de saúde observados em pacientes infectados pelo HIV e tratados por longos períodos (Deeks et al., 2013b).

A efetividade dos ARVs é indiscutível no que diz respeito ao controle da replicação viral. O tratamento da infecção pelo HIV através de medicamentos disponibilizados pelo SUS foi iniciado na década de 1990 no Brasil: em 1991 o SUS oferecia a monoterapia com zidovudina e, a partir de 1996, a terapia combinada passou a ser oferecida aos pacientes (Segurado et al., 2016). A estratégia "testar e tratar" foi introduzida no Brasil em 2013 e visa oferecer tratamento para todas as pessoas vivendo com HIV, independente do estado imunológico. Essa estratégia pode ser considerada como tratamento e prevenção, pois além de ser benéfica aos indivíduos tratados, reduz o número de novos casos de infecção (Segurado et al., 2016). Apesar de avanços como esses, novos tratamentos adjuvantes precisarão ser estabelecidos para controlar a inflamação crônica nos indivíduos infectados. Isso será crucial para que tanto a morbidade quanto a mortalidade entre a população portadora do HIV sejam diminuídas.

Nosso grupo de pesquisa demonstrou em outros trabalhos que indivíduos HIV+ com padrões específicos de progressão clínica apresentam diferentes perfis de citocinas/quimiocinas circulantes (de Medeiros et al., 2016; Valverde-Villegas et al., 2018). Nosso grupo também já demonstrou que variantes genéticas em genes de receptores de quimiocinas impactam a progressão da infecção pelo HIV (Valverde-Villegas et al., 2017a). Nesse sentido, o artigo de revisão apresentado no Capítulo VIII explora de forma ampla os fatores imunogenéticos que influenciam a infecção por outro vírus de grande importância epidemiológica no Brasil, o HCV (Ellwanger et al., 2018a). Entre outros diferentes tópicos, este artigo abordou os aspectos básicos relacionados à resposta imune à infecção pelo HCV, os genes e as variantes genéticas que influenciam a infecção, fatores relacionados à co-infecção HCV/HIV, tratamentos e temas emergentes, bem como o papel dos exossomos na infecção, e patogênese do HCV. Por fim, é evidente que o desfecho da infecção, tanto em termos de suscetibilidade quanto em relação à patogênese, resulta de complexas interações entre fatores genéticos virais e do hospedeiro e também fatores

ambientais. Novamente, a abordagem *One Health* aplica-se adequadamente a essa doença infecciosa.

Apesar de muitos avanços terem sido obtidos no Brasil em termos de controle da infecção e tratamento das doenças relacionadas ao HCV, essa infecção continua representando um grande problema de saúde pública para a população brasileira. Considerando aspectos ambientais e culturais, é necessária uma maior educação da população sobre como esses fatores agravam os problemas de saúde causados pelo HCV nos indivíduos infectados, como o consumo excessivo de álcool, por exemplo. Além disso, os comportamentos de risco envolvidos na exposição ao vírus devem ser explicitados em campanhas voltadas à população com a mesma intensidade com que se promoveram campanhas de prevenção à infecção pelo HIV.

Também há a necessidade de melhor caracterizar epidemiologicamente a circulação das diferentes cepas de HCV encontradas no Brasil, bem como identificar os *hot-spots* de infecção. Esses dados são de grande importância para localizar as regiões onde o cenário epidemiológico é mais crítico e exige intensificação nas campanhas de prevenção, bem como ajudam a conhecer onde se concentram as cepas de mais difícil tratamento. Por fim, devem-se intensificar os estudos de variantes genéticas envolvidas na suscetibilidade à infecção, no desenvolvimento das doenças causadas pelo HCV, bem como daquelas variantes que influenciam a resposta ao tratamento. Esses estudos auxiliam não apenas a identificação das populações que devem receber atenção diferenciada, pois conhecer o efeito de variantes genéticas sobre determinada doença pode fornecer, também, importantes *insights* para o desenvolvimento de novos tratamentos (Hill, 2012).

Além do artigo de revisão abordando especificamente a infecção pelo HCV, esta tese discutiu o efeito de variantes genéticas sobre diferentes infecções virais, dando enfoque principalmente a polimorfismos de genes de microRNAS, um tema ainda pouco explorado (Capítulo IX; Ellwanger et al., 2018b). É evidente que variantes do tipo SNPs e inserções/deleções podem modificar tanto os padrões de resposta imune frente aos patógenos, bem como afetar a expressão de moléculas que aumentam ou diminuem a suscetibilidade dos indivíduos à infecção por um determinado vírus. Juntos, o conjunto de variantes genéticas herdadas, a penetrância de cada variante e as interações gênicas determinarão a suscetibilidade genética de um pessoa a um patógeno específico, conforme representado na Figura 2.

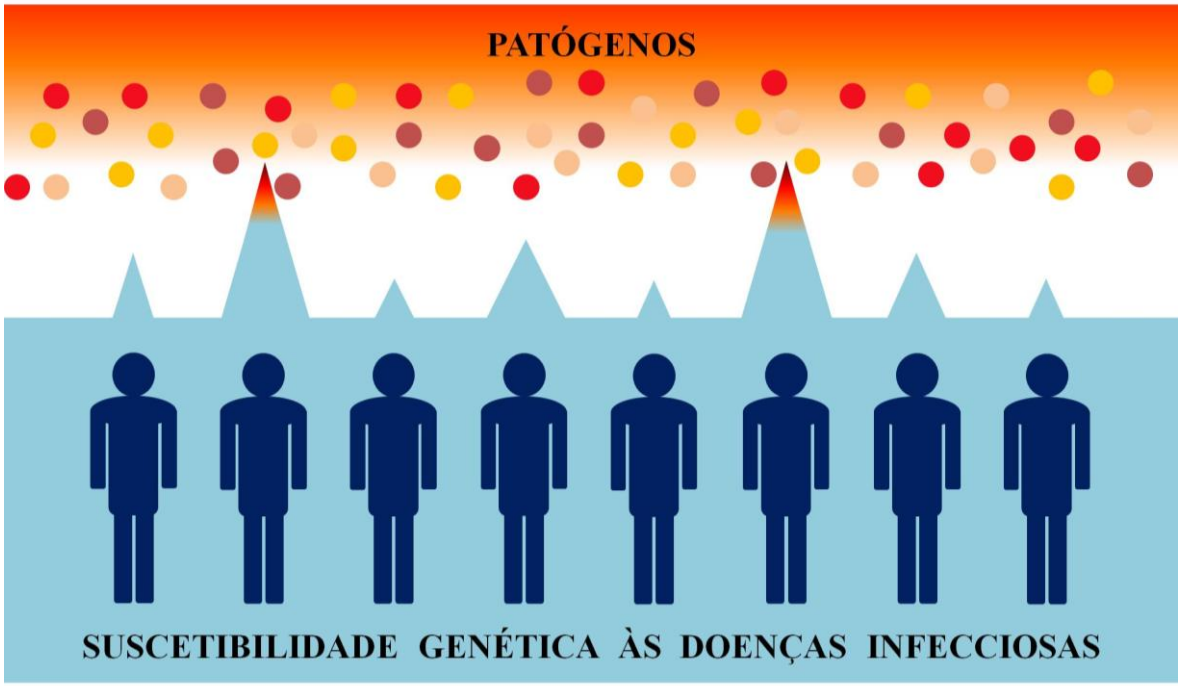

Figura 2. Suscetibilidade genética às doenças infecciosas. A suscetibilidade genética às doenças infecciosas depende do conjunto de variantes genéticas herdadas, da penetrância de cada variante e de interações gênicas. Em conjunto, esses fatores aumentam (indivíduos com triângulos avermelhados) ou diminuem a suscetibilidade individual a um determinado patógeno. Fonte: figura do autor.

Após discutir o efeito de uma ampla variedade de polimorfismos genéticos de fundo imunológico sobre as doenças infecciosas de importância epidemiológica no Brasil, o efeito da variante CCR5Δ32 foi escolhido para ser explorado de forma mais intensa, considerando diferentes contextos biológicos. Essa escolha foi devida ao interesse do autor desta tese em dar continuidade aos trabalhos envolvendo o gene *CCR5* iniciados no então "Laboratório de Imunogenética" no começo dos anos 2000 (Chies e Hutz, 2003; Vargas et al., 2005). Além disso, a influência do CCR5 (gene e proteína) sobre diferentes doenças é cada vez mais evidenciada pela literatura. Durante muitos anos o CCR5 e o CCR5Δ32 eram conhecidos basicamente em razão da relação que possuem com a infecção pelo HIV (O'Brien e Dean, 1997; Parmentier, 2015). Este cenário está se modificando principalmente porque o uso de inibidores de CCR5 (drogas originalmente criadas para tratar e combater o HIV) começa a mostrar efeitos benéficos para tratar diferentes doenças de fundo inflamatório (Brelot e Chakrabarti, 2018; Coppola et al., 2018; Shah e Savjani, 2018; Vangelista e Vento, 2018).

Primeiramente, o Capítulo X abordou os prós e os contras da ausência de expressão de CCR5 em diferentes contextos fisiológicos e patológicos (Ellwanger et al., 2019c). Esta discussão é bastante pertinente, principalmente em razão do já mencionado crescente uso dos inibidores de CCR5 e do recente anúncio da edição do *CCR5* em embriões humanos utilizando a tecnologia CRISPR (Cyranoski e Ledford, 2018). Além da inibição farmacológica do CCR5, é possível que nos próximos anos a modulação da ativação e expressão deste gene possa ser usada de forma valiosa para o tratamento de doenças imunológicas relacionadas ao CCR5.

Apesar da ausência do CCR5 ser benéfica em algumas condições, como a infecção pelo HIV, ela pode ser prejudicial em outros contextos que necessitam deste receptor de quimiocina para respostas adequadas a patógenos. Neste sentido, o Capítulo XI explorou a influência tanto do CCR5 como do CCR5Δ32 na infecção pelo TBEV (Ellwanger e Chies, 2019). Apesar de ainda não existir registro da circulação deste vírus no Brasil, as condições ecológicas do país parecem ser permissivas a ele. Dessa forma, tal discussão é bastante pertinente. Além disso, a encefalite pelo TBEV é uma importante doença infecciosa em diferentes países (Gritsun et al., 2003; Süss, 2011; Zavadska et al., 2013), além de servir como um modelo bastante válido para se estudar os potenciais impactos do CCR5 e do CCR5Δ32 na resposta imune frente as infecções virais.

Os papeis do CCR5 e CCR5Δ32 nas infecções pelo HCV e HBV também representam questões em aberto. Resultados contraditórios em relação ao papel do CCR5Δ32 na infecção pelo HCV foram previamente publicados. Similarmente, poucos estudos envolvendo a variante e a infecção pelo HBV foram conduzidos. A princípio, os trabalhos realizados nesta tese foram os primeiros a investigar tais questões na população brasileira [Capítulos XII (Ellwanger et al., 2018c) e Capítulo XIII (Ellwanger et al., 2019d; em preparação para publicação)].

Os resultados obtidos na investigação do CCR5Δ32 não suportam um papel crucial do CCR5 na infecção pelo HCV (suscetibilidade e doenças relacionadas; Ellwanger et al., 2018c), nem na suscetibilidade à infecção pelo HBV (Ellwanger et al., 2019d). Esses dados são de grande importância, principalmente porque o uso de bloqueadores de CCR5 já é cogitado para o tratamento adjuvante de tais infecções (Gonzalez et al., 2014; Thio et al., 2007). Além disso, é importante mencionar que estudos que apresentam “dados negativos” são tão importantes quanto aqueles que demonstram associações estatisticamente

significativas. Esses estudos indicam para quais áreas de estudo talvez não seja tão promissor continuar investindo recursos e, muitas vezes, apontam as novas direções para o planejamento de novos projetos de pesquisa. Além disso, a publicação de trabalhos com "dados negativos" é importante para que tais dados sejam incluídos em meta-análises, por exemplo. Caso apenas trabalhos com resultados estatisticamente significativos sejam publicados, é possível que os resultados de meta-análises sofram desvios e levem a interpretações equivocadas.

Os trabalhos envolvendo CCR5Δ32 e as infecções pelo HCV, HBV e co-infecções apresentados nesta tese estão entre os estudos abordando CCR5Δ32 com maior número amostral já conduzidos na população brasileira. Dessa forma, é provável que os resultados obtidos representem de forma bastante robusta a influência desta variante sobre as infecções avaliadas, especialmente na população do sul do Brasil. Por fim, tais trabalhos preenchem lacunas do conhecimento em relação à influência do CCR5 nas infecções pelo HCV e HBV.

A patogênese de uma doença infecciosa e a transmissão de um patógeno resultam da interação entre características genéticas do patógeno e do hospedeiro. Além disso, a transmissão do patógeno também é influenciada pelos seguintes fatores: ambiente físico, comportamento do hospedeiro, estrutura populacional e distribuição da infecção entre a população (Metcalf et al., 2015). Dessa forma, fica claro como os fatores sociais, ambientais e biológicos não podem ser dissociados quando a patogênese e a transmissão das doenças infecciosas são avaliadas. Similarmente, o surgimento de novas doenças só pode se entendido de forma completa quando tais fatores são avaliados em conjunto. Em suma, aplicar a abordagem *One Health* no estudo das doenças infecciosas é fundamental, pois ela engloba todos os fatores mencionados. Pelos mesmos motivos, o uso desta abordagem deve ser incentivado em estratégias de prevenção e combate às doenças emergentes e reemergentes.

CONCLUSÕES

Além de abordar problemas científicos específicos, esta tese explorou de forma ampla um tema de grande importância: doenças virais emergentes, reemergentes e negligenciadas no Brasil. Em decorrência da complexidade e variedade de fatores que influenciam essas doenças, a abordagem *One Health* foi usada para guiar as investigações e discussões. Essa estratégia direcionou o estudo de cada doença abordada na tese levando em consideração os fatores humanos, animais e ambientais que afetam a emergência, suscetibilidade e patogênese das doenças infecciosas. Como resultado, foi obtida uma visão ampla do atual cenário de algumas doenças infecciosas do Brasil, além de uma discussão de tópicos específicos de forma abrangente. Por fim, conclui-se que:

- O Brasil possui as condições ecológicas, ambientais e sociais ideais para a emergência e reemergência de diferentes patógenos.

- Levando em consideração a influência do meio ambiente sobre a emergência das doenças infecciosas, as políticas de preservação ambiental devem ser ampliadas e consideradas, também, como estratégias de saúde pública.

- Faz-se necessário intensificar a vigilância das doenças infecciosas no território nacional, principalmente de patógenos já conhecidos, visando a detecção de surtos ou epidemias logo após sua emergência.

- A saúde de animal não humanos é um importante fator na prevenção das doenças infecciosas. Dessa forma, ações que visem o controle da caça, eliminação do tráfico de animais silvestres, sanidade de animais de criação e de companhia, controle de pragas e vetores e preservação de *habitats* são essenciais para o controle de zoonoses.

- Os exossomos representam um tema emergente nas pesquisas envolvendo as interações do tipo patógeno-hospedeiro. Está cada vez mais claro que os exossomos são cruciais na regulação do sistema imune, junto com sub-populações celulares e moléculas solúveis como as citocinas. Os estudos envolvendo o papel de tais microvesículas sobre a suscetibilidade às doenças infecciosas devem ser intensificados.

- A inflamação crônica é um importante problema verificado nos indivíduos portadores do HIV, mesmo sob uso de ARV. Novas estratégias terapêuticas devem ser investigadas para controlar este problema.

- Variantes genéticas modulam de forma importante a suscetibilidade às infecções, assim como o curso clínico das doenças causadas por patógenos. Porém, tais influências podem variar de indivíduo para indivíduo em decorrência de fatores ambientais, devido a características específicas dos patógenos ou em razão de outros fatores genéticos, como a epistasia (Figura 3).

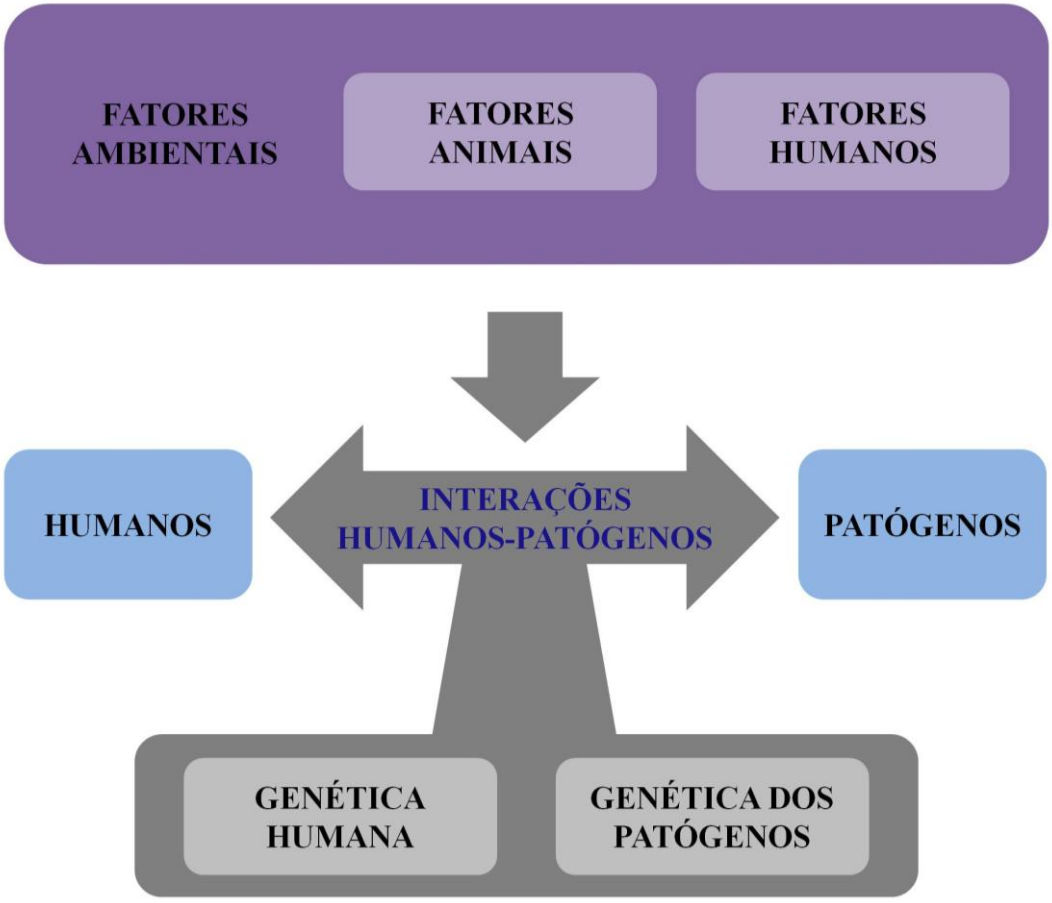

Figura 3. Fatores envolvidos na dinâmica de interações entre patógeno e hospedeiro. Condições ambientais influenciam os desfechos das interações entre humanos e animais, aumentando ou diminuindo o contato dos humanos com patógenos causadores de doenças zoonóticas, por exemplo. As relações entre patógenos e humanos são moduladas por características de ambos. Considerando que humanos e patógenos interagem em um ambiente natural, os fatores derivados de tal ambiente têm participação nas interações patógeno-hospedeiro. Fonte: figura do autor.

- A variante CCR5Δ32 não parece apresentar uma influência importante sobre diferentes aspectos das infecções pelo HCV, HBV e co-infecções destes vírus com o HIV na população do sul do Brasil. Entretanto, fica evidente que esta variante e o receptor

CCR5 apresentam importantes influências sobre outras doenças, incluindo a infecção pelo TBEV.

- O estudo e o enfrentamento das doenças emergentes, reemergentes e negligenciadas devem ganhar papel de destaque no Brasil. Tais estudos devem integrar disciplinas de diferentes áreas do conhecimento, como genética, imunologia, saúde pública, epidemiologia, veterinária, ecologia e sociologia.

PERSPECTIVAS

- Redação de um artigo de revisão em língua portuguesa abordando os tópicos apresentados na introdução e discussão desta tese.

- Continuar a investigação de variantes genéticas potencialmente associadas com padrões diferenciados de progressão clínica da infecção pelo HIV. Este trabalho já se encontra em andamento através da genotipagem de variantes nos genes *MICA* e *NKG2C*.

- Avaliar a expressão de genes candidatos em indivíduos HIV+ com diferentes padrões de progressão clínica.

- Explorar questões em aberto em relação ao CCR5: CCR5 solúvel e CCR5 intracelular.

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ANEXO A – Produção adicional

Abaixo estão listadas publicações selecionadas resultantes de trabalhos com os quais o autor desta tese colaborou durante a realização de seu doutorado:

Kaminski VL, **Ellwanger JH**, Sandrim V, Pontillo A, Chies JAB (2019) Influence of NKG2C gene deletion and CCR5Δ32 in preeclampsia - Approaching the effect of innate immune gene variants in pregnancy. Int J Immunogenet 46: 82-87. doi: 10.1111/iji.12416

Pietrzak B, Sharma V, Wasalathanthri D, **Ellwanger JH**, Sanganyado E, Buschke F, Beardsley FR, Agarwal D, Jensen MM, Easun TL, Lin H, Zhou K, Jordan EJ, Oda FS, MacKay H, Coffey E, Yoho R, Winter M (2018) Nurturing connections to the environment. Science 362: 886-888. doi: 10.1126/science.aav9402

Segal L, Agarwal D, Isaacson KJ, Oehmke TB, Kumar B, Chen JS, Cusimano JM, Negi S, Tiper I, Bakermans AJ, Jensen MM, Sanganyado E, Zaidi SS, Romero-Molina C, Martínez SE, Anderson SM, Santos GM, De Lella Ezcurra AL, Farragher J, Sharma V, Duncan G, Dutton-Regester K, Kim SA, Yu S, Schwendimann BA, Reichardt JKV, Halder A, Dennis AF, **Ellwanger JH**, Chiu YH (2018) NextGen Voices: Quality mentoring. Science 362: 22-24. doi: 10.1126/science.aav5914

Kaminski VL, **Ellwanger JH**, Matte MCC, Savaris RF, Vianna P, Chies JAB (2018) IL-17 blood levels increase in healthy pregnancy but not in spontaneous abortion. Mol Biol Rep 45: 1565-1568. doi: 10.1007/s11033-018-4268-7

Valverde-Villegas JM, de Medeiros RM, **Ellwanger JH**, Santos BR, Melo MG, Almeida SEM, Chies JAB (2018) High CXCL10/IP-10 levels are a hallmark in the clinical evolution of the HIV infection. Infect Genet Evol 57: 51-58. doi: 10.1016/j.meegid.2017.11.002

Franke SIR, Molz P, Mai C, **Ellwanger JH**, Zenkner FF, Horta JA, Prá D (2018) Influence of hesperidin and vitamin C on glycemic parameters, lipid profile, and DNA damage in rats treated with sucrose overload. An Acad Bras Cienc 90(2 suppl 1): 2203-2210. doi: 10.1590/0001-3765201820170751

Kaminski V, **Ellwanger JH**, Chies JAB (2017) Down-regulation of HLA-G gene expression as an immunogenetic contraceptive therapy. Med Hypotheses 102: 146-149. doi: 10.1016/j.mehy.2017.03.030

Müller TE, **Ellwanger JH**, Michita RT, Matte MCC, Renner JDP (2017) CYP2B6 516 G>T polymorphism and side effects of the central nervous system in HIV-positive individuals under Efavirenz treatment: Study of a sample from southern Brazil. An Acad Bras Cienc 89(1 Suppl 0): 497-504. doi: 10.1590/0001-3765201720160355

Franke SIR, Molz P, Mai C, **Ellwanger JH**, Zenkner FF, Horta JA, Prá D (2017) High consumption of sucrose induces DNA damage in male Wistar rats. An Acad Bras Cienc 89: 2657-2662. doi: 10.1590/0001-3765201720160659

da Silva AL, Karnopp TE, Weber AF, Goulart CD, Scheneiders PB, Cardoso DM, Carvalho LL, **Ellwanger JH**, Possuelo LG, Valim AR (2016) DNA damage and repair capacity in lymphocyte of chronic obstructive pulmonary diseases patients during physical exercise with oxygen supplementation. Multidiscip Respir Med 11: 43. doi: 10.1186/s40248-016-0079-7

Molz P, **Ellwanger JH**, Zenkner FF, Campos D, Prá D, Putzke MT, Franke SIRF (2016) Recognition memory and DNA damage in undernourished young rats. An Acad Bras Cienc 88(3 Suppl): 1863-1873. doi: 10.1590/0001-3765201620150608

ANEXO B – Aspectos éticos

Abaixo estão litados os artigos desta tese que utilizaram dados de voluntários humanos, com os respectivos comitês de ética que aprovaram os projetos aos quais os artigos estão vinculados:

Ellwanger JH, Crovella S, Dos Reis EC, Pontillo A, Chies JAB (2016) Exosomes are possibly used as a tool of immune regulation during the dendritic cell-based immune therapy against HIV-I. Med Hypotheses 95: 67-70.

Comitê de ética do Hospital das Clínicas (São Paulo, Brasil).

Ellwanger JH, Leal BK, Valverde-Villegas JM, Simon D, Marangon CG, Mattevi VS, Lazzaretti RK, Sprinz E, Kuhmmer R, Chies JAB (2018) CCR5Δ32 in HCV infection, HCV/HIV co-infection, and HCV-related diseases. Infect Genet Evol 59: 163-166.

Commitês de ética da Universidade Federal do Rio Grande do Sul, Hospital de Clínicas de Porto Alegre (Porto Alegre, Brasil) e Universidade Luterana do Brasil (Canoas, Brasil).

Ellwanger JH, Valverde-Villegas JA, Kaminski VL, de Medeiros RM, Almeida SEM, Santos BR, Melo MG, Hackenhaar FS, Chies JAB (2019). Increased IL-8 levels in HIV-infected individuals on ART - A potential hallmark of chronic inflammation. Submetido para publicação.

Commitês de ética da Universidade Federal do Rio Grande do Sul e do Hospital Nossa Senhora da Conceição (Porto Alegre, Brasil).

Ellwanger JH, Leal BK, Wolf JM, Michita RT, Simon D, Lunge VR, Chies JAB (2019) CCR5Δ32 in HBV infection and HBV/HIV co-infection. Em preparação para publicação.

Comitê de ética da Universidade Luterana do Brasil (Canoas, Brasil).