UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL – UFRGS

Imunobiologia e Imunogenética de Desordens Gestacionais e Transtorno do Espectro Autista

Valéria de Lima Kaminski

Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS como requisito parcial para a obtenção do grau de Doutor em Ciências (Genética e Biologia Molecular).

Orientador: Dr. José Artur Bogo Chies

Porto Alegre
Janeiro de 2020

INSTITUIÇÕES E FONTES FINANCIADORAS

Este trabalho foi desenvolvido no Laboratório de Imunobiologia e Imunogenética da UFRGS. A autora desta tese contou com o apoio de uma bolsa de doutorado da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). O orientador deste trabalho recebeu bolsa de produtividade em pesquisa do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Ambas bolsas foram fundamentais para a dedicação da aluna e do supervisor às atividades relacionadas a esta tese. A parte experimental deste trabalho foi financiada por recursos do Laboratório de Imunobiologia e Imunogenética e do Programa de Pós-Graduação em Genética e Biologia Molecular (PPGBM) da UFRGS.

Pesquisadores e pesquisadoras de outras instituições brasileiras também contribuíram para a realização dos trabalhos incluídos nesta tese. A seguir, encontram-se listadas tais instituições: Hospital de Clínicas de Porto Alegre (HCPA), Universidade Estadual de São Paulo (UNESP) e Universidade de São Paulo (USP). Os nomes dos pesquisadores e os detalhes de suas filiações estão descritos nos trabalhos publicados (ou em estágio de preparação para publicação) incluídos nesta tese.

À memória de Josefa Straub Kaminski.

AGRADECIMENTOS

Um dos momentos mais marcantes do final de um doutorado é, certamente, escrever os agradecimentos. Aos meus pais Antônio Kaminski e Ligia Lima, e irmãos Vanessa e João Miguel, eu poderia dedicar páginas e páginas para demonstrar minha gratidão. Vocês são as bases de tudo que eu me tornei, são meu porto seguro, e estar junto de vocês ou saber que "em breve iremos nos reunir" é o que, de fato, dá sentido para minha vida. Além de minha vó Cecília Gonçalves e meus tios Erwin e Odete Lenzi pelo apoio de sempre, agradeço a todos os meus familiares pelo incentivo nessa jornada.

Em várias áreas do conhecimento, principalmente na ciência, é comum que novatos apoiem-se em "ombros de gigantes", como sabiamente ressaltou Issac Newton há um tempinho. No meu caso, os primeiros grandes ombros em que me escorei foram os de meu primeiro orientador, durante a iniciação científica e o mestrado. Foi com o incentivo do Professor Dr. Elgion Lúcio da Silva Loreto, na Universidade Federal de Santa Maria, que eu descobri meu gosto por trabalhar dentro de um laboratório e meu encanto pela ciência. Por isso, meus agradecimentos acadêmicos iniciais são dedicados ao Professor Elgion, sem esquecer, é claro, de deixar meu muito obrigada ao Dr. Gabriel da Luz Wallau, pela co-orientação que recebi durante a iniciação científica e pela amizade e colaboração em trabalhos que duram até hoje.

Ingressar no Programa de Pós-Graduação em Genética e Biologia Molecular da UFRGS (PPGBM) era um sonho que nasceu assim que descobri a existência desse programa. A proximidade de meu primeiro mentor a esse PPG facilitou a admiração crescente pelo Departamento de Genética da UFRGS. Em um evento comemorativo ao aniversário de 50 anos do PPGBM, assisti a uma palestra sobre Imunologia da Gestação, ministrada pelo Professor Dr. José Artur Bogo Chies, o Zéca. O desfecho do que eu senti nesse dia está em vossas mãos nesse momento, sob a forma de uma Tese de Doutorado. Ser aluna do PPGBM e ter a honra de me apoiar nos ombros gigantes do Zéca passaram a ser meus objetivos profissionais. Aqui cabe o meu grande agradecimento à Drª. Francis Maria Báo Zambra, de quem fui colega de laboratório ainda na UFSM e a pessoa que mais me incentivou a buscar o contato inicial com o chefe do Laboratório de Imunobiologia e Imunogenética da UFRGS. Obrigada, Francis, pelo incentivo, pela amizade, pelo coleguismo e pelo companheirismo antes e durante minha estadia em Porto Alegre.

Preciso agradecer a paciência inicial que o Prof. Zéca teve comigo até minha aprovação no processo seletivo. Obrigada pela confiança em mim, por não ter desistido de me receber como sua aluna e pela oportunidade de ter crescido como pessoa e como profissional em seu laboratório. Serei sempre muitíssimo grata, também, pela liberdade que senti em desenvolver os meus trabalhos (de bancada e teóricos) sob sua supervisão. Certamente, a orientação que recebi durante o meu doutorado reverberará positivamente por toda a minha carreira na ciência. Obrigada.

A sorte andou ao meu lado nos últimos quatro anos. Sinto-me extremamente privilegiada por ter conhecido, convivido e colaborado com o Dr. Joel Henrique Ellwanger, por quem carrego admiração pessoal e profissional indescritíveis. Joel, muito obrigada por ter sido, praticamente, meu co-mentor durante minha estadia no laboratório. Sem o teu apoio, ou melhor, sem me apoiar também nos teus ombros gigantes, essa tese seria bem diferente. Através da tua parceira, foi divertido passar até mais de 14 horas por dia no laboratório, como tantas vezes o fizemos. Acima de tudo, obrigada pela amizade que cultivamos e que tanto enriqueceu minha vida. Sou grata por cada taça de vinho e cada xícara de café compartilhada que, muitas vezes, foram gatilhos para termos brindado nossos artigos com bons espumantes. Ter cruzado o teu caminho foi, para mim, uma das manifestações mais belas do acaso!

À Dr.ª Jacqueline María Valverde Villegas, primeira pessoa em cujos experimentos me envolvi ao chegar ao laboratório, serei sempre muito grata pela receptividade, pelos ensinamentos iniciais em citometria de fluxo, pelas colaborações nos trabalhos e, mais importante ainda, pela amizade que ficou. Em especial também, deixo meu agradecimento ao meu colega e amigo Dr. Rafael Michita, pelas colaborações que firmamos, pelo tempo compartilhado em laboratório, pelas discussões sobre ciência e pelo companheirismo.

A Bruna Kulmann Leal, Brenda Pedron e Giovana Cechim, agradeço pela parceria nos artigos, nos trabalhos de bancada, na manutenção cotidiana do ambiente de trabalho, pelas conversas descontraídas e, claro, pela companhia em chás no “Chics” e eventuais drinks que fomentaram a nossa amizade e coleguismo.

Aos queridos ICs que tive a oportunidade de acompanhar, Andressa Rodrigues, Guilherme Nunes e Marina Ziliotto: vocês foram essenciais na minha formação e essenciais para o meu autoconhecimento. Obrigada pela parceria nos trabalhos e por, sempre educadíssimos, me aturarem no dia-a-dia do laboratório.

Várias pessoas participaram de forma direta ou indireta da minha jornada acadêmica como doutoranda. São amigos queridos, colegas, ex-colegas, colaboradores dos trabalhos e/ou pessoas que, por algum período, de maneira pessoal ou à distância, foram importantes nesses

quatro anos. Eu poderia escrever um ou mais parágrafos para agradecer a cada pessoa, mas devo limitar-me apenas a citar nomes: Lilian Benchimol, Luís e Aimée Knak, Larissa Bernardo, Tiago Minuzzi, Pedro Fonseca, Francine Cenzi, Sinara Jardim, Paloma Rubin, Michele Antunes, Gabriela Malaquias, Matheus Gutierres, Tiago Veit, Gustavo Fioravanti Vieira, Pedro Brum, Maria Cristina Matte, Lian Troncoso, Martiela Freitas, Marcelo Bragatte, Mauro Ortiz, Pabulo Rampelotto, Alexandre Copês e Lílian Caesar.

É importante, também, que eu deixe meu ‘muito obrigada’ aos profissionais colaboradores dos trabalhos que compõe esta tese, Dr.ª Priscila Vianna, Dr.ª Valeria Sandrim, Dr.ª Alessandra Pontillo, Dr.ª Jaqueline Schuch, Dr.ª Tatiana Roman, Dr.ª Lavinia Schüler-Faccini, Dr. Rudimar Riesgo e Dr. Ricardo Savaris. Sem o apoio desses pesquisadores não seria possível a realização dos experimentos. Ao Prof. Dr. Claiton Bau e seus alunos, obrigada por diponibilizarem a infraestrutura do laboratório para parte dos experimentos.

Agradeço a todos os professores do PPGBM que, através de suas aulas, seminários ou ‘conversas de corredor’ contribuíram para a minha formação. Muito obrigada ao relator dessa tese, Prof. Dr. Nelson da Rosa, que também contribuiu imensamente sendo membro da banca da qualificação desse doutorado, em agosto de 2018.

À banca examinadora titular: Prof.ª Dr.ª Alessandra Peres, Prof.ª Dr.ª Ana Veiga e Prof.ª Dr.ª Maria Teresa Sanseverino: é uma honra ter meu trabalho avaliado por mulheres da ciência, excelentes profissionais em seus cargos e grandes exemplos para todos os cientistas. Obrigada, também, à banca examinadora suplente, composta pela Prof.ª Dr.ª Fernanda Sales Luiz Vianna e pelos pesquisadores Dr. Maurício Rigo e Dr. Vinicius Sortica pelo aceite e disponibilidade em avaliar este trabalho. Ainda, deixo um agradecimento especial ao Sr. Elmo, secretário do PPGBM, pela eficiência, agilidade e cordialidade diários. A gratidão ao PPGBM chega, também, à coordenação do programa, cujas duas últimas gestões permearam minha passagem como aluna do departamento.

Por fim, agradeço aos pacientes que, nobremente, forneceram as amostras biológicas que possibilitaram a execução dos trabalhos aqui apresentados; ao governo brasileiro, que financiou a realização do meu doutorado através do Conselho de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), e à Universidade Federal do Rio Grande do Sul pela infraestrutura de que usufruí.

"Três ideias profundamente desestabilizadoras ricochetearam por todo o século XX e se dividiram em três partes desiguais: o átomo, o byte e o gene. (…) Cada uma começou a vida como um conceito científico muito abstrato, mas acabou por invadir numerosos discursos humanos e, com isso, transformou a cultura, a sociedade, a política e a linguagem. No entanto, incomparavelmente, o paralelo mais crucial entre essas ideias é conceitual: cada uma representa a unidade irredutível – o tijolo construtor, a unidade básica – de um todo maior: o átomo, da matéria; o byte (ou "bit"), da informação digitalizada; o gene, da hereditariedade e informação biológica. (...) Não podemos explicar o comportamento da matéria – por que o ouro brilha, por que o hidrogênio entra em combustão com o oxigênio – sem invocar a natureza atômica da matéria. Não podemos entender as complexidades da computação – a natureza dos algoritmos, a armazenagem ou corrupção de dados – sem compreender a anatomia estrutural da informação digitalizada. (...) De maneira análoga, (...) é impossível entender a biologia de organismos e células ou a evolução – ou ainda a patologia, o comportamento, temperamento, doença, raça, identidade ou destino dos seres humanos – sem primeiro lidar com o conceito de gene." [1]

Siddhartha Mukherjee

[1]Texto extraído do livro "O Gene: uma história íntima" (Mukherjee, Siddhartha. 1ª ed. São Paulo: Companhia das Letras, 2016, p. 20-21).

SUMÁRIO

LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

Abreviaturas

ASD: *Autism Spectrum Disorder*

CAPES: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico

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 differentiation 4*/Grupamento de diferenciação 4

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

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

DNA: *deoxyribonucleic acid*/ácido desoxirribonucleico

EUA: Estados Unidos da América

HLA: *human leukocyte antigen*/antígeno leucocitário humano

IL: *interleukin*/interleucina

MIA: *Maternal Immune Activation*/ativação imune materna

MICA: *MHC-I Chain Related Protein A*/Proteína A Relacionada ao MHC de Classe I

NIH: *National Institutes of Health*/ Institutos Nacionais da Saúde

NK: *Natural killer (cells)/* células *Natural Killer*

NKG2A*: Natural Killer Group 2 Member A/* Receptor de células NK Grupo 4 membro A

NKG2C*: Natural Killer Group 2 Member C/* Receptor de células NK Grupo 4 membro C

NKG2D: *Natural Killer Group 2 Member D/* Receptor de células NK Grupo 4 membro D

PE: *preeclampsia*/pré-eclâmpsia

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

RNA: *ribonucleic acid*/ácido ribonucleico

SUS: Sistema Único de Saúde

TEA: Transtorno do Espectro Autista

Símbolos e unidades

Δ: delta

kb: kilobase

µm: micrômetro

nm: nanômetro

LISTA DE FIGURAS E TABELAS

RESUMO

Esta tese apresenta artigos de dados e trabalhos teóricos envolvendo imunologia da gestação, desordens gestacionais e imunogenética do Transtorno do Espectro Autista (TEA). Este trabalho está dividido em duas partes, a primeira sendo composta por quatro capítulos, e a segunda parte, por três capítulos totalizando sete artigos diferentes. Uma introdução geral precede as duas partes principais, onde são apresentados conceitos importantes abordados ao longo dos textos. A gestação humana é um processo complexo que envolve diferentes sistemas fisiológicos que sofrem influência do sistema imune materno. Complicações gestacionais e seus possíveis gatilhos ambientais são apresentados nesta tese, com destaque para desequilíbrios nos níveis de citocinas nessas condições. Além disso, é abordado o componente genético das respostas imunes aos diferentes desafios ambientais enfrentados durante a gravidez pela mãe e pelo feto em desenvolvimento. O TEA é uma condição que impacta o neurodesenvolvimento fetal, além de afetar as habilidades cognitivas e sociais dos indivíduos acometidos, geralmente manifestando-se antes dos três anos de idade. Sabe-se que o TEA possui um forte componente genético com altas taxas de herdabilidade e de concordância entre gêmeos monozigóticos. Além disso, o TEA possui um importante componente ambiental. Sabe-se, ainda, que distúrbios imunológicos são um componente do TEA. Nesta linha, são abordados polimorfismos em genes relacionados ao sistema imunológico no contexto do TEA. Um dos fatores ambientais fortemente sugeridos como risco para TEA são algumas complicações gestacionais, principalmente aquelas com fundo inflamatório. Assim, diferentes aspectos imunológicos e ambientais durante a gravidez podem ser fatores-chave para o desenvolvimento de distúrbios gestacionais e/ou para a incidência de problemas neurológicos na prole. Na “Parte I” desta tese são abordados aspectos imunológicos da gestação humana, juntamente com discussões sobre o papel das vesículas extracelulares no contexto de gravidez bem-sucedida e de complicações gestacionais e em diferentes doenças infecciosas. Ao longo da “Parte II”, são apresentados genes relacionados ao sistema imunológico, nos quais diferentes polimorfismos, especificamente variantes genéticas pró-inflamatórias, variantes genéticas do MHC e variantes genéticas imunometabólicas, já foram estudados no contexto do autismo e TEA. Diferentes gatilhos

inflamatórios durante a gravidez que já foram indicados como fatores de risco para a manifestação de TEA em crianças nascidas dessas gestações são também aqui discutidos. Nesse contexto, destacam-se as consequências da ativação imune materna (MIA) e sua influência no feto em desenvolvimento. Além disso, é proposta uma conexão mecanicista entre os principais distúrbios relacionados à inflamação na gravidez e risco para autismo considerando o “universo das vesículas extracelulares”. Por fim, são apresentados resultados de estudos imunogenéticos envolvendo a deleção do gene *NKG2C* e variantes nos genes *NKG2D* e *NKG2A* em pacientes com TEA e em seus respectivos pais biológicos.

Palavras-chave: Genética; Imunologia; Gestação; Autismo; Polimorfismo; Inflamação.

ABSTRACT

This thesis presents data and theoretical studies involving gestational immunology, gestational disorders, and immunogenetics of Autism Spectrum Disorder (ASD). This work is divided into two parts, each consisting of four chapters, totaling eight different articles. A general introduction precedes these two main parts, where important concepts covered throughout the texts are presented. Human pregnancy is a complex process that involves different physiological systems, which are influenced by the maternal immune system. Gestational complications and their possible environmental triggers are also presented in this thesis, highlighting imbalances in cytokine levels under these conditions. In addition, the genetic component of immune responses to the different environmental challenges faced during pregnancy by the mother and the developing fetus is addressed. ASD is a condition that impacts both fetal neurodevelopment and the cognitive and social abilities of affected individuals, usually manifesting before the age of three. ASD is known to have a strong genetic component with high heritability and agreement rates between monozygotic twins. In addition, it has an important environmental component. Immune disorders are also known to be a component of ASD. In this line, polymorphisms in genes related to the immune system in the context of ASD are addressed. Strongly suggested environmental risk factors for ASD are gestational complications, especially those with an inflammatory background. Thus, different immunological and environmental aspects during pregnancy may be key factors for the development of gestational disorders and/or for the incidence of neurological problems in the offspring. In “Part I” of this thesis, immunological aspects of human pregnancy are discussed, along with discussions about the role of extracellular vesicles in the contexts of both successful and complicated pregnancies and in different infectious diseases. “Part II” presentes genes related to the immune system, in which different polymorphisms have already been studied in the context of autism and ASD, specifically proinflammatory genetic variants, MHC genetic variants, and immunometabolic genetic variants. Different inflammatory triggers during pregnancy that were already suggested as risk factors for ASD in children born of these pregnancies are also discussed. In this context, the consequences of maternal immune activation (MIA) and its influence on the developing fetus are highlighted. In addition, a mechanistic connection between major inflammation-related disorders in pregnancy and risk for autism

considering the “extracellular vesicle universe” is proposed. Finally, results of immunogenetic studies involving deletion of the *NKG2C* gene and variants in the *NKG2D* and *NKG2A* genes in ASD patients and their respective biological parents are presented.

Keywords: Genetics; Immunology; Pregnancy; Autism; Polymorphism; Inflammation

APRESENTAÇÃO E ESTRUTURAÇÃO DA TESE

No tópico **Introdução Geral e Objetivos** são apresentados os temas abordados em detalhe nas **Partes I** e **II** desta tese, que trata sobre imunobiologia e imunogenética de desordens gestacionais e transtorno do espectro autista.

Na **Parte I**, encontram-se quatro capítulos, cada um correspondente a uma publicação. O papel da interface materno-fetal na tolerância imune em relação ao feto e os processos que impedem infecções nessa região, com ênfase no papel de exossomos e outras vesículas extracelulares, é abordado no **Capítulo II**. O **Capítulo III** discute o papel da molécula imunotolerogênica HLA-G e, dada a importância da mesma no período gestacional em que é altamente expressa pela placenta, é apresentada uma hipótese para o desenvolvimento de um método contraceptivo baseado em micro RNAs que afetam a sua expressão. A importância de citocinas na adequada manutenção e coordenação das respostas imunes durante a gestação é abordada no **Capítulo IV**, por meio de um artigo de dados que avaliou o perfil de citocinas no plasma sistêmico de mulheres que sofreram aborto espontâneo idiopático e em mulheres gestantes sem apresentação de intercorrências. Aspectos imunogenéticos da gestação humana são discutidos no **Capítulo V**, o qual consiste em um artigo de dados que avaliou o impacto da deleção completa do gene *NKG2C* e da variante CCR5Δ32 sobre o desenvolvimento da pré-enclâmpsia. Estes genes foram escolhidos como alvo do estudo dado o potencial dessas moléculas em modificar o comportamento de células inflamatórias. O estudo foi realizado no contexto de uma intercorrência gestacional de caráter complexo e multifatorial, a pré-eclâmpsia, em que abordagens imunogenéticas têm potencial para contribuir no entendimento de suas causas, agravantes e/ou gatilhos.

A **Parte II** desta tese é baseada em aspectos imunogenéticos do Transtorno do Espectro Autista (TEA), apresentando quatro capítulos correspondentes a dois artigos de revisão e outros dois artigos de dados, todos em fase final de preparação para publicação. Além disso, este eixo da tese conecta-se com a **Parte I** através da discussão acerca dos gatilhos inflamatórios gestacionais que são potenciais fatores de risco para o desenvolvimento de autismo. O **Capítulo VI** apresenta um trabalho de revisão sobre polimorfismos em genes relacionados ao sistema imune já investigados no contexto do

TEA. O impacto de processos inflamatórios durante a gestação e o risco de desenvolvimento de autismo nas crianças nascidas dessas gestações é discutido no **Capítulo VII**, em que vesículas extracelulares são indicadas como um possível elo mecanístico negligenciado nos processos inflamatórios abordados. O **Capítulo VIII** é composto por um estudo em que a deleção completa do gene *NKG2C* foi avaliada em indivíduos autistas e em seus respectivos pais biológicos, juntamente com o papel de diferentes SNPs dos genes *NKG2D* e *NKG2A*. Por fim, o **Capítulo IX** de uma discussão geral e conclusão, conectando brevemente os temas abordados nesta tese, além de apresentar um fechamento deste trabalho.

Capítulo I

Introdução e Objetivos

INTRODUÇÃO

1. *Imunogenética*

Imunogenética é a área do conhecimento que trata da base genética das respostas imunes. Além disso, ela inclui o estudo das vias imunológicas consideradas “normais” e o estudo de variações genéticas que resultam em respostas imunes defectivas ou ineficientes. Além de serem importantes *per se*, os estudos no ramo da imunogenética têm um grande potencial para a descoberta de novos alvos terapêuticos para diversas doenças relacionadas ao sistema imunológico (Nature, 2019).

1.1. *Imunobiologia e Imunogenética da Gestação Humana*

A gestação humana é um processo complexo e finamente regulado pelo sistema imune materno mesmo antes da implantação do embrião até após o momento do parto. Após a fecundação, a fusão das membranas do ovócito e do espermatozóide induz alterações químicas que impedem a penetração e fecundação por outros espermatozóides. Os cromossomos parentais são pareados e as primeiras divisões celulares ocorrem cerca de 24h após a fecundação. Essas primeiras divisões celulares são controladas por componentes citoplasmáticos do óvulo, pois até esse momento não há síntese de mRNA. O desafio imunológico da gestação inicia-se mais efetivamente de dois a três dias após a fertilização, com a chamada “ativação gênica do zigoto”, quando os antígenos de origem paterna começam a ser expressos. A partir desse momento, o sistema imune da mãe é posto em contato com antígenos não-próprios e, no decorrer de uma gestação de sucesso, o ambiente uterino deve configurar-se de forma a evitar a rejeição do feto em desenvolvimento. Nesse contexto, a placenta começa a se desenvolver, com a expressão de diferentes moléculas, recrutamento de diversas células imunes e uma intensa produção de exossomos e outras vesículas celulares. Todos esses componentes atuam de forma conjunta e, em última instância, possibilitam o adequado desenvolvimento do feto (Braude et al., 1988; Capmany et al., 1996; Hedlund et al., 2009; Stenqvist et al., 2013).

A placenta, órgão temporário derivado do feto, além de mediar as trocas gasosas e de nutrientes, atua como um importante regulador imune. Além disso, nesse órgão são observadas

estratégias que evitam a passagem de patógenos e o estabelecimento de infecções na interface materno-fetal (Gude et al., 2004; Kaminski et al., 2019a). A placenta torna-se um hemocorial após o remodelamento dos vasos sanguíneos maternos e o desenvolvimento das artérias espirais. Nesse contexto, o reconhecimento de antígenos paternos expressos pela placenta envolve tanto respostas imunes locais, na interface materno-fetal, quanto sistêmicas. O contato íntimo entre células fetais e células e tecidos imunes maternos representa um substancial desafio imunológico para o feto em desenvolvimento. Assim, diferentes células e moléculas atuam em sincronia culminando em um ambiente altamente imunomodulado, de forma a tolerar o desenvolvimento do feto, que pode ser comparado a um enxerto semi-alogênico (Trowsdale e Betz, 2006; Vianna et al., 2011; Svensson-Arvelund et al., 2015; Kaminski et al., 2019a). Diferentes estratégias para evitar um eventual "ataque" do sistema imune materno à placenta em desenvolvimento podem ser observadas na interface materno-fetal.

É importante salientar que, embora o ambiente central das reações imunológicas durante a gestação seja a interface materno-fetal, é possível que padrões de respostas imunes sejam também detectados na circulação sistêmica de gestantes. Ainda, os primeiros estudos acerca da imunologia da gestação e o histórico das descobertas ao longo das décadas foram realizados no contexto de caracterização do perfil de citocinas na circulação periférica de gestantes. A Figura 1 apresenta um histórico resumido dos estudos sobre a imunologia da gestação humana. Detalhes dos aspectos históricos das descobertas estão descritos na Parte I desta tese, principalmente no Capítulo I.

As primeiras inferências sobre a importância da imunologia na gestação foram realizadas pelo grupo de pesquisa do Sir Peter Medawar, nos anos 1950, quando a gestação humana era ainda vista como um fenômeno possível devido à separação anatômica entre a mãe e o feto, à imaturidade antigênica do feto e à inércia do sistema imune da mãe em relação ao feto (Billingham et al., 1953). No entanto, pesquisas subsequentes investigando o processo de reprodução humana postularam que as propostas de Medawar não eram compatíveis com a realidade e a totalidade do período gestacional de mamíferos, ou não eram suficientes para explicar o sucesso do processo gestacional (revisado em Kaminski et al., 2019a).

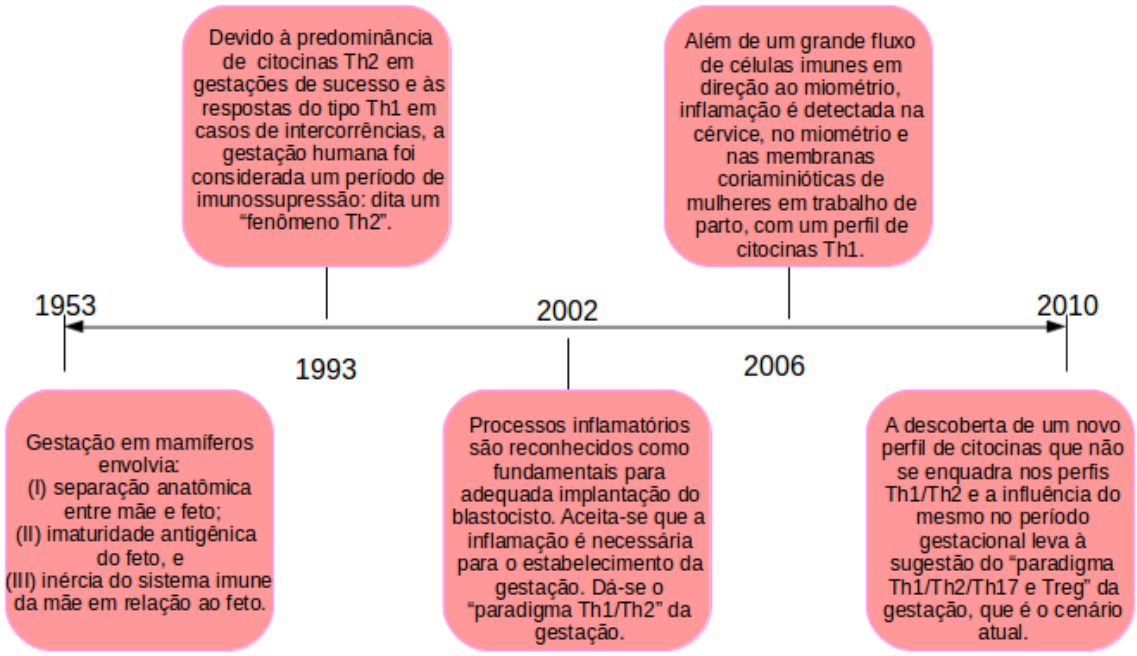

Figura 1. Histórico resumido dos estudos sobre a imunologia da gestação humana. As distâncias entre os anos das descobertas plotadas na figura não representam escala temporal correspondente.

Citocinas são pequenas proteínas secretadas pelas células que participam das interações e comunicações celulares (Zhang e An, 2007). Considerando o predomínio de citocinas antiinflamatórias na circulação sistêmica de gestantes, nos anos 1990 a gestação humana era vista como um "fenômeno Th2" (Wegmann et al., 1993). Na época, essa ideia foi reforçada devido à predominância de citocinas do tipo Th1, pró-inflamatórias, na circulação de gestantes que passavam por intercorrências gestacionais, como o aborto e a pré-eclâmpsia (Piccini et al., 1998; Raghupathy et al., 2000).

No entanto, após as descobertas de que tanto o processo de implantação do blastocisto quanto o parto são eventos fisiológicos em que respostas pró-inflamatórias são fundamentais, estabeleceu-se o "paradigma Th1/Th2 da gestação" (Chaoaut et al., 2002; Hill et al., 1995; Mor et al., 2011; Piccinni, 2002; Raghupathy et al., 2000; Wegmann et al., 1993). Assim, a complexidade da regulação imunológica durante a gestação começou a ser melhor compreendida. Porém, a descoberta de outras citocinas que não se enquadravam nem no perfil Th1 nem no perfil Th2 e a demonstração da importância de tais citocinas na gestação mexeu,

novamente, com os conceitos até então estabelecidos na imunologia gestacional (Chaoaut et al., 2002; Zenclussen, 2013).

O papel de células T regulatórias (Tregs) e Th17 e das citocinas produzidas por essas células na gestação (Wu et al., 2014) levou ao estabelecimento do chamado "Paradigma Th1/Th2/Th17 e Treg" da gestação, sendo este o cenário atualmente aceito (Saito et al., 2010). Discutido em maiores detalhes no Capítulo III, o papel de células (e citocinas) Th17 está relacionado à manutenção de períodos gestacionais prolongados (Pongcharoen et al., 2007; Martínez-García et al., 2011; Chavan et al., 2017; Kaminski et al., 2018).

Considerando o contexto da regulação imunológica para uma gestação de sucesso, além das citocinas abordadas acima, quimiocinas e receptores de quimiocinas são também importantes. Quimiocinas são citocinas quimiotáticas que direcionam a migração celular em diferentes contextos imunológicos. Por meio da ligação às quimiocinas via receptores de quimiocinas presentes em suas membranas, as células do sistema imune migram da corrente sanguínea em direção aos sítios de inflamação. Esse processo de migração se dá em resposta ao gradiente de quimiocinas estabelecido, cuja concentração aumenta em direção ao local em que o processo inflamatório está localizado (Ellwanger et al., 2019). Polimorfismos nos genes das quimiocinas e seus receptores são abordados em estudos imunogenéticos. Considerando a imunogenética de desordens gestacionais, diferentes trabalhos já evidenciaram o impacto de variantes em genes do sistema imune sobre os desfechos da gestação humana (Michita et al., 2016; Michita et al., 2018; Kaminski et al., 2019b).

Além das citocinas e seus receptores, diferentes células e moléculas residentes na decídua, ou derivadas da placenta, atuam ao longo do período gestacional. Tais componentes agem controlando diferentes aspectos e etapas da gestação, desde a implantação do embrião até o desenvolvimento completo do feto e o trabalho de parto. A decídua corresponde ao tecido interno de um útero gravídico. Esse tecido é formado por glândulas endometriais, vasos sanguíneos e pelo estroma. De todas as células que compõem a decídua, em número, os leucócitos representam de 15 a 30%, podendo estar distribuídos em grupos (*cell clusters*), em regiões subepiteliais ou distribuídos de maneira aleatória. Desses leucócitos, a maioria são células *natural killer* (NK) uterinas, células T do tipo αβ e γδ, células dendríticas (DCs) e macrófagos; células B são raras ou ausentes (Mincheva-Nilson et al., 1994; Moffet-King, 2002; Lash et al., 2010). A **Tabela 1** apresenta uma visão geral das moléculas envolvidas no controle das respostas imunes durante a gestação abordadas na presente tese.

Tabela 1. Visão geral das moléculas envolvidas no controle das respostas imunes durante a gestação.

Moléculas	Funções	Referências
Estradiol, progesterona e gonadotrofina coriônica humana	No contexto da receptividade uterina ao blastocisto a ser implantado, regulam fatores de transcrição, produção de citocinas e a expressão de moléculas de adesão.	Gude et al., 2004; Makrigiannakis et al., 2017
HLA-C, HLA-E, HLA-F e HLA-G	A expressão de HLA-E por células do trofoblasto extraviloso as permite evadir do ataque de células NK. HLA-G atua na modulação de células NK e APCs, também induzindo-as a secretarem citocinas e fatores pró-angiogênicos.	King et al., 2000; Moffett-King, 2002; Hackmon et al.,2017; van der Meer et al., 2004; Li et al., 2009; LeMaoult et al., 2004
Indoleamina - 2,3 dioxigenase (IDO)	Detectada ao longo da gestação e, no blastocisto, a partir do dia 6, IDO é uma enzima que cataliza a síntese do triptofano. Dessa maneira, induz células T à inanição, impedindo sua proliferação.	Kudo et al., 2004; Shayda et al., 2009
Fas e FasL	Receptor e ligante, respectivamente. Moléculas envolvidas em vias apoptóticas, podem ser detectadas no citotrofoblasto viloso e no sinciciotrofoblasto. Junto de outras moléculas, atuam na regulação das respostas imunes maternas na interface materno-fetal.	Uckam et al., 1997; Pongcharoen et al., 2004; Abrahams et al., 2004; Frängsmyr et al., 2005; Stenqvist et al., 2013
Ligante Indutor de Apoptose Relacionado a TNF (TRAIL)	TRAIL é expresso pela placenta de forma constitutiva. Em cooperação com o sistema Fas/FasL, TRAIL induz apoptose em linfócitos ativados na interface materno-fetal. São, assim, importantes durante a invasão e diferenciação do trofoblasto, promovendo um ambiente de imunotolerância.	Huppertz et al., 1998; Mor et al., 2002; Wiley et al., 1995; Bai et al., 2009

Early pregnancy factor (EPF)	Molécula imunossupressora, é detectada no soro de gestantes.	Fan and Zheng, 1997
Fator Indutor de Leucemia (LIF)	LIF é uma glicoproteína da família da Interleucina-6, com efeitos pleiotróficos, entre eles a indução de proliferação, diferenciação e sobrevivência de células do trofoblasto.	Aghajanova, 2004
CD59, Proteína Cofator de Membrana (MCP) e Fator de Aceleração de Decaimento (DAF)	São proteínas regulatórias do Complemento. São expressas pela placenta a partir da 6ª semana da gestação, evitando ou minimizando a ativação do complemento. A presença delas na interface materno-fetal é fundamental para a manutenção da gestação, evitando respostas imunes mediadas pelo Complemento.	Holmes et al., 1992
Receptor de células NK Grupo 2 membro D (NKG2D) e seus ligantes: Proteínas Relacionadas ao MHC-I (MIC) e Proteínas ligantes de UL16 (ULBP)1-6	NKG2D é um receptor ativatório expresso por células NK, NKT, Tαβ e Tγδ CD8+. O sistema receptor-ligante é um forte indutor de citotoxicidade, direcionado à eliminação de células estressadas, estrangeiras, invasoras ou infectadas. Os ligantes MICA/B e ULBP são expressos pela placenta em suas formas solúveis, associados à exossomos, resultando na regulação negativa do receptor cognato, suprimindo potenciais eventos de citotoxicidade na interface materno-fetal, promovendo a tolerância das células maternas em relação aos tecidos fetais/placentários.	Bauer et al., 1999; Stern-Ginossar e Mandelboim, 2009; Hedlund et al., 2009

Além das células e moléculas do sistema imune, vesículas extracelulares são extremamente abundantes na interface materno-fetal e evidências têm demonstrado que são de fundamental importância nos processos envolvendo uma gestação de sucesso. Dentre essas vesículas, destacam-se os exossomos, que são nanovesículas formadas por uma bicamada lipídica, com capacidade de transportar diferentes moléculas tanto em seu interior quanto na própria membrana, constituindo um importante mecanismo de comunicação celular à longa distância (Théry et al., 2002). A placenta ativamente produz e secreta exossomos, o que leva à formação de uma "nuvem" dessas nanovesículas na interface materno-fetal, cuja concentração diminui conforme a distância dessa região. Por meio de estudos de caracterização do perfil de moléculas associadas a esses exossomos, é sabido que os mesmos atuam na promoção de um ambiente imunossupressor, controlando respostas imunes maternas que podem ser nocivas ao feto e à placenta (Hedlund et al., 2009; Mincheva-Nilson, 2010; Stenqvist at al., 2013).

Exossomos derivados da placenta podem ser detectados na circulação periférica de gestantes, e sabe-se que aumentam em número ao longo do período gestacional (Salomon et al., 2014). Além disso, em casos de pré-eclâmpsia, por exemplo, o número dessas nanovesículas é muito maior em comparação ao encontrado em uma gestação normotensa, sendo a avaliação do perfil de exossomos uma potencial estratégia para monitorar o risco de intercorrências gestacionais (Kshirsagar et al., 2012; Tannetta et al., 2017a; 2017b).

1.2. *Imunogenética do Transtorno do Espectro Autista*

O autismo é caracterizado como uma desordem do neurodesenvolvimento humano, composta por um conjunto de condições clínicas de início precoce que se manifestam na infância. Atualmente esta condição está inserida em um conjunto de características neurocomportamentais referidas como "Transtorno do Espectro Autista" (TEA). Mencionado pela primeira vez em 1911 pelo psiquiatra alemão Eugen Bleuler, somente algumas décadas mais tarde Leo Kanner definiu o termo autismo, após a observação de um grupo de crianças com comportamento estereotipado (Kanner e Eisenberg, 1957).

Dentre as manifestações típicas de indivíduos com TEA encontram-se déficit de interação social e da comunicação verbal e não verbal, presença de comportamento repetitivo e estereotipado e interesses e atividades restritos. A manifestação deste quadro

clínico ocorre antes dos três anos de idade, afetando mais meninos do que meninas em uma relação de 4:1, o que remete à conhecida relação entre autismo e muitas doenças ligadas ao cromossomo X. Indivíduos diagnosticados com TEA podem apresentar diversos sintomas e comorbidades, caracterizando uma grande heterogeneidade de manifestações clínicas, com fenótipos que variam de leve a extremamente severos (O'Hare, 2009).

Os critérios diagnósticos do TEA são identificados através do Manual Diagnóstico e Estatístico dos Transtornos Mentais, que se encontra em sua 5ª edição revisada (DSM-V-TR). O TEA é um novo transtorno do DSM-V que engloba o transtorno autista do DSM-IV (autismo), transtorno de Asperger, transtorno desintegrativo da infância, transtorno de Rett e transtorno invasivo do desenvolvimento sem outra especificação (PDD-NOS) (Baio et al., 2018).

Devido à heterogeneidade na manifestação destes transtornos, o diagnóstico do TEA requer manifestação de déficits em dois domínios centrais: 1) déficits na comunicação social e interação social e 2) padrões repetitivos de comportamento, com interesses e atividades restritos. Ambos os domínios independem da manifestação de prejuízos intelectuais e de linguagem, sendo classificados em níveis de gravidade/severidade da manifestação (APA, 2013).

Em razão de se apresentar como uma doença com alta herdabilidade, um dos principais focos de pesquisa envolvendo o TEA é o seu provável fundo genético (Bai et al., 2019). Considerando que outras doenças genéticas se mostram associadas ao TEA, atualmente é amplamente aceito que o transtorno seja o resultado de interações entre fatores genéticos, epigenéticos e ambientais, incluindo os fatores associados ao sistema imunológico (Ivanov et al., 2015). Especificamente, idade paterna avançada (Wu et al., 2017), mudanças epigenéticas (Loke et al., 2015; Duffney et al., 2018), intercorrências gestacionais (Meltzer e Van der Water, 2017), uso de medicamentos como ácido valpróico (Nicolini e Fahnestock, 2018) e exposição a alergenos (Singer et al., 2016) durante a gestação, assim como desbalanços do microbioma (Kraneveld et al., 2016) são potenciais fatores contribuintes para a manifestação do TEA.

Como anteriormente citado, é atualmente consenso a contribuição de fatores genéticos para o desenvolvimento do TEA (Muhle et al., 2004; Michaelsonn et al., 2012; Vorstman et al., 2017). Segundo um trabalho recente envolvendo cinco países e mais de dois milhões de indivíduos diagnosticados com TEA, a contribuição de fatores genéticos

para essa condição é de aproximadamente 80% (Bai et al., 2019). Embora muitos estudos evidenciem a contribuição de muitos genes e variantes genéticas nos casos de autismo, até o momento nenhum padrão genético que, isoladamente, explique o quadro clínico foi identificado. Nesse contexto, abordagens envolvendo estudos genéticos de associação em larga escala (GWAS – *Genome Wide Association Studies*) têm sido realizados na tentativa de encontrar variantes em genes que podem atuar como fatores de risco para o TEA (Jiang et al., 2013). Salienta-se, ainda, que mutações genéticas únicas contribuem para 1-2% dos casos de TEA (Abrahams e Geschwind, 2008). Dessa forma, estudos genéticos em trios de famílias de diferentes populações abordando genes candidatos são, também, uma excelente ferramenta para avaliar a influência de polimorfismos nessa condição clínica complexa e multifatorial.

Considerando as alterações imunes presentes no TEA, o estudo de variantes em genes relacionados ao sistema imune contribui para o entendimento tanto da susceptibilidade quanto das diferentes manifestações clínicas da doença. Nesse contexto, diversos estudos abordando imunogenética e o TEA já foram realizados e estão apresentados detalhadamente no Capítulo V desta tese.

1.2.1. *Alterações Imunológicas na Gestação e Transtorno do Espectro Autista*

Originalmente, o TEA é referenciado como um transtorno neurocomportamental, porém, cada vez mais evidências indicam uma forte participação do sistema imune nesta condição (Masi et al. 2017; Meltzer e Van de Water, 2017). Assim, as interações entre o sistema imune e o ambiente parecem ser importantes não apenas após o nascimento do indivíduo, mas também ao longo do desenvolvimento fetal, situação já descrita como MIA (do inglês *maternal immune activation*, ativação imune materna), a qual já foi testada em modelos experimentais com camundongos e macacos rhesus (Meltzer e Van de Water, 2017).

Alterações nos níveis de citocinas no sangue de mães de crianças com TEA durante a gestação destacam-se entre os componentes imunes já descritos no contexto do TEA. Além disso, intercorrências gestacionais com fundo imunológico já foram associadas com a manifestação aumentada de TEA nos filhos de gestantes afetadas por tais intercorrências (Zerbo et al., 2017; Meltzer e Van de Water, 2017; Maher et al., 2018). Nesse contexto, já

foram relatados níveis aumentados de IFN-γ, IL-4 e IL-5 durante a gestação (Goines et al., 2011), além de um maior histórico de doenças auto-imunes em mães de filhos com TEA (Croen et al., 2005). Além disso, auto-anticorpos maternos contra proteínas cerebrais da criança já foram encontrados durante o período de gravidez. Experimentos com esses auto-anticorpos já foram conduzidos em camundongos. Fêmeas gestantes foram injetadas com auto-anticorpos provenientes de fêmeas que haviam gerado prole com características autistas. Os resultados mostraram que tal exposição provoca alterações no comportamento exploratório e motor na prole das fêmeas teste (Dalton et al., 2003). No mesmo modelo utilizando camundongos, a exposição pré-natal a auto-anticorpos humanos provenientes de mães de filhos autistas provocou alterações de ansiedade, reflexos de sobressalto e alterações de sociabilidade nos filhotes nascidos das fêmeas expostas (Singer et al., 2009). Estudos posteriores, avaliando moléculas IgG de camundongos que tiveram prole com traços autistas, demonstraram a especificidade desses auto-anticorpos em relação a proteínas cerebrais de 37 e 73 kDa (Braunschweig et al., 2012). Atualmente, sabe-se que auto-anticorpos com este padrão de reconhecimento são encontrados em 12% das mulheres que tiveram filhos autistas. Corroborando esta observação, um estudo utilizando macacos rhesus como modelo demonstrou alterações no comportamento social e no tamanho cerebral da prole de fêmeas que foram expostas a essa classe de IgGs contra as proteínas cerebrais do feto (Bauman et al., 2013).

Também é importante salientar que a inflamação, seja ela subclínica, crônica, aguda ou patológica, é cada vez mais observada como envolvida na patogênese de transtornos psiquiátricos. Embora não seja reconhecido como uma doença autoimune, o TEA compartilha diversas características com essa classe de doenças, como a predisposição genética a anormalidades imunes e grandes disparidades em relação ao sexo dos afetados (Lyall et al., 2017). Por fim, considerando que vesículas extracelulares já foram implicadas no contexto do autismo e de outras doenças psiquiátricas (Tsilioni e Theoharides, 2018; Saeedi et al., 2019) e considerando o papel dessas vesículas nas respostas inflamatórias e modulações do sistema imune, é possível que as vesículas extracelulares desempenhem um papel chave na susceptibilidade e/ou nas diferentes manifestações clínicas do TEA.

Considerando que alterações em respostas inflamatórias faz parte tanto (I) do quadro clínico de problemas gestacionais, (II) das possíveis causas para o desenvolvimento de TEA e (III) do quadro clínico do TEA, justifica-se o desenvolvimento da presente tese.

OBJETIVOS

Objetivo geral

Abordar de forma integrada os principais fatores imunogenéticos e ambientais que contribuem para o desenvolvimento de uma gestação de sucesso e/ou de intercorrências gestacionais, além de discutir como fatores inflamatórios e imunogenéticos podem ser fatores de risco para o desenvolvimento do Transtorno do Espectro Autista.

Objetivos específicos

- Correlacionar e resumir o papel da interface materno-fetal na tolerância do sistema imune materno em relação ao feto e os processos que modulam o trânsito de patógenos na interface materno-fetal, com ênfase na participação de exossomos em ambos processos.

- Abordar a molécula HLA-G e sua importância no estabelecimento e manutenção da gestação através da proposta de uma prova de conceito de um método contraceptivo baseado na regulação negativa dessa molécula no ambiente uterino.

- Comparar os níveis de citocinas Th1/Th2/Th17 presentes na circulação periférica de mulheres que sofreram aborto com os níveis dessas moléculas em um grupo de mulheres grávidas sem intercorrências gestacionais.

- Avaliar o impacto da deleção completa do gene *NKG2C* em mulheres que sofreram pré-eclâmpsia, em comparação com mulheres que tiveram gestação normotensiva.

- Avaliar o impacto da variante genética CCR5Δ32 em mulheres que sofreram pré-eclâmpsia, comparado com mulheres que tiveram gestação normotensiva.

- Revisar, apresentar e discutir o papel de variantes de genes relacionados ao sistema imune no contexto do Transtorno do Espectro Autista.

- Descrever e relacionar os fatores imunológicos envolvidos na inflamação durante a gestação que são considerados fatores de risco para o desenvolvimento do Transtorno do Espectro Autista.

- Avaliar o impacto da deleção completa do gene *NKG2C* em indivíduos diagnosticados com Transtorno do Espectro Autista e seus respectivos pais biológicos, abordando a sintomatologia dos pacientes e a transmissão dessa deleção dos pais para os filhos.

- Avaliar o impacto de polimorfismos nos genes *NKG2D* e *NKG2A* em indivíduos diagnosticados com Transtorno do Espectro Autista e seus respectivos pais biológicos, abordando a sintomatologia dos pacientes e a transmissão dessa deleção dos pais para os filhos.

Parte I

Capítulos I, II, III e IV

Capítulo II

Extracellular vesicles in host-pathogen interactions and immune regulation—exosomes as emerging actors in the immunological theater of pregnancy

Valéria de Lima Kaminski, Joel Henrique Ellwanger, José Artur Bogo Chies

Artigo publicado na revista científica Heliyon.

Heliyon 5 (2019) e02355

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Heliyon

Review Article

Extracellular vesicles in host-pathogen interactions and immune regulation — exosomes as emerging actors in the immunological theater of pregnancy

Valéria de Lima Kaminski, Joel Henrique Ellwanger, José Artur Bogo Chies *

Laboratório de Imunobiologia e Imunogenética, Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Exosomes
Extracellular vesicles
Viruses
Bacteria
Fungi
Parasites
Prion
Placenta
Maternal-fetal
Gestation
Infection
Vertical transmission
Genetics
Immunology
Microbiology

ABSTRACT

This review correlates and summarizes the role of the maternal-fetal interface in the immune tolerance of the fetus and the processes that lead to infection avoidance, emphasizing the participation of exosomes and other extracellular vesicles in both situations. Exosomes are released into the extracellular medium by several cell types and are excellent carriers of biomolecules. Host-derived exosomes and the transport of pathogen-derived molecules by exosomes impact infections in different ways. The interactions of exosomes with the maternal immune system are pivotal to a favorable gestational outcome. In this review, we highlight the potential role of exosomes in the establishment of an adequate milieu that enables embryo implantation and discuss the participation of exosomes released at the maternal-fetal interface during the establishment of an immune-privileged compartment for fetal development. The placenta is a component where important strategies are used to minimize the risk of infection. To present a contrast, we also discuss possible mechanisms used by pathogens to cross the maternal-fetal interface. We review the processes, mechanisms, and potential consequences of dysregulation in all of the above-mentioned phenomena. Basic information about exosomes and their roles in viral immune evasion is also presented. The interactions between extracellular vesicles and bacteria, fungi, parasites and proteinaceous infectious agents are addressed. The discovery of the placental microbiota and the implications of this new microbiota are also discussed, and current proposals that explain fetal/placental colonization by both pathogenic and commensal microbes are addressed. The comprehension of such interactions will help us to understand the immune dynamics of human pregnancy and the mechanisms of immune evasion used by different pathogens.

1. Introduction

In humans, recognition of self and nonself antigens, tissues or even whole organisms encompasses both local and systemic immune reactions. In the context of pregnancy, the intimate contact of fetal cells and maternal immune cells and tissues represents a substantial immune challenge. The maternal immune system must be shaped to tolerate the developing fetus, which can be compared to a semiallogeneic graft (Trowsdale and Betz, 2006; Vianna et al., 2011; Svensson-Arvelund et al., 2015). The search for factors involved in such immune adaptation has led many researchers in the field of reproductive immunology to examine the new universe of extracellular vesicles.

Extracellular vesicles (EVs) are secreted by cells from all eukaryotes and by prokaryotic organisms through shedding mechanisms (Colombo et al., 2014). Various biological fluids contain EVs, which can cross physical and physiological barriers and perform essential roles in cell-to-cell communication. Thus, EVs are critical modulators of the immune response under normal and pathological conditions (Nair and Salomon, 2018). EVs are usually classified according to their size and tissue or cell of origin (Colombo et al., 2014). However, it is difficult to assume the origin of a specific EV unless it is captured at the time of shedding by adequate imaging techniques. Therefore, it is now strongly recommended that operational terms encompassing size, shape, and biochemical composition be used for identifying EV subtypes (reviewed in Théry et al., 2018).

The term "EVs" encompasses microparticles, microvesicles (MVs), nanovesicles, nanoparticles, ectosomes, exosomes, exovesicles, and exosome-like vesicles (Colombo et al., 2014). The diversity of EVs, in terms of origin and function, makes an individual classification difficult for each type, and EVs have usually been differentiated based on their size, cargo, and origin (Nair and Salomon, 2018). Although we are in agreement with the recommendation of MISEV2018 (Minimal

* Corresponding author.
E-mail address: jabchies@terra.com.br (J.A.B. Chies).

https://doi.org/10.1016/j.heliyon.2019.e02355
Received 5 November 2018; Received in revised form 30 June 2019; Accepted 19 August 2019

information for studies of extracellular vesicles 2018) that "terms such as exosome and microvesicle that are historically burdened by both manifold, contradictory definitions and inaccurate expectations of unique biogenesis" (Théry et al., 2018), the purpose of this review is to present an overview of the role of exosomes in host-pathogen interactions and immune regulation in human pregnancy; therefore, the nomenclature for the EVs will be identical to that used by the authors of the original articles.

One specific group of EVs, exosomes, has received special attention, mainly due to its reported roles in both normal pregnancies as well as in pregnancy-related disorders (Mitchell et al., 2015). Exosomes are released into the extracellular space by virtually all types of viable cells and have a prominent function in intercellular and intracellular communication and as biomolecule carriers (Théry et al., 2002a). Different cell types release distinct types of exosomes under healthy and pathological conditions (Corrado et al., 2013). Exosomes in healthy pregnancy are known for their ability to induce and maintain, at least partially, a local immunosuppressive environment at the maternal-fetal interface (Hedlund et al., 2009). This ability is fundamental to the control of the maternal immune responses that would otherwise be harmful to the "semiallogeneic" fetus (Mincheva-Nilsson and Baranov, 2010; Mitchell et al., 2015).

It seems plausible that such intimate contact between mother and fetus, allied to local immune modulation, creates a scenario that favors infection of both individuals; however, with some exceptions, viral infections during pregnancy have been considered low-risk conditions until recently (Silasi et al., 2015). Even with placental control of both the mother's immune reactions and pathogen infections, microbes have found ways to bypass the placenta (Silasi et al., 2015; Coyne and Lazear, 2016). In addition, the findings revealing a healthy, normal microbiota in the placenta (Aagaard et al., 2014; Parnell et al., 2017; Seferovic et al., 2019) led to the following question: how do these organisms or their genetic material get in contact with the fetus before birth? The mechanisms that modulate the immune system and the universe of extracellular vesicles will probably offer some hints to the answer.

It is no wonder that an intensely immunomodulated environment at the maternal-fetal interface can open a window to relatively easy development of viral or bacterial infections. Nevertheless, placental tissues have physiological characteristics that hinder the entry of pathogens (Arora et al., 2017). Such strategies, whether physical or molecular in nature, make the placenta a complex organ where nutrient and gas exchanges occur while immune responses must be carefully regulated. In this review, we address important aspects of the local immune adjustments that enable embryo implantation and acceptance, minimizing the risk of abortion/rejection, and call attention to the placental microbiota and its implications during pregnancy. In addition, some aspects of exosomes produced by cells from the male genital tract and specific facts about sexually transmitted infections during pregnancy will be discussed.

2. Main text

Immunomodulation – is there relevant systemic immune suppression in pregnancy?

That the maternal immune system is largely suppressed in pregnancy is an oversimplified concept. Several studies indicate that immune system activation is a crucial step for healthy pregnancy development. Thus, it is inappropriate to think that human pregnancy could develop better without immune responses even in the absence of pathogens (Mor et al., 2011). The first studies on the immunology of pregnancy were conducted in the 1950s when Sir Peter Medawar first questioned that the fetus was accepted by the maternal immune system. His group postulated that fetal acceptance by the maternal immune system was due to an anatomical separation of the fetus from the mother, antigenic immaturity of the fetus, and immunological indolence/inertness of the mother toward the fetus (Billingham et al., 1953). Since then, various publications have addressed immune functions and dynamics during the gestational period. Nevertheless, in the 1990s, human pregnancy was still viewed as a period of immune inertness. Since T helper type 2 (Th2) cells are classically considered to be involved in anti-inflammatory responses, it seemed plausible to classify, at that time, human pregnancy as a "Th2 phenomenon" (Wegmann et al., 1993). In this line, as T helper type 1 (Th1) cells and cytokines were predominantly linked to inflammatory situations, Th1 cell responses were classically related to pregnancy complications (Raghupathy et al., 2000; Piccinni, 2002), and inadequate physiological outcomes of pregnancy or unsuccessful pregnancies were considered the result of a "Th1 response."

A subtle inflammatory process that involves the presence of numerous immune cells is essential for successful implantation. However, inflammation at the implantation site, more than a response against the fetus, promotes tissue remodeling and enables embryo implantation (Mor et al., 2011). Additionally, it is important to emphasize that the blastocyst is highly adhesive and travels throughout the fallopian tube to the implantation site. The endometrium is extensively covered by molecules that avoid blastocyst implantation, and it has been hypothesized that cytokines and chemokines produced by macrophages and dendritic cells (DCs) promote the degradation of these molecules covering the implantation site (Mor et al., 2011).

Considering that inflammation is a process characterized by the presence of a large number of Th1 cells and molecules derived from these cells, parturition was considered to be a pro-inflammatory phenomenon (Romero et al., 2006). Supporting this idea, inflammation has been detected in the cervix, myometrium, chorioamniotic membranes, and amniotic cavity of women in labor. Prior to parturition, there is a large influx of immune cells to the myometrium, creating an inflammatory profile and culminating in uterine contractions and delivery (Romero et al., 2006). Initially, it was believed that if such an inflammatory profile happened early in the gestational period, preterm delivery, miscarriage or other pregnancy complications would follow as a logical consequence (Wegmann et al., 1993; Raghupathy et al., 2000; Piccinni, 2002), but later, the need for a more complex Th1/Th2 balance was observed (Chaouat et al., 2002). These observations led to a new paradigm suggesting that slight inflammation at the beginning of pregnancy is followed by a longer period featured mainly by anti-inflammatory characteristics. Finally, signaling created by high levels of inflammation was expected near and at delivery. Since the description of this theory, several studies have supported it, but others have also contradicted the described dichotomy (Wegmann et al., 1993; Hill et al., 1995; Piccinni, 2002; Raghupathy et al., 2000; Chaouat et al., 2002; Mor et al., 2011).

Given such a dynamic balance at all phases of pregnancy, systemic immune suppression in pregnancy was doubted. How could an immune-suppressed organism control the dynamic fluctuations of cytokines and the migration, differentiation, and proliferation of so many immune cell types? The answer to this question is based on the absence of strong systemic immune suppression in pregnancy. Instead, tight immune regulation involves numerous cell types and molecules. Currently, human pregnancy is considered a very complex and meticulously regulated immune process. The description of a new set of cytokine-producing cells that did not fit the Th1/Th2 profile was essential to changing this pre-established paradigm (Chaouat et al., 2002; Zenclussen et al., 2002). Studies revealing the role of T regulatory (Treg) and Th17 cells and the molecules they produce during the gestational period are examples of recent discoveries that challenged the dichotomous Th1/Th2 view of human gestation (Saito et al., 2010). In this context, we highlight Interleukin-17 (IL-17), a pro-inflammatory cytokine that induces the expression of several inflammatory mediators (Witowski et al., 2004) and is produced mostly by T cells (Th17 cells) (Fu et al., 2014). Importantly, IL-17 has been shown to induce the production of proangiogenic molecules and to favor neovascularization (Numasaki et al., 2003). Concerning the human maternal-fetal interface, it was already been demonstrated that decidual cells recruit peripheral Th17 cells into the decidua by secreting CCL2 (Wu et al., 2014). At this location, Th17 cells

promote the proliferation and invasion of human trophoblast cells through the secretion of IL-17, which also inhibits apoptosis during the first trimester of pregnancy (Wu et al., 2014). Moreover, it had already been observed that IL-17 levels continuously increase throughout pregnancy (Martínez-García et al., 2011; Kaminski et al., 2018). In the context of mammalian pregnancy evolution, IL-17 can be assumed to be one of the molecules responsible for the maintenance of prolonged periods of gestation (Fu et al., 2014). The absence of IL-17A in marsupials suggests that it is an essential signaling molecule for the maintenance of the prolonged pregnancy observed in eutherian mammals, which differs from that of marsupials (Chavan et al., 2017). Of note, a new "Th1/Th2/Th17 and Treg" cell paradigm of pregnancy has been suggested and is the current trend. In this context, Th17 cells favor implantation and induce a protective immune response against microbes by the induction of inflammation, and Tregs, in contrast, are important for the immunoregulation and induction of tolerance (Saito et al., 2010).

Exosomes

As previously described, the term EV refers to exosomes (30–150 nm in diameter), microvesicles (0.1–1 μm) and apoptotic bodies (0.5–5 μm) released during both pathologic and healthy physiological situations (De Toro et al., 2015; Lo Cicero et al., 2015; Yáñez-Mó et al., 2015). Exosomes originate from multivesicular bodies (MVBs) and are formed by a lipid bilayer derived from their cells of origin. Various biomolecules, such as proteins and nucleic acids, are found attached to the lipid bilayer and/or inside the exosomes (Théry et al., 2002a). The secretion of proteins and nucleic acids through exosomes confers interesting features and advantages to this process: (I) the three-dimensional structure and biological role of the cargo molecules are preserved; (II) the delivery of molecular signals can occur independently of direct cell-cell contact; (III) the concentration of specific proteins inside exosomes can be maintained at high levels; (IV) the accurate delivery of biomolecules to the target (due to specific surface markers) is assured and can be achieved with long distances between the cells; and (V) *de novo* secretion in the target cell is not necessary (Mincheva-Nilsson and Baranov, 2010).

Due to the diversity of the cargos and target cells, exosomes can interfere with distinct pathways and affect different body systems. Taking into account the interests specific to the present review, it is important to emphasize that exosomes can act as modulators of immune responses. In this sense, exosomes derived from antigen-presenting cells have immune-activating properties (Théry et al., 2002b; Hwang et al., 2003). Additionally, syncytiotrophoblast-derived exosomes from non-pathological human placenta seem to participate in pathogen infection resistance pathways, although they can be immune suppressive or tolerogenic, such as exosomes from the majority of tumors and epithelial cells (Karlsson et al., 2001; Andreola et al., 2002; Mincheva-Nilsson and Baranov, 2010).

There is great debate over the most appropriate methods for isolating and characterizing exosomes. Diverse EV isolation techniques can be found in the original articles cited throughout this review. These methods are mainly based on differential and/or density gradient ultracentrifugation, size-based isolation techniques, coprecipitation, and immunoaffinity enrichment.

The most widely used exosome isolation technique is ultracentrifugation, which is considered the gold standard method. Ultracentrifugation isolation is based on the weight and size of the exosomes, and its low cost presents a major advantage over the other available methods; however, the exosome recovery is low. Size-based methodologies (which also consider molecular weight) produce a high yield through rapid processing; however, they lack specificity and require specific equipment, which are disadvantages. Based on the surface proteins present in the exosomes, the fastest and easiest method to isolate them is coprecipitation, which is characterized by high cost, low recovery, and a relative lack of specificity. At high cost and with low recovery capacity, the method of immunoaffinity enrichment recovers many exosomes of high purity (Bu et al., 2019).

Such techniques vary in adequacy depending on the sample of interest and are in continuous need of improvement (Bu et al., 2019). Considering these variables, we highlight the importance of following the latest proposals from the International Society for Extracellular Vesicles that are featured in MISEV2018 (Théry et al., 2018), the gold standard reference that presents the latest scientific advances for better handling of samples, from collection to storage, and are quite suitable for use with cell culture, biological fluids, or tissues.

An overview of exosome isolation methods is shown in the studies addressing placental exosomes from maternal circulation. For example, enriched fractions of these specific nanovesicles with minimal "contamination" from other EVs can be obtained through methods based on the proposed use of buoyant density centrifugation (Salomon et al., 2014; Sarker et al., 2014) and immunoaffinity capture using antibody-conjugated agarose beads (Lai et al., 2018). Alternatively, some studies have obtained exosomes from the supernatant of placental explant cultures using sequential centrifugation and ultracentrifugation, followed by identification and characterization by Western blotting, immune electron microscopy, and immuno-flow cytometry based on the proteins expressed on the surface of the isolated placental exosomes (Hedlund et al., 2009; Stenqvist et al., 2013). It is also important to consider the following limitation: most isolation methods cannot ensure the complete purity of the obtained vesicles, and it is possible to coisolate other nontargeted EVs and viral particles with the desired exosomes (Ellwanger et al., 2017).

To date, the most studied exosome markers are ALIX, TSG101, CD9, CD63, and CD81 (Ellwanger et al., 2017). However, the list of exosome markers is continuously revised, with new markers being incorporated at the same time that previously established markers are considered not sufficiently specific for exosomes. The direct consequence of such a dynamic research field is that different studies use different markers to identify exosomes. Thus, it is always a challenge to know when the authors actually worked with exosomes or with another type of extracellular vesicle (Ellwanger et al., 2017). Taking this into consideration, although we use the term "exosomes" throughout this review, it is important to emphasize that the data discussed here can possibly extend to the other types of EVs described in the literature collectively as "exosomes." In an attempt to clarify this situation, web portals have been organized through which researchers are working together to better classify the different subsets of extracellular vesicles, including exosomes (Kim et al., 2015).

The maternal-fetal interface: a site of intense immune regulation accounting for exosomes and other EVs

Just after fertilization of the oocyte by the spermatozoon, the binding and fusion of the sperm cell and oocyte membranes promote oocyte changes that block polyspermic fertilization and drive the resumption of oocyte meiosis (Capmany et al., 1996). At 24h post fertilization, parental chromosomes have intermixed, and the first cellular division occurs. Messenger RNA (mRNA) synthesis is absent as the initial cells divide, apparently driven exclusively by the maternal cytoplasmic signals, an event designated as the 'maternal legacy' (Braude et al., 1988). Such maternal signals could originate from maternal mitochondrial DNA, which replicates during early embryonic cell division. The point at which the paternal genome is activated and undergoes transcription is called zygotic gene activation and is first detected in embryos 2–3 days after fertilization (Braude et al., 1988). From this moment, the mother's immune system addresses the emergence of nonself antigens (those of paternal origin) to enable adequate fetal development. During pregnancy, the uterine environment promotes tolerance in relation to the developing fetus, avoiding maternal rejection of the fetal allograft, and it has been suggested that exosomes may be pivotal in the establishment of such an immune-privileged environment (Hedlund et al., 2009; Stenqvist et al., 2013).

The uterine receptiveness to blastocyst implantation is modulated by cyclic secretion of estradiol, progesterone, and human chorionic gonadotropin — the first known hormonal signals of the conceptus. These hormones regulate growth factors, cytokine production and the expression of the adhesion molecules that alter the endometrial surface, openning an implantation window (Gude et al., 2004; Makrigiannakis et al., 2017). The blastocyst is composed of two cell types with an inner and an outer cell mass. The inner cell mass develops into the fetus. The outer cell mass consists of undifferentiated trophoblast stem cells that form the cytotrophoblast (CTB) and the syncytiotrophoblast (STB). Before attachment, the zona pellucida is lost, and the trophoblast cell layer rapidly proliferates and differentiates into an inner layer, the CTB, and in an outer multinucleated mass, the STB (Gude et al., 2004). Subsequently, the STB extends into the endometrial epithelium and invades the connective tissue, breaking through the endometrial surface, provoking the natural tissue damage that ultimately enables implantation. Thus, the uterine endothelium and vascular smooth muscles of the mother's blood vessels are gradually replaced by trophoblast cells, creating the optimal conditions for initiating and developing the placental-fetal blood supply (Gude et al., 2004; Mor et al., 2011).

After the development of spiral arteries due to remodeling of the mother's blood vessels, the human placenta becomes a hemochorial villous organ. Such proximity enables the maternal blood to come into direct contact with the placental trophoblast cells (Gude et al., 2004). The functions of the placenta range from the exchange of nutrients, gases and metabolic residues to the production of regulatory molecules, which reveals its role as an immunomodulatory organ. The formation of the placenta starts at the implantation of the blastocyst into the uterine mucosa within 5–6 days post fertilization (Cross et al., 1994). The placenta is composed of two types of villi, the floating villi, which comprise an inner layer of CTB covered by the STB, which is bathed in maternal blood at the intervillous space, and the anchoring villi, which attach to the decidual tissue by highly invasive CTB cells referred to as extravillous trophoblasts (EVTs) (Hamilton and Boyd, 1960). The placental villous surface is in direct contact with maternal blood through the STB in a compartment that enables nutrient and oxygen supplementation and metabolic residue and cell debris removal and that, at the same time, hinders the passage of potential pathogens from the maternal circulation to the fetus (Gude et al., 2004; Delorme-Axford et al., 2013; Arora et al., 2017). Of note, in the STB, it is possible to find physical and molecular functions that ultimately block immune activation at the maternal-fetal interface (Robbins and Bakardjiev, 2012).

In this environment of intimate contact between the uterine region and the placenta, the exosomes secreted in the maternal blood are possibly the most abundant in the intervillous space of the chorionic villi. Moreover, the continuous release of exosomes by the STB creates an exosomal concentration gradient, accounting for stronger protection against an exacerbated maternal immune response at the maternal-fetal interface. It is said that the fetus, together with the placenta, is surrounded by a “cloud of exosomes” (Mincheva-Nilsson, 2010). Importantly, the concentration of the placenta-derived exosomes in the maternal blood increases during a healthy pregnancy (Salomon et al., 2014). In this context, it has been suggested that the concentration of placenta-derived exosomes in maternal blood could also be a potential marker of abnormal placentation (Kshirsagar et al., 2012). Trophoblast-derived exosomes could regulate the recruitment and differentiation of monocytes into tissue macrophages by inducing them to secrete the cytokines and chemokines required for trophoblast growth and survival (Atay et al., 2011). Placenta-derived exosomes lack the classical major histocompatibility complex (MHC) expression, presenting with the same characteristics as their tissue of origin. Instead, they express the nonclassical molecules MICA/B and RAE-T1/ULBP1–5, ligands of the activated Natural Killer (NK) cell receptor NKG2D (Mincheva--Nilsson et al., 2006; Hedlund et al., 2009).

The decidua is the mucosal layer of the resulting pregnant uterus. It comprises the endometrial/decidual glands, blood vessels, and the decidual stroma. Furthermore, leukocytes represent approximately 15–30% of all the cells in the early pregnant decidua of humans (Mincheva-Nilsson et al., 1994). The organization of decidual lymphoid tissue is unique and includes lymphoid cell clusters, subepithelial lymphoid cells, and individual immune cells that are randomly distributed. B cells are absent or rare; instead, there are abundant uterine NK (uNK) cells, αβ T and γδ T cells, DCs, and macrophages. Interestingly, uNK cells represent up to 70% of decidual leukocytes in the first trimester (Moffett-King, 2002), while there is no consensus regarding the distribution and number of uNK cells in later stages of gestation (Lash et al., 2010).

Immunomodulation in human pregnancy: tolerance is enhanced by exosomes and other EVs

Early studies on human preimplantation embryos reported the absence of expression of MHC class I or II genes (Roberts et al., 1992). The villous trophoblast, exposed to maternal blood, seems to lack expression of both MHC class I and class II proteins. However, the EVTs, which invade the uterus, express a particular combination of four MHC class I molecules: the classical HLA-C and the nonclassical class I molecules, HLA-E, HLA-F, and HLA-G (Moffett-King, 2002; Hackmon et al., 2017). The expression of HLA-E in EVTs enables them to evade NK cell-mediated cytotoxicity (King et al., 2000). Regarding HLA-G, it was first proposed that its expression in trophoblast cells also protected the fetus from maternal NK cell cytotoxicity, but this proposal is being debated, as HLA-G can induce secretion of cytokines and proangiogenic factors from decidual NK cells and human decidual antigen-presenting cells (APCs) *in vitro* (van der Meer et al., 2004; Li et al., 2009). Thus, it seems that HLA-G contributes to fetal tolerance by modulating decidual NK cell and APC responses (LeMaoult et al., 2004). Such nonclassical MHC molecules are of key importance not only in the establishment of the pregnancy but also in its maintenance, as revealed by studies in both healthy and pathological pregnancies (Tripathi et al., 2006; Michita et al., 2016; Persson et al., 2017; Meuleman et al., 2018). Notably, it has been shown that immunomodulatory molecules from the B7 family and the soluble HLA-G isoform HLA-G5 are secreted from the placenta during the first trimester and at term via exosomes (Kshirsagar et al., 2012).

As previously described, blastocyst implantation requires an inflammatory environment (Fest et al., 2007). The implantation process is achieved via the proper interaction of the innate uterine immune cells with the invading trophoblast (Huppertz et al., 1998; von Rango et al., 2003; Shih et al., 2006). During implantation, the uNK cells are pivotal for trophoblast invasion, and their absence is a predictor of poor vascularization of the placenta and pregnancy interruption (Hanna et al., 2006). Additionally, depletion of DCs leads to blastocyst implantation failure and prevents decidual development, likely due to failure in uterine receptivity; in healthy pregnancy, DCs orchestrate uterine receptivity through the regulation of tissue remodeling and angiogenesis (Placks et al., 2008).

In addition, equal to the importance of the role played by decidual leukocytes, cellular receptors and other molecules are important for fetal tolerance. For example, indoleamine 2,3-dioxygenase (IDO) is an enzyme that catalyzes the degradation of tryptophan, leading to cell starvation and inhibition of T cell proliferation. Interestingly, in the human blastocyst, IDO is detected from day 6 (Kudo et al., 2004). Furthermore, immunohistochemical analysis of mice revealed the presence of IDO throughout pregnancy (Shayda et al., 2009).

Genetic variants have also been reported as important in the likelihood of different pregnancy outcomes. For example, different gene polymorphisms can impact human gestation in such ways that can enhance or attenuate inflammation-related pregnancy disorders (Michita et al., 2016, 2018; Kaminski et al., 2019).

Membrane-associated and secreted immune regulatory factors are widely produced and secreted by placental tissues and are also detected in the maternal serum during the gestation period. For example, expression of Fas and FasL can be detected in the villous CTB and in the

STB (Pongcharoen et al., 2004; Frängsmyr et al., 2005); early pregnancy factor (EPF) activity was detected in sera from pregnant women (Fan and Zheng, 1997); progesterone-induced blocking factor (PIBF), which blocks lytic NK cell activity, was observed in term placentas (Anderle et al., 2008); and a variety of cytokines are expressed and tightly regulated both locally and systemically throughout pregnancy (von Rango et al., 2003). An important role for the Leukemia Inducing Factor (LIF) in implantation was shown in *LIF*-knockout mice, in which embryos failed to implant (Stewart et al., 1992). Notably, LIF is a secreted glycoprotein belonging to the interleukin 6 (IL-6) family, with pleiotropic effects that include the induction of the proliferation, differentiation, and survival of trophoblast cells (Aghajanova, 2004).

Complement activation at the maternal-fetal interface is avoided, or at least minimized, by the expression of the complement regulatory proteins CD59, MCP, and DAF in the placenta. These proteins are expressed on the trophoblast from at least 6 weeks of gestation, and their presence at the maternal-fetal interface (Holmes et al., 1992) implies a pivotal role in the maintenance of pregnancy, probably by protecting the developing fetus against maternal complement-mediated immune responses.

As previously stated, the human placenta constitutively expresses Fas and Fas-ligand (FasL) (Abrahams et al., 2004; Stenqvist et al., 2013). These molecules participate with other factors that regulate the maternal immune system. Fas is a type I membrane protein that is a member of the tumor necrosis factor (TNF) receptor family and is expressed by a wide variety of cells. FasL is a type II transmembrane protein expressed by activated T cells, some tumor cells and epithelial and other cells at immune-privileged sites (Ferguson and Griffith, 2006). Cross-linking of Fas expressed on the cell surface with its natural ligand FasL (also called CD95L) induces apoptosis (Nagata and Golstein, 1995). The FasL protein and mRNA transcripts were detected in human term placenta, with higher expression being detected in the STB layer (Uckan et al., 1997). It has been suggested that Fas determinants are expressed in lymphocytes when maternal lymphocytes are activated by fetal antigens. In this case, the interaction of the activated Fas-expressing T lymphocytes with the Fas-expressing STB cells would lead to apoptosis of the activated maternal lymphocytes, thus mitigating or preventing inflammatory responses towards the fetus (Uckan et al., 1997). Subsequent to this proposal, more studies confirmed the role of Fas and FasL in the human placenta. Apoptotic leukocytes, mainly T lymphocytes, can be seen at the maternal-fetal interface, strongly suggesting that these cells are negatively affected by immune suppression (Mor et al., 1998; Hammer and Dohr, 2000). In this context, it was proposed that clonal deletion of the activated immune cells through the Fas/FasL apoptotic pathway is involved in the establishment of the immune-privileged maternal-fetal interface (Runic et al., 1996; Uckan et al., 1997; Ohshima et al., 2001). In summary, the activated maternal lymphocytes that express the Fas receptor will undergo apoptosis when they interact with the FasL-expressing trophoblast. Experimental data demonstrate the importance of such molecules in human pregnancy. Abrahams et al. (2004) showed that, despite the absence of membrane-associated FasL in isolated first-trimester trophoblast cells, a cytoplasmic form of FasL is expressed in association with a specialized secretory lysosomal pathway (Abrahams et al., 2004). Later, this association was further investigated, and the abovementioned FasL association was found to be related to placenta-derived exosomes (Stenqvist et al., 2013).

TNF-Related Apoptosis-Inducing Ligand (TRAIL) is a type II membrane protein detected in a variety of tissues. TRAIL is constitutively expressed by the placenta, and its receptors DcR1 and DcR2 are located predominantly in the STB, and DR4 and DR5 are preferentially found in the CTB (Bai et al., 2009). DcR1 and DcR2 act as decoy receptors, while DR4 and DR5 are death receptors that are responsible for activating the apoptotic pathway (Truneh et al., 2000). TRAIL activates intracellular apoptotic pathways in a way similar to that of FasL, indicating a potential functional redundancy between these two ligands. Thus, TRAIL has been proposed, together with FasL, to be a cooperating factor for inducing apoptosis in activated lymphocytes (Wiley et al., 1995; Bai et al., 2009). These two molecules represent the most important apoptosis pathways and can be observed in the human placenta throughout pregnancy, where they participate in important processes such as trophoblast invasion and differentiation (Huppertz et al., 1998; Mor et al., 2002) and in the development of maternal immune tolerance towards the fetus (Phillips et al., 1999; Mincheva-Nilsson et al., 2000; Clark, 2005).

Importantly, the intracellular localization of FasL and TRAIL in the human placenta is intimately connected to the biogenesis of exosomes. These molecules, in their membrane form, are associated with induction of apoptosis. The observation of a constitutive release of FasL- and TRAIL-expressing exosomes from the apical microvillous surface of the STB suggests an important role of such structures in the protection of the fetus from maternal lymphocytes (Stenqvist et al., 2013). This finding is in accordance with the first demonstrations showing that FasL is targeted to the MVB of the secretory lysosomes and is expressed on exosome-like microvesicles (Martínez-Lorenzo et al., 1999; Jodo et al., 2000; Mincheva-Nilsson et al., 2000; Monleón et al., 2001; Andreola et al., 2002; Smith et al., 2003; Frängsmyr et al., 2005).

It is also important to consider the regulation of NK cell activity during pregnancy. In this context, both activating and inhibitory receptors should be taken into account. For example, the receptor Natural Killer Group 2 Member D (NKG2D) is a type II transmembrane protein belonging to the C-type lectin-like family and is expressed on the surface of NK, NKT, αβ T, and CD8+ γδ T cells. In these cells, NKG2D acts as an activating receptor (Bauer et al., 1999). NKG2D ligands are divided into two families: the MHC chain-related proteins A and B (MICA and MICB, respectively) and the UL16-binding protein (ULBP) 1–6, which is also known as retinoic acid early transcript 1 (RAET1). These ligands are distantly related to MHC class I molecules and are themselves signals of cellular stress instead of antigen-presenting molecules (Stern-Ginossar and Mandelboim, 2009). NKG2D stands out as a major activating NK cell receptor, and its ligand/receptor system is a potent inducer of cytotoxicity through a mechanism directed to the elimination of stressed, foreign, transformed or infected cells.

NKG2D ligands are expressed at low levels in normal cells. However, NKG2D ligands are upregulated or expressed *de novo* in response to a great variety of biological stress signals, such as those triggered by DNA damage, irradiation, oxidative stress, and inflammation, as a strategy to display stress, danger or pathological conditions in the cell (Raulet, 2003). Soluble NKG2D ligands downregulate the cognate receptor, suppress cytotoxicity and, upon release from tumors, protect tumor cells from host immune attack through an evasion strategy (Groh et al., 2002; Song et al., 2006). Interestingly, the release of soluble NKG2D ligands has been associated with exosomes in the context of cancer (Clayton et al., 2008) and pregnancy (Mincheva-Nilsson et al., 2006). MIC proteins A and B, the human ligands of the receptor NKG2D, are expressed by the placenta, delivered to the MVB of the STB and released via MIC-bearing exosomes into the circulating blood. In sera from pregnant women, a constitutive MIC is produced and released in its soluble form by the STB. It was suggested that this MIC release is associated with placenta-derived exosomes. Notably, the soluble MIC is able to downregulate the NKG2D receptor on peripheral blood NK cells and T cells, impairing NKG2D-mediated cytotoxicity (Mincheva-Nilsson et al., 2006). The second family of human NKG2D ligands, ULBP, is also expressed by the placenta (Hedlund et al., 2009). Immunoelectron microscopy revealed that ULBP1–5 are produced and retained in the MVB of the STB on microvesicles/exosomes. In addition, it has been confirmed that exosomes bearing NKG2D ligands are released by the human placenta. The isolation of placental exosomes indicated their ability to carry ULBP1–5 and MIC on their surface and to induce the downregulation of NKG2D on NK, CD8+ and γδ T cells, which culminated in the reduction of their cytotoxic effects without affecting the perforin-mediated lytic apoptosis pathway *in vitro* (Hedlund et al., 2009). Placental delivery of NKG2D ligands via exosomes suggests a bioactive role for the soluble forms of these ligands (Hedlund et al., 2009). Such discoveries emphasize a role

for NKG2D ligand-bearing placental exosomes in the evasion of the fetus from the maternal immune responses and reinforce the view of the placenta as an important temporary immune organ.

It is noteworthy that placental EVs can also be pro-inflammatory (Holder et al., 2016; Tannetta et al., 2017a, 2017b). In agreement with the slight inflammation required in early pregnancy, the syncytiotrophoblast-derived EVs from the initial gestational periods have more inflammation-inducing characteristics than has been observed for EVs secreted by the term placenta (Tannetta et al., 2017a). During normal healthy pregnancy, the exosome concentration in plasma can be as much as 50-fold greater in pregnant women than in nonpregnant women, with levels increasing significantly with gestational age. Such an increase is observed for both placenta- and nonplacenta-derived exosomes (Salomon et al., 2014). Since the characteristics of EVs resemble the cell type from which they were derived, Tannetta et al. (2017b) reviewed and called attention to the potential use of STB-derived EVs from the maternal circulation in pregnancy monitoring. According to this suggestion, alterations in cellular responses would likely alter the EV content, thus enabling the identification of potential imbalances in tissues located in regions of the body where an optimal biopsy cannot be performed. Moreover, EV levels could be measured throughout gestation and in a personalized manner.

With regard to other important features worth noting, EVs are very transitory in the maternal circulation and do not accumulate such that the analysis of a sample would represent an up-to-date picture of "placental well-being" in terms of EV levels and molecular fingerprints. One great example of the applicability of this proposed monitoring tool involves cases of preeclampsia, which is an important heterogeneous pregnancy disorder with symptoms, which include systemic inflammation, that are triggered by the placenta because of its impaired functioning. Notably, it has been shown that release of microvesicles and nanovesicles from the placenta is greatly augmented in preeclampsia, and all fractions of such EVs from preeclamptic placenta can induce activation of endothelial cells, likely via sequestration of Vascular Endothelial Growth Factor (VEGF) by fms-kinase 1, a vasoactive factor (Tong et al., 2017); VEGF is a component of the EVs isolated from normal gestation (Tong et al., 2016) and is also found in high levels in the circulation of women with preeclampsia (Tannetta et al., 2017b).

Avoiding the vertical transmission of pathogens at the maternal-fetal interface

The defense mechanisms by which the placenta limits microbial access to the fetus are still unknown. Notably, the intervillous space could contain as much as 500 mL of maternal blood, exposing the villous surfaces to microbes present in the mother (Arora et al., 2017). In addition to the place where the placenta implants, the decidua basalis is also the location where the semiallogeneic fetal trophoblast is in direct contact with these maternal cells and acts on immune tolerance. It is believed that the decidua keeps its immune privileged condition because of its immune cell components. This composition limits lymphocyte access, and precise regulation of chemokine expression is responsible for controlling cell traffic (Red-Horse et al., 2004; Nancy et al., 2012). This regulated immune environment is maintained due to constant maternal-fetal cross-talk between the invading fetal trophoblast cells and various maternal immune cell subsets (Mor and Cardenas, 2010; Arck and Hecher, 2013; Erlebacher, 2013; Zenclussen, 2013).

The syncytial surface of the human placenta acts as a first line of protection with unique physical properties, such as the presence of dense, branched microvilli at the apical surface and a complex cortical actin network that might limit microbial invasion (Cantle et al., 1987; Fisher et al., 2000; Koi et al., 2001; McDonagh et al., 2004; Maidji et al., 2010; Robbins et al., 2010; Zeldovich et al., 2011, 2013). In this context, it was demonstrated that disruption of the actin cytoskeleton subtly facilitates the invasion of *Listeria monocytogenes* (Zeldovich et al., 2013), indicating the existence of direct physical barriers that restrict pathogen infections (Arora et al., 2017). In a healthy pregnancy, the STB layer is greatly resistant to infection by viruses such as human cytomegalovirus (HCMV), herpes simplex virus-1 (HSV1), and Zika virus (ZIKV), and other pathogens such as *L. monocytogenes* and *Toxoplasma gondii* (Fisher et al., 2000; Koi et al., 2001; Maidji et al., 2006, 2010; Robbins et al., 2010; Delorme-Axford et al., 2013; Bayer et al., 2015, 2016). In addition to relying on physical barriers, resistance could be acquired by transfer from the STB of a full-term placenta to the nonplacental cells in a paracrine manner. Experiments have shown that this transfer involves placenta-specific microRNAs (miRNAs) and type III interferons that are both packaged within exosomes (Delorme-Axford et al., 2013; Bayer et al., 2015, 2016; Ouyang et al., 2016).

In an interesting experiment, primary human trophoblast cells were infected with different RNA and DNA viruses — coxsackievirus B3 (CVB), poliovirus (PV), vesicular stomatitis virus (VSV), vaccinia virus (VV), HSV-1, and HCMV. The cells showed high resistance to these infections. Additionally, when nonplacental cells, normally permissive to these viruses, were cultured with a medium containing material isolated from naïve primary human trophoblast cells, the nonplacental cells also presented some degree of resistance to the infection. In fact, the authors showed that this antiviral profile was due to exosomes released by primary human trophoblast cells (Delorme-Axford et al., 2013). These exosomes contain miRNA members of the chromosome 19 miRNA cluster (C19MC) that are almost exclusively expressed in the human placenta (Noguer-Dance et al., 2010; Donker et al., 2012). The trophoblast-derived exosomes packing these miRNAs from C19MC are capable of attenuating viral replication in target cells by inducing autophagy, thus representing a striking evolutionary adaptation that enhances protection of the fetus against viral infections (Delorme-Axford et al., 2013). Another study demonstrated this attenuated infection by the human immunodeficiency virus (HIV)-1, varicella zoster, rubella and other togaviruses in nonplacental cells previously exposed to the same abovementioned trophoblast-conditioned medium, emphasizing that human trophoblast cells can confer resistance to viruses implicated in perinatal infection (Bayer et al., 2015).

Other molecules important to the immune response to pathogens should also be cited here. Interferons (IFNs) are pro-inflammatory cytokines that enhance adaptive immunity and antiviral responses (Schneider et al., 2014). According to Bayer et al. (2016), primary human trophoblast cells isolated from full-term placenta are resistant to infection caused by two strains of ZIKV. Exposure to the conditioned medium isolated from these cells conferred resistance against these same ZIKV strains to nontrophoblast cells, likely due to the release of IFNλ1; as a result, the ZIKV must evade this strong antiviral response or bypass these cells and use another mechanism to access the fetal compartment *in vivo* (Bayer et al., 2016).

Defensins are part of a large family of antimicrobial peptides (Ganz, 2003) directed against specific gram-negative and gram-positive bacteria, yeasts, filamentous-phase fungi, and enveloped viruses (Svinarich et al., 1997). At the transcriptional level, defensins are also present in the human placenta, amnion, and chorion, suggesting their participation in the protection of the fetus against pathogen infection (Svinarich et al., 1997).

Pathogen-associated molecular patterns (PAMPs) are microbe-derived molecules which act as critical regulators of the innate immune response (Medzhitov and Janeway, 1997) and can be a threat to the development of a healthy pregnancy. In this context, Koh et al. (2014) addressed the release of pro-inflammatory cytokines and the expression of *NF-kβ* gene by JEG-3 and BeWo human choriocarcinoma cell lines under the influence of lipopolysaccharide (LPS), a common PAMP recognized by the immune system. Interestingly, an elevated inflammatory response was observed in JEG-3 cells in comparison to the BeWo cell line, indicating that LPS influence trophoblast cells in different ways. Moreover, despite the lack of NF-kβ response in BeWo cells, this study corroborates that bacterial products such as LPS can trigger an inflammatory response in trophoblast cells, thus representing a risk factor for

pregnancy disorders like preterm labor. Toll-like receptors (TLRs) are, in humans, a family of ten molecules that recognize and respond to PAMPs. Both TLR-2 and TLR-4 are expressed by amniotic epithelial cells (Kim et al., 2004; Adams et al., 2007), and TLR-2 expression is limited to the basolateral side of these cells (Kim et al., 2004). In situations where inflammation occurs, this expression pattern is lost, and both TLR-2 and TLR-4 are upregulated. Decidual cells, decidual macrophages, and neutrophils also express TLR-2 and TLR-4 (Kim et al., 2004). Decidual cells from the first and second trimester express TLR-2 and TLR-4 (Krikun et al., 2007), and at term, these cells express TLR-1 and TLR-6 (Canavan and Simhan, 2007). Regarding mRNA expression, all ten TLRs have been identified in term placentas (Zarember and Godowski, 2002; Abrahams, 2008). In the first trimester, EVTs and villous CTB cells highly express TLR-2 and TLR-4. The STB lacks expression of TLRs; however, in the third trimester, expression of TLR-2 and TLR-4 can be found in the outer STB layer and in intermediate and EVT cells (Holmlund et al., 2002; Kumazaki et al., 2004; Ma et al., 2007; Rindsjö et al., 2007). This change in the TLR expression pattern shows the ability of the placental villi to promptly respond to an infection at the placental surface. Additionally, this shift in TLR expression could reflect changes in placental function throughout the gestational period and might suggest how infection can impact pregnancy at each trimester (Abrahams, 2008).

The trophoblast also expresses cytoplasmic-based Nod-like receptors (NLRs) (Costello et al., 2007). Nucleotide-binding Oligomerization Domain (NOD) proteins recognize peptides derived from the degradation of bacterial peptidoglycans during normal bacterial growth or destruction (Girardin et al., 2003). NOD proteins are thought to be a second line of defense in cases where TLR signaling is defective, reduced, absent or has been evaded (Abrahams, 2008). In the first trimester of pregnancy, NOD1 and NOD2 proteins are detected in the CTB and STB (Costello et al., 2007); in term placentas, only NOD1 expression has been observed (Abrahams, 2011). In the decidual stroma and glandular epithelium, NOD1 and NOD2 are also expressed (King et al., 2000).

Interestingly, transplacental trafficking of EVs from the mother to the fetus was also demonstrated. Holder et al. (2016) showed that macrophage-derived exosomes are internalized by the human placenta. This process likely occurs in a time- and dose-dependent manner via clathrin-dependent endocytosis. Such internalized exosomes have the ability to prompt the secretion of proinflammatory cytokines, thus potentially enhancing the responses to maternal inflammation and infection and thereby thwarting harm to the developing fetus. This is an important finding that indicates the existence of a highly controlled and bidirectional extracellular vesicle-mediated transfer of protein and nucleic acids that accounts for the balance of immune responses at the maternal-fetal interface (Holder et al., 2016).

The different pathways discussed here highlight the diverse immune mechanisms that protect the developing fetus from pathogen infections without invoking harmful immune responses. This process results in an immune uterine environment that must undertake controlled responsiveness such that a slight inflammatory state is created for embryo implantation. In a second, concomitant task of the uterine immune environment, immune responses are developed towards potential infections in such a careful manner that the first task is not disrupted.

Pathogens bypassing maternal-fetal immune defenses: an arms race in the biological world

The STB has been shown to be refractory to several infections. However, this feature seems to be almost exclusive to this cell type, since neither the amniotic epithelium and CTB cells of the chorionic villi isolated from mid- and late-gestation placentas nor explants from the first trimester showed such resistance (Tabata et al., 2016). Interestingly, experiments with ZIKV showed that early trophoblasts are quite susceptible to infection, but this susceptibility is lost as the STB is formed, with the trophoblast cells becoming increasingly resistant to ZIKV infection (Sheridan et al., 2017).

The classic ways of vertical infection of a developing fetus by pathogens are (I) infection of endothelial cells in the maternal microvasculature that spread to invasive extravillous trophoblasts (EVTs); (II) trafficking of infected maternal immune cells across the placental barrier; (III) paracellular or transcellular transport from maternal blood across the villous trees and into the fetal capillaries; (IV) damage to the villous tree and breaks in the STB layer; and (V) transvaginal ascending infection (Coyne and Lazear, 2016). However, except for the infections caused by some specific pathogens included in the TORCH group (toxoplasmosis, "other," Rubella, CMV, and HSV), viral infections during pregnancy are often considered of little concern from a clinical point of view. Women are frequently infected by viruses during pregnancy without severe consequences to their developing progeny (Silasi et al., 2015). Nonetheless, the recent ZIKV epidemic and the developmental problems related to ZIKV infection in newborns reveal the possible consequences of neglecting pathogens in pregnant women, including undesirable gestational outcomes and a concomitant high cost in terms of health services (Schuler-Faccini et al., 2016; Ellwanger and Chies, 2018).

When pathogens breach the STB and reach the underlying villous core, inflammation of the placental villous is the result. Such inflammation eventually induces monocyte binding to the syncytial surface through ICAM-1 (Juliano et al., 2006). This reaction can cause an immune-mediated breakdown of the STB, which creates damage that could predispose the individual to infections mediated by other pathogens (Mor and Cardenas, 2010).

As already discussed, there are two anatomical interfaces between maternal cells and fetal cells — the trophoblasts, which constitute the villous region where maternal blood bathes the STB for nutrient exchange, and the maternal decidua, where the EVT anchors the villous region to the uterus. Using first-trimester human placental explants, it was shown that the interface composed of the EVT is significantly more vulnerable to infections, despite having a much smaller surface area (Koi et al., 2001; McDonagh et al., 2004; Robbins et al., 2012). Furthermore, it has been shown that EVT cells are not as resistant to infections as STB cells, and distinct studies suggested EVTs as favorite targets of some pathogens (Robbins et al., 2010, 2012; Tabata et al., 2016). In this context, an experiment demonstrated the preference of *L. monocytogenes* for infecting EVTs by penetrating the intrauterine space (Robbins et al., 2010). This is probably due to the lack of E-cadherin expression by the STB, which is the receptor for internalin, a surface protein required for the entry of *L. monocytogenes* into epithelial cells (Mengaud et al., 1996). Additionally, a study used cultures from first trimester placentas to define where and how *L. monocytogenes* breaches the maternal-fetal barrier and demonstrated that the EVT is the preferred site for the initial placental infection (Robbins et al., 2010). A cell culture model system of primary human EVT was used to study the intracellular life cycle of *L. monocytogenes* inside EVTs. Isolated EVTs were able to restrict intracellular bacterial growth and spread, preventing vacuolar escape, and were also capable of guiding vacuolated bacteria towards lysosomes for degradation. This finding suggested that the EVT has effective defense mechanisms against intracellular pathogens and is a significant bottleneck to transplacental infections (Zeldovich et al., 2011).

Among the different strategies of immune system evasion, an interesting example comes from *T. gondii*. It was suggested that a fetal infection from *T. gondii* starts with maternal immune cells of the decidua acting as "Trojan horses" (Oz, 2017). Subsequently, the pathogen is transferred from the infected leukocytes to susceptible EVT cells or even to other cell types. Once successful in those two steps, *T. gondii* bypasses the villous core and infects fetal vascular tissues such that it reaches the central nervous system (Arora et al., 2017). The use of immune cells as Trojan horses by *T. gondii* facilitates parasite entry into immune-privileged sites. Additionally, *T. gondii*-derived exosomes have the ability to change the cytokine profile of the macrophages to modulate their activation *in vitro*. A positive aspect of this mode of infection is that *T. gondii*-derived exosomes are excellent therapeutic candidates since they have been shown to trigger humoral and cellular immune responses

and induce partial protection against acute parasitic infection in mice (Li et al., 2018).

The HCMV replicates in the underlying CTB of the floating and anchored villi, which is a place where STB is sparse. Therefore, this virus must first breach the STB and its defenses. As suggested, HCMV may bypass the STB through transcytosis of the virions in an antibody-mediated manner throughout the neonatal Fc receptor that serves as an IgG transporter instead of through direct infection (Arora et al., 2017). In addition, studies using decidual tissue cultures with clinically derived and laboratory-derived viral strains *ex vivo* showed that the HCMV could also target the EVT as well as the microvasculature and leukocytes to reach the CTB (Weisblum et al., 2011).

Immune evasion of pathogens mediated by exosomes and other EVs: a double-edged sword

The constant clash described as "pathogens *versus* immune system" involves a multifaced and very complex process. Several immune evasion mechanisms were selected during viral evolution, and many viruses usurp both exosomal trafficking and budding pathways with distinct consequences in terms of infectivity and viral spread (Gould et al., 2003; Anderson et al., 2016; Raab-Traub and Dittmer, 2017; Sadeghipour and Mathias, 2017). For instance, exosomes packing viral particles can promote viral persistence, increasing the potential for viral infection, since the viral material is "masked." Viruses, namely those that enter cells by endocytosis, can usurp endosomal/exosomal pathways to advance their infectivity and spread (Nour and Modis, 2014; Anderson et al., 2016). Dengue virus (DENV), West Nile virus, hepatitis C virus (HCV), and ZIKV are examples of pathogens that enter cells with mechanisms related to endosome formation, and exosomes also have an endosomal origin such that their proximity may facilitate the accumulation of viral antigens in exosomes, thus increasing their spread and infection capacities (Smit et al., 2011; Anderson et al., 2016). When secreted as exosomes, intraluminal vesicles (ILVs) containing viral genomes can target uninfected cells and then penetrate them via endocytic pathways. Evidence of this process has already been observed in cases of HCV, the genome of which can be secreted in ILVs as infectious particles (Liu et al., 2014). Thus, viruses such as HCV can hijack components of the vesicular trafficking machinery and thereby integrate viral components into exosomes (Liu et al., 2014; Raab-Traub and Dittmer, 2017). Therefore, it is likely that other viruses with genomes that can be found in endosomal ILVs are also trafficked between cells via exosomes (Raab-Traub and Dittmer, 2017; Sadeghipour and Mathias, 2017). Recently, many studies have addressed the interaction of exosomes with different viruses. Some viruses reportedly interact with exosomes, such as bunyavirus, cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis A virus (HAV),

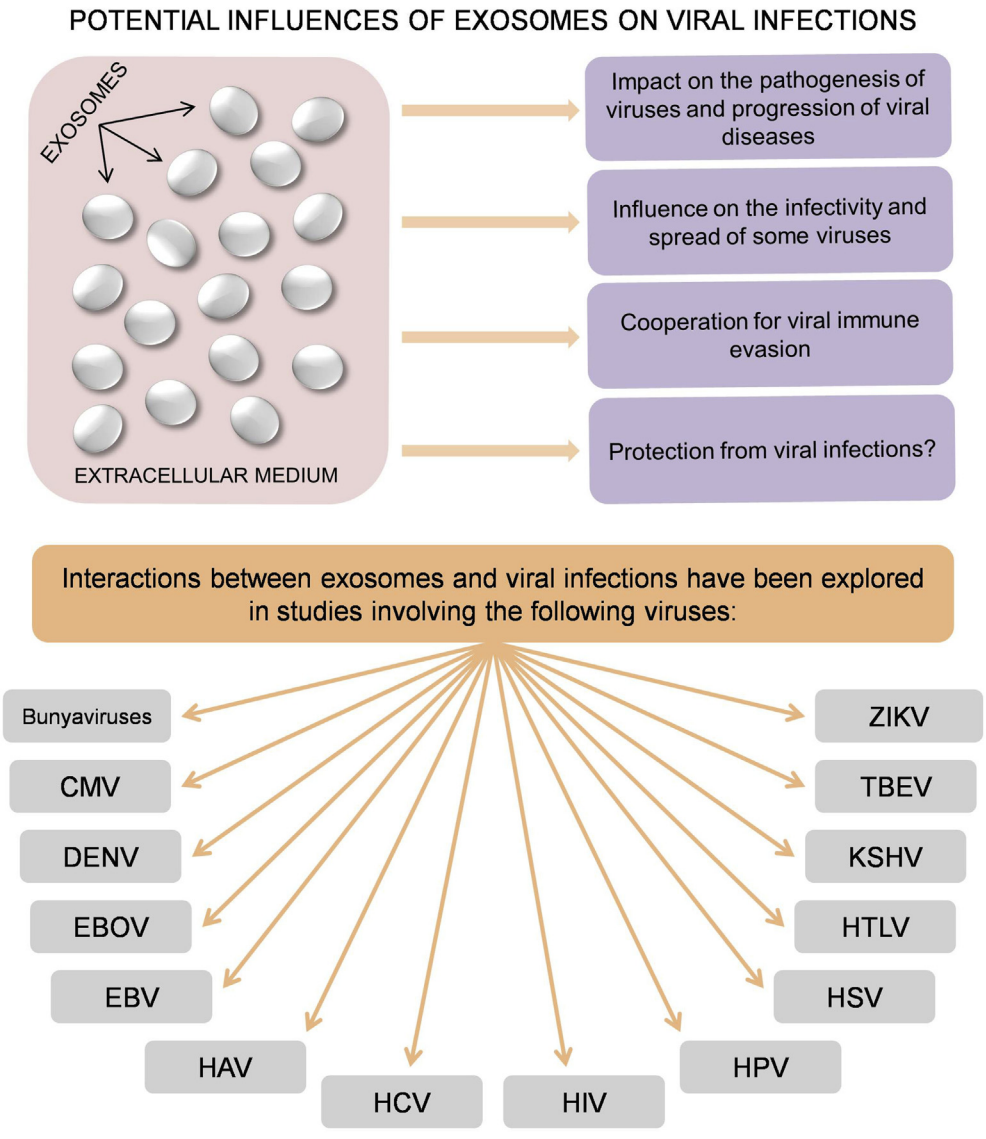

Fig. 1. Potential influences of exosomes on viral infections and examples of viruses for which their interaction with exosomes have been investigated using different methodological approaches. CMV: Cytomegalovirus; DENV: Dengue virus; EBOV: Ebola virus; EBV: Epstein-Barr virus; HAV: Hepatitis A virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; HSV: Herpes simplex virus; HTLV: Human T cell lymphotropic virus; KSHV: Kaposi's sarcoma-associated herpesvirus; TBEV: Tick-borne encephalitis virus; ZIKV: Zika virus. References are cited throughout the text.

herpes simplex virus 1 (HSV-1), HIV, human papillomavirus (HPV), human T cell lymphotropic virus (HTLV), DENV, ZIKV, tick-borne encephalitis virus (TBEV), and Kaposi's sarcoma-associated herpesvirus (KSHV) (Anderson et al., 2016; Ellwanger et al., 2017; Raab-Traub and Dittmer, 2017; Sadeghipour and Mathias, 2017; Martins et al., 2018; Zhou et al., 2019; Ellwanger and Chies, 2019; Reyes-Ruiz et al., 2019). In addition to the viruses covered in these studies, Ebola virus (EBOV) also appears to be associated with exosomes, in particular, those that package the viral protein VP40, causing immune cell dysfunction (Pleet et al., 2016). Furthermore, a prominent research field to be explored stems from the antiviral action of exosomes (Li et al., 2013; Madison et al., 2014). Fig. 1 presents a summary of some interactions between exosomes and viral infections that have been suggested to date.

As stated in previous sections, EVs can influence other pathogens besides viruses. The interaction between exosomes and pathogens can modify the outcomes of the infections. Such modifications caused by the action of EVs can enhance the infectivity of a particular microorganism via the delivery of infective particles and pathogen-derived molecules to distant sites from the primary focus of infection. Alternatively, EVs can favor the immune evasion of the pathogens from the host (Zhang et al., 2018). For example, *Staphylococcus aureus*-derived exosomes can transfer virulence factors, such as α-toxin, to cells found in distant physiological sites from the original location of the bacteria (Husmann et al., 2009). Also, cells infected by *Bacillus anthracis* secrete exosomes containing pathogen-derived toxins, thus enabling the action of virulence factors at long-distances (Abrami et al., 2013). In the context of *Helicobacter pylori* infection, exosomes have been associated to the secretion of cytotoxins by the host gastric epithelial cells, promoting extragastric complications (Shimoda et al., 2016).

Intrauterine infections by bacteria also represent a major threat to pregnancy when they gain access to gestational tissues. Bacterial infections can take place via the maternal circulation, in the peritoneal cavity, or ascend into the uterus from the lower tract (Espinoza et al., 2006). Regarding *L. monocytogenes,* a successful infection in the EVT could rely on the capacity of this bacterium to produce extracellular vesicles, called bacterial membrane vesicles (MVs). Studies have shown that MVs help bacteria survive inside mouse embryonic fibroblasts *in vitro* (Vdovikova et al., 2017). Considering opportunistic infections of the genitourinary tract, Group B *Streptococcus*-derived MVs loaded with virulence factors led to up-regulation of pro-inflammatory cytokines and inflammation related-symptoms of chorio-amnionitis in mice. These observations suggested that these bacterial-derived MVs are capable of triggering events at the maternal-fetal interface associated with preterm birth or even fetal death (Surve et al., 2016).

Intracellular pathogens such as *Mycobacterium tuberculosis*, which primarily infect macrophages in the lungs, also interact with microvesicles. It is known that infection of macrophages with mycobacteria leads to the release of EVs by the infected host cells. These released EVs contain numerous mycobacterial lipoglycans, lipoproteins, and antigens that modulate the immune response of the host (Bhatnagar et al., 2007). Moreover, electron microscopy images of *M. bovis* BCG-infected macrophages suggested that these EVs are indeed exosomes (Giri and Schorey, 2008). However, a study brought important evidence regarding the origin of EVs in the context of *M. tuberculosis* infection. It was demonstrated that some immune modulations related to TLR2 signaling provoked by this pathogen in the host are derived from the bacterial membrane vesicles rather than exosomes derived from the infected macrophages. In summary, the study suggested that the impairments on immune effector functions are primarily driven by the exportation of *M. tuberculosis* lipoglycans and lipoproteins released from the pathogen cell membrane (Athman et al., 2015).

Other infectious agents besides viruses and bacteria present interesting links with EVs. Prions are proteinaceous infectious particles that spread in the host cells due to the ability of these proteins to interact and shape the quaternary structure of nascent proteins. The resulting quaternary structure is an exact copy of the proteinaceous infectious proteins, which can also modify other proteins, thus further spreading the infection. Prions are responsible for transmissible spongiform encephalopathies in humans and other mammals. These infections are fatal and commonly known as prion diseases (Prusiner, 1982; Watts et al., 2006). Interestingly, exosomes can contain and transport prion proteins, contributing to the spread of these proteins in the infected organism (Robertson et al., 2006; Fevrier et al., 2004; Hartmann et al., 2017). Moreover, a study demonstrated that mouse neuroblastoma cells transmit cytosolic prions in association with membrane-bound vesicles besides the classical transmission by direct cell contact. The presence of flotillin, Alix-1, and Tsg101 and cup-shaped appearance of the EVs indicated that these vesicles indeed represented exosomes (Liu et al., 2016).

Regarding parasites, interesting features involving the pathogenesis of malaria and EVs secretion by host cells were addressed. Red blood cells infected by *Plasmodium falciparum* can secrete EVs containing molecules involved in the silencing of gene expression in endothelial cells. Also, these EVs were efficiently internalized by the endothelial cells and can disrupt the mechanisms responsible for hindering the entry of pathogens, thus enhancing the infection of the parasite in the host target cells (Mantel et al., 2016).

Besides the previously mentioned aspects regarding EVs and *T. gondii* infection in pregnancy, the pathogenesis of this parasite can be further affected by exosomes through the transference of molecules that alter the host cell cycle. Such alterations eventually decrease host cell proliferation, favoring the parasite because it invades cells in the S stage more easily than in other phases of the host cell cycle (Kim et al., 2016).

The bloodstream form of *Trypanosoma brucei* secrete EVs that interact with the cell membrane of host erythrocytes. Thus, it was postulated that fusogenic EVs derived from the trypanosome may act as vehicles for pathogen-to-host cell transfer of membrane proteins. Of note, this fusion between EVs from the pathogen and host cells results in the transfer of lipids and antigens derived from the parasite to the host cells. Such traffic of molecules has the potential to cause host anemia, a clinical outcome due to modifications in the structure of the host erythrocytes probably related to the incorporation of lipids from the parasite via EV fusion (Szempruch et al., 2016).

The first discovery regarding the role of EVs in fungal infection was made addressing *Cryptococcus neoformans* (Rodrigues et al., 2007). It was demonstrated that the *Cryptococcus*-derived virulence factor glucuronoxylomannan was produced inside the cell and then released in the extracellular environment inside EVs. Of note, EVs released by *C. neoformans* facilitate the pathogen passage through the blood-brain barrier and modulate the host immune responses, enhancing *C. neoformans* pathogenesis (Huang et al., 2012; Bielska and May, 2019).

Subsequently, various studies addressing associations between fungi and EVs have emerged (Rodrigues et al., 2014; Coakley et al., 2015; Peres da Silva et al., 2015; Joffe et al., 2016; Bielska and May, 2019). Like EVs derived from other species, fungal EVs transport proteins, lipids, pigments, polysaccharides, and genetic cargoes (Joffe et al., 2016). Thus, fungal EVs can induce strong and different impacts on host immunity, including stimulation of pro- and anti-inflammatory cytokine production (Bielska and May, 2019). Therefore, it can be speculated that fungi-derived EVs may have some impact on the host's inflammatory status, triggering other health problems not directly related to the fungal infection, but due to unbalanced inflammation. Importantly, fungi EVs can also interact with other pathogenic microorganisms in co-infected hosts, generating an even more complex immune landscape. Also, considering that approximately three hundred species of fungi are pathogenic for humans and only eleven species have their EVs addressed, more studies in this field are necessary (Bielska and May, 2019).

Parasitic helminths are metazoan organisms that also produce and secrete exosomes. In this context, studies addressing trematodes demonstrated intact exosomes in the parasites' teguments, indicating that these vesicles could also reach the host environment. Initially, it was speculated that exosomes derived from these parasites participated in the

down-regulation of the host immune responses, a common feature of helminth infections. The immune manipulation of the host immune responses by these parasites eventually ensures the survival of the parasites, mainly by exporting a range of immuno-modulatory mediators that interact with host cells and tissues. Evidence for the role of exosomes in the host immune modulation by helminths was demonstrated by the internalization of helminth-derived exosomes by host intestinal epithelial cells (Coakley et al., 2016).

Open questions and emerging topics

Seminal exosomes — friends or foes in sexually transmitted infections?

The role of semen in sexually transmitted viral infections is another interesting topic that connects exosomes, infectious diseases, and reproduction. Semen is a complex fluid composed of cells and seminal plasma. Human semen contains immunosuppressive components with the ability to drive tolerance towards paternal antigens, consequently maximizing the chances of successful fertilization. This immune suppression is likely derived from the low incidence of antibodies against sperm and the soluble components of semen in the woman body (Johansson et al., 2004). However, the immunosuppressive properties of semen could also contribute to the evolutionary success of sexually transmitted viruses; that is, they may take advantage of the immune suppressed environment that follows from exposure to semen (Sabatté et al., 2011). Seminal plasma has a high concentration of subcellular lipid-bound microparticles that are morphologically and molecularly consistent with exosomes that originate from multiple cellular sources of the male genital tract (Renneberg et al., 1997). These microparticles are, in general terms, called "seminal exosomes" (Vojtech et al., 2014). In summary, the immunosuppressive properties of seminal plasma seem to be related to its exosome fraction, and therefore, exposure to seminal exosomes could facilitate the establishment of viral infections (Vojtech et al., 2014).

The wide variety of cells that secrete exosomes also dictates the spectrum of biological fluids from which they can be isolated: amniotic fluid, breast milk, bronchoalveolar lavage fluid, cerebrospinal fluid, malignant ascites, plasma, saliva, synovial fluid, urine, vaginal fluid, and semen, among others (Ellwanger et al., 2017). Considering semen, each ejaculate contains trillions of exosomes with an average of 2.2×10^{13} particles (Vojtech et al., 2014, 2016). Seminal exosomes are considered immunosuppressive particles due to their inhibitory action during lymphoproliferative responses (Kelly et al., 1991), phagocytic cells (Skibinski et al., 1992), and NK cell function (Tarazona et al., 2011). Seminal exosomes are efficiently and rapidly captured by peripheral and vaginal DCs, whereas seminal exosomes are captured by T cells in the vaginal environment at a lower rate and efficacy (Vojtech et al., 2016). The immunosuppressive properties of semen are predominantly restricted to the seminal plasma since isolated sperm cells can induce immune responses and alterations in the uterine environment, and seminal plasma alone induces tolerance to paternal antigens and confers benefits to the offspring mainly in early pregnancy (Robertson et al., 2009; Bromfield, 2014).

Although there is some evidence suggesting that semen-derived exosomes have anti-HIV activity, exosomes apparently do not have an effect on the replication of other viruses (Madison et al., 2014). Recently, it was shown that Herpesviruses hijack host exosomes, which contributes to their viral pathogenesis (Sadeghipour and Mathias, 2017). Thus, once viruses take advantage of the local altered/suppressed immune responses induced by exposure to semen, semen immunosuppressive properties can contribute to the prevalence of sexually transmitted viral infections. In addition, as described above, the tremendous number of exosomes present in semen could facilitate viral spread.

Do exosomes facilitate transplacental and sexually transmitted viral infections?

The role of exosomes as facilitators of (I) transplacental and (II) sexually transmitted viral infections (Fig. 2) should be considered based on the following four premises:

1st) Trojan exosomes: Gould et al. (2003) hypothesized that retroviruses such as HIV and HTLV could usurp the machinery that causes the budding and trafficking of exosomes to infect new cells without being recognized by the immune system. Although the Trojan exosome hypothesis is still debated, some experimental evidence supporting the cellular mechanisms consistent with this theory has been published (Nguyen et al., 2003; Booth et al., 2006; Gan and Gould, 2012; Kadiu et al., 2012). Interestingly, the presence of HCV particles in exosomes has already been demonstrated (Liu et al., 2014). Moreover, the detailed cellular mechanisms of the budding/trafficking of exosomes that could be used by HCV, HAV, HIV, EBV, and KSHV to spread from cell to cell were recently revised (Raab-Traub and Dittmer, 2017). Thus, the mechanism that leads to the budding/trafficking of exosomes may also be employed by viruses to cross the maternal-fetal barrier.

2nd) Immunomodulation in pregnancy: Pregnancy is considered a challenge to the woman's immune system. In fact, fifty percent of the fetal genome, and consequently the antigens and other immune molecules present, are of paternal origin. Thus, the immune system of a pregnant woman must be readjusted during pregnancy to avoid perturbing the developing fetus (Mincheva-Nilsson, 2010; Mor et al., 2011; Stenqvist et al., 2013). When immune adaptation fails, abortion is a likely consequence (Trowsdale and Betz, 2006). The local downregulation of the maternal immune system, especially in the first trimester of pregnancy, could favor transplacental viral infection.

3rd) The cloud of exosomes at the maternal-fetal interface: Taking into consideration the immune system adjustments during pregnancy that promote a tolerogenic environment for the fetus, the general suppression of the immune system during the entire gestational period would be expected. However, as previously discussed, systemic immunosuppression would not be desirable because the blastocyst would not implant, and the pregnant woman would be highly susceptible to a variety of infections. Here, we present a series of studies showing that placenta-derived exosomes play important roles in this tolerogenic process by carrying immunomodulatory molecules through the maternal-fetal interface (Hedlund et al., 2009; Mincheva-Nilsson, 2010; Stenqvist et al., 2013). It is believed that exosomes contribute to this process by forming a "cloud of exosomes", which would protect the fetus from exacerbated maternal immune responses (Mincheva-Nilsson, 2010). Of note, this process would not compromise the woman's immune defenses as a whole.

4th) Seminal exosomes: Semen has trillions of exosomes (Karlsson et al., 2001; Vojtech et al., 2014). Furthermore, although semen is an immune privileged biological fluid, some viruses have been detected in this fluid months after host infection (Madison et al., 2014; Abbate et al., 2016; Anderson et al., 2016; D'Ortenzio et al., 2016; Uyeki et al., 2016). Exosomes present in semen could facilitate sexual transmission of viruses through semen-derived immunosuppression and hide, in some cases, viral components from the host immunological system.

The placental/fetal microbiota

Aagaard et al. (2014) reported that placental and amniotic fluid from healthy human placenta are not sterile. Historically, the uterus was considered a sterile environment, but it is currently viewed by some researchers as a compartment where the microbiota starts to be established (Stinson et al., 2017). Lactic acid bacteria and other commensal bacteria were isolated from meconium obtained from healthy neonates born either by labor or cesarean section, indicating that mother-to-child efflux of commensal bacteria may exist through the placenta (Martín et al., 2004; Jiménez et al., 2008). In 2005, Jiménez et al. (2005) also found commensal bacteria in the umbilical cord blood of healthy neonates born by cesarean section. In addition, a study using placental tissues from low-gestational-age neonates showed that almost one-half of second-trimester placentas harbor organisms within the chorionic plate

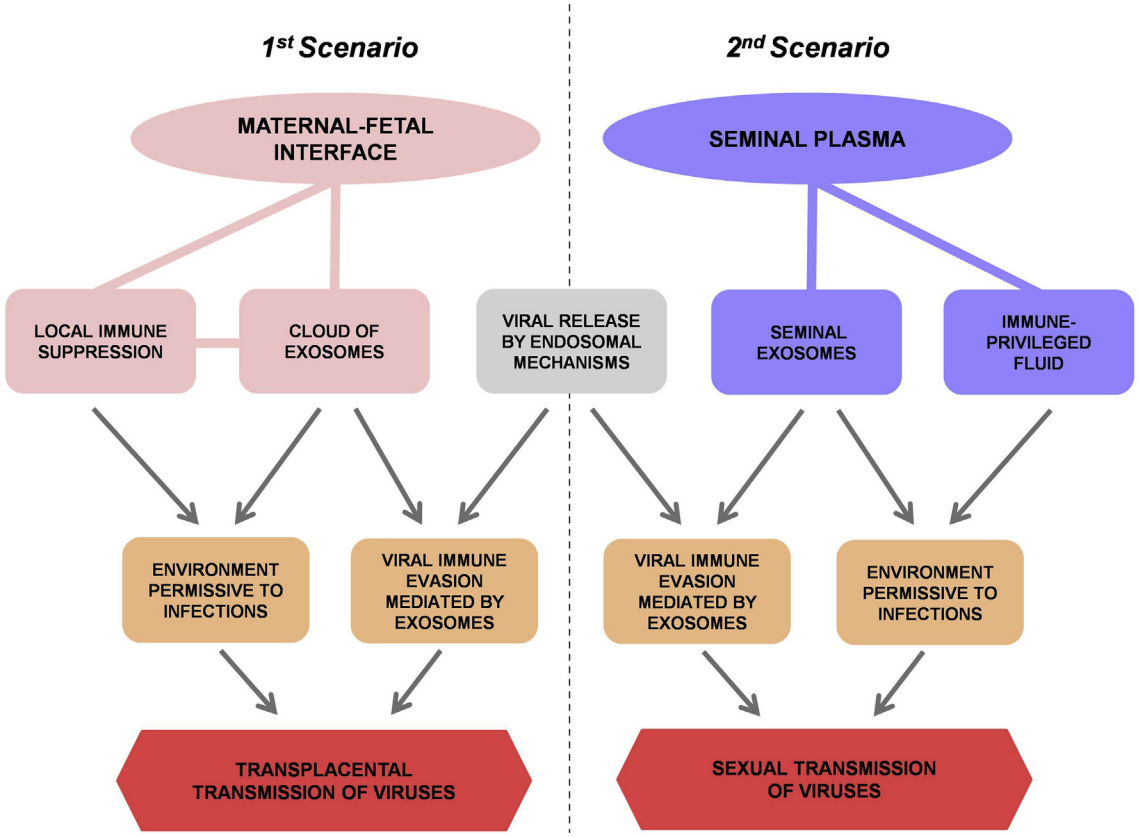

Fig. 2. Potential roles of exosomes in transplacental (1st scenario) and sexually transmitted (2nd scenario) viral infections. References are cited throughout the text.

(Onderdonk et al., 2008a). Another study showed that the chorion of placentas from preterm labor pregnancies (not related to preeclampsia) had a much higher rate of microorganism recovery than that of placentas from increasingly severe preeclampsia pregnancies (Onderdonk et al., 2008b). The presence of microorganisms in the placental parenchyma was associated with the presence of neutrophils in the fetal stem vessels of the chorion and umbilical cord, strongly indicating that the presence of microorganisms within the placental parenchyma is biologically important (Onderdonk et al., 2008b). The presence of microorganisms may correlate with the high number of neutrophils recruited to the maternal-fetal interface, as they may create an inflammatory environment similar to that necessary for the labor process but at the wrong time.

The following main question arises from these observations: how do microorganisms, or their genetic material, make contact with the fetus before birth? To date, three different origins for the fetal/placental microbiota have been proposed: maternal gut microbiota, vaginal microbiota, and oral microbiota (Stinson et al., 2017). Regarding the transference of microorganisms from the maternal gut, it is possible that the microorganisms are translocated into the maternal bloodstream from the gut epithelium, and the DCs could be quite important for this process. The intestinal epithelial barrier prevents bacteria from entering into the bloodstream. However, DCs can take up bacteria from the intestinal lumen by penetrating the gut epithelium. DCs packing bacteria could traffic to mesenteric lymph nodes via intestinal lymphatics and thus spread the bacteria to other body compartments (Stinson et al., 2017). Of note, maternal intestinal bacteria can also be found in breast milk, reportedly through the same DC-based dissemination pathway hypothesized for the delivery to the fetal tissues (Fernández et al., 2013).

The vaginal pathway by which microbes would reach the placenta is not well known, but it has been well established that microbes may ascend from the vagina and reach the amniotic cavity. One suggested mechanism involves the microbial colonization of the decidua, through mechanisms previously discussed, from which the microorganisms spread to fetal membranes and invade the amniotic fluid. Another suggested pathway involves direct microbial invasion of the amniotic fluid by penetration of a discontinuous section of fetal membranes (Stinson et al., 2017). Whatever the pathways of infection, DNA from vaginal microbes in the amniotic fluid (DiGiulio, 2012), fetal membranes (Steel et al., 2005) and the placenta (Aagaard et al., 2014) have already been found in both normal and complicated pregnancies. For example, pathogenic oral species of bacteria have been found in the placenta and amniotic fluid of pregnant women with periodontal disease (Barak et al., 2007; Katz et al., 2009). This finding indicates an opportunistic migration of bacteria from the oral cavity to the uterine environment and has been extensively correlated with preterm birth. Comparing the placental microbiome with the microbiome derived from different body compartments, the oral cavity showed the greatest similarity in terms of bacterial composition (Aagaard et al., 2014). Despite this finding, these studies were performed with the microbiota of healthy nonpregnant individuals, which makes it difficult to infer routes of transmission (Stinson et al., 2017). The effect of sexual practices in the transfer of oral and gut bacteria to the intrauterine cavity also needs further investigation. In this context, oral or anal sex preceding vaginal sex may present a mechanism of microbial transfer. The resolution of these tangled issues could not only create the possibility for studying, measuring, and mapping healthy placental/fetal microbiota from its precise beginning but could also provide an additional basis for the establishment of new public health strategies and improvement of clinical practices for complicated pregnancies with the aim of reducing the cases of newborns with severe sequelae.

However, a recent study addressing hundreds of placental samples stated that healthy placentas do not display a microbiome. This study represents the largest sample number in this field of research and was composed of 537 placental samples. de Goffau et al. (2019) performed such elegant experiments that allowed the identification of even possible contaminants from DNA extraction kits, and the results showed the

presence of only one type of microorganism in 5% of placentas: *Streptoccocus agalactiae*. Of note, this microorganism is one of the main concerns regarding the risk of neonatal sepsis, and is probably transmitted from the mother's genital tract. Besides revealing possible routes of contamination during the experimental procedure in previous related studies, these findings revealed a possible way of early detection of potential harmful agents during pregnancy. In summary, this study presented convincing evidence to support that healthy placentas lack a microbiome. The study reinforces that, despite the lack of a placental microbiome, pathogens may eventually be found in the placenta, although bacterial infection of this transient organ is not a common cause of gestational complications (de Goffau et al., 2019).

Taking together, we believe that future discussions and experimentation should consider the role of exosomes and other EVs as potential vehicles used by microorganisms in the establishment of the newborn microbiome. Finally, it is possible that the observed first microbiome in meconium samples is a result of EV-mediated traffic of bacteria from the vaginal tract, placenta or uterine cavity towards the fetus during the first signals of labor or even during delivery, thus representing the early seeds of the neonatal microbiome. Considering the emergence of studies addressing the establishment of the neonate microbiome, these hypotheses should be investigated.

3. Conclusion

The immune system of a pregnant woman, far from being in a resting state, undergoes several changes throughout the entire gestation period, encompassing distinct organs, tissues, cellular, and molecular profiles. Over the years, studies in the reproductive biology field have elegantly approached the multiple interactions at the maternal-fetal interface, which can result in normal or pathological pregnancies. Firstly considered a threat to a successful pregnancy, inflammation is currently recognized as an essential step to pregnancy establishment and maintenance, although such an immune response should be regulated. Exacerbated inflammation can cause abortion and other pregnancy complications, but the absence of inflammation precludes effective implantation due to inadequate tissue remodeling. A shift to a less inflammatory environment occurs during pregnancy, enabling fetal development. Finally, by the end of the third trimester, near parturition, a range of physiological alterations occurs, and a pro-inflammatory milieu is again predominant.

Additionally, when implantation takes place, the paternal antigens are expressed, and the maternal immune system meets two challenges: avoiding immune activation and rejection of the developing fetus while simultaneously inducing immune activation to avoid pathogen infection. Fetal tolerization is a complex process that transpires during the entire gestation period and involves modulation of local immune responses towards an anti-inflammatory profile. Interestingly, the placenta is a vigorous producer of exosomes, extracellular vesicles that have been described as key players in the regulation of maternal immune responses. The syncytiotrophoblast has important physical and molecular mechanisms that prevent microbes from bypassing the placenta and reaching the fetus, and these features range from dense, branched microvilli at the apical surface to soluble receptors carried by exosomes. Recent studies have revealed the importance of exosomes to a successful pregnancy, namely, as partners of the immune system at the maternal-fetal interface.

The trafficking of molecules, cells and even pathogens between mother and fetus during pregnancy is currently seen as a natural phenomenon. In this context, exosomes can be important mediators of transplacental infections. Additionally, the immunosuppression induced by seminal exosomes can help explain the persistence of the many viruses found in semen. In addition, this review revisited the discussion about the processes that enable viruses (and possibly other pathogens) to overcome the maternal-fetal barrier through sexual transmission. Taking into consideration the particularities of each cell type and virus *per se,* we call urgent attention to the role of exosomes and other microvesicles in viral infectivity and spread. Finally, the traditional view that establishes serious potential complications to the fetus should a microorganism succeed in crossing the placenta has been revised. In this regard, bacteria found in the normal gut, oral cavity, and vagina were detected in the amniotic cavity and in the placenta of normal pregnancies. Despite the recent emergence of controversial findings regarding this aspect, such discoveries raised important discussions about potential routes for the establishment of the newborn microbiota. Current studies are now trying to elucidate the distinct pathways used by microbes to colonize the developing fetus. Thus, we expect this review to provide insights for future investigations and new studies on all the topics addressed, since the universe of extracellular vesicles is similar to an iceberg from which, at the present moment, we are able to see only the portion that lies above the water. In this sense, a submerged “EV world” awaits our discovery, and with new methods, approaches and the establishment of connections among the several scientific fields involved, we will be able to uncover the immersed portions that will help us comprehend the immunology of gestation, fetal microbiome, and even the transplacental and sexual transmission of pathogens.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil). Valéria de Lima Kaminski and Joel Henrique Ellwanger received a doctoral scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). Currently, Joel Henrique Ellwanger receives a postdoctoral fellowship from CAPES (Brazil). José Artur Bogo Chies receives a fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors thank the funding agencies that made this work possible: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

References

Aagaard, K., Ma, J., Antony, K.M., Ganu, R., Petrosino, J., Versalovic, J., 2014. The placenta harbors a unique microbiome. Sci. Transl. Med. 6 (237), 237ra65.

Abbate, J.L., Murall, C.L., Richner, H., Althaus, C.L., 2016. Potential impact of sexual transmission on Ebola virus epidemiology: Sierra Leone as a case study. PLoS Neglected Trop. Dis. 10 (5), e0004676.

Abrahams, V.M., 2008. Pattern recognition at the maternal-fetal interface. Immunol. Investig. 37 (5), 427–447.

Abrahams, V.M., 2011. The role of the Nod-like receptor family in trophoblast innate immune responses. J. Reprod. Immunol. 88 (2), 112–117.

Abrahams, V.M., Straszewski-Chavez, S.L., Guller, S., Mor, G., 2004. First trimester trophoblast cells secrete Fas ligand which induces immune cell apoptosis. Mol. Hum. Reprod. 10 (1), 55–63.

Abrami, L., Brandi, L., Moayeri, M., Brown, M.J., Krantz, B.A., Leppla, S.H., van der Goot, F.G., 2013. Hijacking multivesicular bodies enables long-term and exosome-mediated long-distance action of anthrax toxin. Cell Rep. 5 (4), 986–996.

Adams, K.M., Lucas, J., Kapur, R.P., Stevens, A.M., 2007. LPS induces translocation of TLR4 in amniotic epithelium. Placenta 28 (5-6), 477–481.

Aghajanova, L., 2004. Leukemia inhibitory factor and human embryo implantation. Ann. N. Y. Acad. Sci. 1034, 176–183.

Anderle, C., Hammer, A., Polgár, B., Hartmann, M., Wintersteiger, R., Blaschitz, A., Dohr, G., Desoye, G., Szekeres-Barthó, J., Sedlmayr, P., 2008. Human trophoblast cells express the immunomodulator progesterone-induced blocking factor. J. Reprod. Immunol. 79 (1), 26–36.

Anderson, M., Kashanchi, F., Jacobson, S., 2016. Exosomes in viral disease. Neurotherapeutics 13 (3), 535–546.

Andreola, G., Rivoltini, L., Castelli, C., Huber, V., Perego, P., Deho, P., Squarcina, P., Accornero, P., Lozupone, F., Lugini, L., Stringaro, A., Molinari, A., Arancia, G., Gentile, M., Parmiani, G., Fais, S., 2002. Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. J. Exp. Med. 195 (10), 1303–1316.

Arck, P.C., Hecher, K., 2013. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. Nat. Med. 19 (5), 548–556.

Arora, N., Sadovsky, Y., Dermody, T.S., Coyne, C.B., 2017. Microbial vertical transmission during human pregnancy. Cell Host Microbe 21 (5), 561–567.

Atay, S., Gercel-Taylor, C., Suttles, J., Mor, G., Taylor, D.D., 2011. Trophoblast-derived exosomes mediate monocyte recruitment and differentiation. Am. J. Reprod. Immunol. 65 (1), 65–77.

Athman, J.J., Wang, Y., McDonald, D.J., Boom, W.H., Harding, C.V., Wearsch, P.A., 2015. Bacterial membrane vesicles mediate the release of Mycobacterium tuberculosis lipoglycans and lipoproteins from infected macrophages. J. Immunol. 195 (3), 1044–1053.

Bai, X., Williams, J.L., Greenwood, S.L., Baker, P.N., Aplin, J.D., Crocker, I.P., 2009. A placental protective role for trophoblast-derived TNF-related apoptosis-inducing ligand (TRAIL). Placenta 30 (10), 855–860.

Barak, S., Oettinger-Barak, O., Machtei, E.E., Sprecher, H., Ohel, G., 2007. Evidence of periopathogenic microorganisms in placentas of women with preeclampsia. J. Periodontol. 78 (4), 670–676.

Bhatnagar, S., Shinagawa, K., Castellino, F.J., Schorey, J.S., 2007. Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response in vitro and in vivo. Blood 110 (9), 3234–3244.

Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J.H., Lanier, L.L., Spies, T., 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 285 (5428), 727–729.

Bayer, A., Delorme-Axford, E., Sleigher, C., Frey, T.K., Trobaugh, D.W., Klimstra, W.B., Emert-Sedlak, L.A., Smithgall, T.E., Kinchington, P.R., Vadia, S., Seveau, S., Boyle, J.P., Coyne, C.B., Sadovsky, Y., 2015. Human trophoblasts confer resistance to viruses implicated in perinatal infection. Am. J. Obstet. Gynecol. 212 (1), 71.e1-e8.

Bayer, A., Lennemann, N.J., Ouyang, Y., Bramley, J.C., Morosky, S., Marques Jr., E.T., Cherry, S., Sadovsky, Y., Coyne, C.B., 2016. Type III interferons produced by human placental trophoblasts confer protection against Zika virus infection. Cell Host Microbe 19 (5), 705–712.

Bielska, E., May, R.C., 2019. Extracellular vesicles of human pathogenic fungi. Curr. Opin. Microbiol. 52, 90–99.

Billingham, R.E., Brent, L., Medawar, P.B., 1953. 'Actively acquired tolerance' of foreign cells. Nature 172 (4379), 603–606.

Booth, A.M., Fang, Y., Fallon, J.K., Yang, J.M., Hildreth, J.E.K., Gould, S.J., 2006. Exosomes and HIV Gag bud from endosome-like domains of the T cell plasma membrane. J. Cell Biol. 172 (6), 923–935.

Braude, P., Bolton, V., Moore, S., 1988. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 332 (6163), 459–461.

Bromfield, J.J., 2014. Seminal fluid and reproduction: much more than previously thought. J. Assist. Reprod. Genet. 31 (6), 627–636.

Bu, H., He, D., He, X., Wang, K., 2019. Exosomes: isolation, analysis, and applications in cancer detection and therapy. Chembiochem 20 (4), 451–461.

Canavan, T.P., Simhan, H.N., 2007. Innate immune function of the human decidual cell at the maternal-fetal interface. J. Reprod. Immunol. 74 (1-2), 46–52.

Cantle, S.J., Kaufmann, P., Luckhardt, M., Schweikhart, G., 1987. Interpretation of syncytial sprouts and bridges in the human placenta. Placenta 8 (3), 221–234.

Capmany, G., Taylor, A., Braude, P.R., Bolton, V.N., 1996. The timing of pronuclear formation, DNA synthesis and cleavage in the human 1-cell embryo. Mol. Hum. Reprod. 2 (5), 299–306.

Chaouat, G., Zourbas, S., Ostojic, S., Lappree-Delage, G., Dubanchet, S., Ledee, N., Martal, J., 2002. A brief review of recent data on some cytokine expressions at the materno-foetal interface which might challenge the classical Th1/Th2 dichotomy. J. Reprod. Immunol. 53 (1-2), 241–256.

Chavan, A.R., Griffith, O.W., Wagner, G.P., 2017. The inflammation paradox in the evolution of mammalian pregnancy: turning a foe into a friend. Curr. Opin. Genet. Dev. 47, 24–32.

Clark, D.A., 2005. Tolerance signaling molecules. Chem. Immunol. Allergy 89, 36–48.

Clayton, A., Mitchell, J.P., Court, J., Linnane, S., Mason, M.D., Tabi, Z., 2008. Human tumor-derived exosomes down-modulate NKG2D expression. J. Immunol. 180 (11), 7249–7258.

Coakley, G., Buck, A.H., Maizels, R.M., 2016. Host parasite communications-Messages from helminths for the immune system: parasite communication and cell-cell interactions. Mol. Biochem. Parasitol. 208 (1), 33–40.

Coakley, G., Maizels, R.M., Buck, A.H., 2015. Exosomes and other extracellular vesicles: the new communicators in parasite infections. Trends Parasitol. 31 (10), 477–489.

Colombo, M., Raposo, G., Théry, C., 2014. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell Dev. Biol. 30, 255–289.

Corrado, C., Raimondo, S., Chiesi, A., Ciccia, F., De Leo, G., 2013. Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. Int. J. Mol. Sci. 14 (3), 5338–5366.

Costello, M.J., Joyce, S.K., Abrahams, V.M., 2007. NOD protein expression and function in first trimester trophoblast cells. Am. J. Reprod. Immunol. 57 (1), 67–80.

Coyne, C.B., Lazear, H.M., 2016. Zika virus – reigniting the TORCH. Nat. Rev. Microbiol. 14 (11), 707–715.

Cross, J.C., Werb, Z., Fisher, S.J., 1994. Implantation and the placenta: key pieces of the development puzzle. Science 266 (5190), 1508–1518.

de Goffau, M.C., Lager, S., Sovio, U., Gaccioli, F., Cook, E., Peacock, S.J., Parkhill, J., Charnock-Jones, D.S.3., Smith, G.C.S., 2019. Human placenta has no microbiome but can contain potential pathogens. Nature.

D'Ortenzio, E., Matheron, S., Yazdanpanah, Y., de Lamballerie, X., Hubert, B., Piorkowski, G., Maquart, M., Descamps, D., Damond, F., Leparc-Goffart, I., 2016. Evidence of sexual transmission of Zika virus. N. Engl. J. Med. 374 (22), 2195–2198.

De Toro, J., Herschlik, L., Waldner, C., Mongini, C., 2015. Emerging roles of exosomes in normal and pathological conditions: new insights for diagnosis and therapeutic applications. Front. Immunol. 6, 203.

Delorme-Axford, E., Donker, R.B., Mouillet, J.F., Chu, T., Bayer, A., Ouyang, Y., Wang, T., Stolz, D.B., Sarkar, S.N., Morelli, A.E., Sadovsky, Y., Coyne, C.B., 2013. Human placental trophoblasts confer viral resistance to recipient cells. Proc. Natl. Acad. Sci. U.S.A. 110 (29), 12048–12053.

DiGiulio, D.B., 2012. Diversity of microbes in amniotic fluid. Semin. Fetal Neonatal Med. 17 (1), 2–11.

Donker, R.B., Mouillet, J.F., Chu, T., Hubel, C.A., Stolz, D.B., Morelli, A.E., Sadovsky, Y., 2012. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol. Hum. Reprod. 18 (8), 417–424.

Ellwanger, J.H., Chies, J.A.B., 2018. Zoonotic spillover and emerging viral diseases – time to intensify zoonoses surveillance in Brazil. Braz. J. Infect. Dis. 22 (1), 76–78.

Ellwanger, J.H., Chies, J.A.B., 2019. Host immunogenetics in tick-borne encephalitis virus infection-The CCR5 crossroad. Ticks Tick Borne Dis. 10 (4), 729–741.

Ellwanger, J.H., Veit, T.D., Chies, J.A.B., 2017. Exosomes in HIV infection: a review and critical look. Infect. Genet. Evol. 53, 146–154.

Erlebacher, A., 2013. Immunology of the maternal-fetal interface. Annu. Rev. Immunol. 31, 387–411.

Espinoza, J., Erez, O., Romero, R., 2006. Preconceptional antibiotic treatment to prevent preterm birth in women with a previous preterm delivery. Am. J. Obstet. Gynecol. 194 (3), 630–637.

Fan, X.G., Zheng, Z.Q., 1997. A study of early pregnancy factor activity in preimplantation. Am. J. Reprod. Immunol. 37 (5), 359–364.

Ferguson, T.A., Griffith, T.S., 2006. A vision of cell death: fas ligand and immune privilege 10 years later. Immunol. Rev. 213, 228–238.

Fernández, L., Langa, S., Martín, V., Maldonado, A., Jiménez, E., Martín, R., Rodríguez, J.M., 2013. The human milk microbiota: origin and potential roles in health and disease. Pharmacol. Res. 69 (1), 1–10.

Fest, S., Aldo, P.B., Abrahams, V.M., Visintin, I., Alvero, A., Chen, R., Chavez, S.L., Romero, R., Mor, G., 2007. Trophoblast-macrophage interactions: a regulatory network for the protection of pregnancy. Am. J. Reprod. Immunol. 57 (1), 55–66.

Fevrier, B., Vilette, D., Archer, F., Loew, D., Faigle, W., Vidal, M., Laude, H., Raposo, G., 2004. Cells release prions in association with exosomes. Proc. Natl. Acad. Sci. U.S.A. 101 (26), 9683–9688.

Fisher, S., Genbacev, O., Maidji, E., Pereira, L., 2000. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. J. Virol. 74 (15), 6808–6820.

Frängsmyr, L., Baranov, V., Nagaeva, O., Stendahl, U., Kjellberg, L., Mincheva-Nilsson, L., 2005. Cytoplasmic microvesicular form of Fas ligand in human early placenta: switching the tissue immune privilege hypothesis from cellular to vesicular level. Mol. Hum. Reprod. 11 (1), 35–41.

Fu, B., Tian, Z., Wei, H., 2014. TH17 cells in human recurrent pregnancy loss and pre-eclampsia. Cell. Mol. Immunol. 11 (6), 564–570.

Gan, X., Gould, S.J., 2012. HIV Pol inhibits HIV budding and mediates the severe budding defect of Gag-Pol. PLoS One 7 (1), e29421.

Ganz, T., 2003. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 3 (9), 710–720.

Girardin, S.E., Boneca, I.G., Carneiro, L.A., Antignac, A., Jéhanno, M., Viala, J., Tedin, K., Taha, M.K., Labigne, A., Zähringer, U., Coyle, A.J., DiStefano, P.S., Bertin, J., Sansonetti, P.J., Philpott, D.J., 2003. Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science 300 (5625), 1584–1587.

Giri, P.K., Schorey, J.S., 2008. Exosomes derived from M. Bovis BCG infected macrophages activate antigen-specific CD4+ and CD8+ T cells in vitro and in vivo. PLoS One 3 (6), e2461.

Gould, S.J., Booth, A.M., Hildreth, J.E.K., 2003. The Trojan exosome hypothesis. Proc. Natl. Acad. Sci. 100 (19), 10592–10597.

Groh, V., Wu, J., Yee, C., Spies, T., 2002. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 419 (6908), 734–738.

Gude, N.M., Roberts, C.T., Kalionis, B., King, R.G., 2004. Growth and function of the normal human placenta. Thromb. Res. 114 (5-6), 397–407.

Hackmon, R., Pinnaduwage, L., Zhang, J., Lye, S.J., Geraghty, D.E., Dunk, C.E., 2017. Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. Am. J. Reprod. Immunol. 77 (6).

Hamilton, W.J., Boyd, J.D., 1960. Development of the human placenta in the first three months of gestation. J. Anat. 94, 297–328.

Hammer, A., Dohr, G., 2000. Expression of Fas-ligand in first trimester and term human placental villi. J. Reprod. Immunol. 46 (2), 83–90.
Hanna, J., Goldman-Wohl, D., Hamani, Y., Avraham, I., Greenfield, C., Natanson-Yaron, S., Prus, D., Cohen-Daniel, L., Arnon, T.I., Manaster, I., Gazit, R., Yutkin, V., Benharroch, D., Porgador, A., Keshet, E., Yagel, S., Mandelboim, O., 2006. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat. Med. 12 (9), 1065–1074.
Hartmann, A., Muth, C., Dabrowski, O., Krasemann, S., Glatzel, M., 2017. Exosomes and the prion protein: more than one truth. Front. Neurosci. 11, 194.
Hedlund, M., Stenqvist, A.C., Nagaeva, O., Kjellberg, L., Wulff, M., Baranov, V., Mincheva-Nilsson, L., 2009. Human placenta expresses and secretes NKG2D ligands via exosomes that down-modulate the cognate receptor expression: evidence for immunosuppressive function. J. Immunol. 183 (1), 340–351.
Hill, J.A., Polgar, K., Anderson, D.J., 1995. T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. J. Am. Med. Assoc. 273 (24), 1933–1936.
Holder, B., Jones, T., Sancho Shimizu, V., Rice, T.F., Donaldson, B., Bouqueau, M., Forbes, K., Kampmann, B., 2016. Macrophage exosomes induce placental inflammatory cytokines: a novel mode of maternal-placental messaging. Traffic 17 (2), 168–178.
Holmes, C.H., Simpson, K.L., Okada, H., Okada, N., Wainwright, S.D., Purcell, D.F., Houlihan, J.M., 1992. Complement regulatory proteins at the feto-maternal interface during human placental development: distribution of CD59 by comparison with membrane cofactor protein (CD46) and decay accelerating factor (CD55). Eur. J. Immunol. 22 (6), 1579–1585.
Huang, S.H., Wu, C.H., Chang, Y.C., Kwon-Chung, K.J., Brown, R.J., Jong, A., 2012. Cryptococcus neoformans-derived microvesicles enhance the pathogenesis of fungal brain infection. PLoS One 7 (11), e48570.
Husmann, M., Beckmann, E., Boller, K., Kloft, N., Tenzer, S., Bobkiewicz, W., Neukirch, C., Bayley, H., Bhakdi, S., 2009. Elimination of a bacterial pore-forming toxin by sequential endocytosis and exocytosis. FEBS Lett. 583 (2), 337–344.
Holmlund, U., Cebers, G., Dahlfors, A.R., Sandstedt, B., Bremme, K., Ekström, E.S., Scheynius, A., 2002. Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta. Immunology 107 (1), 145–151.
Huppertz, B., Frank, H.G., Kingdom, J.C., Reister, F., Kaufmann, P., 1998. Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. Histochem. Cell Biol. 110 (5), 495–508.
Hwang, I., Shen, X., Sprent, J., 2003. Direct stimulation of naive T cells by membrane vesicles from antigen-presenting cells: distinct roles for CD54 and B7 molecules. Proc. Natl. Acad. Sci. U.S.A. 100 (11), 6670–6675.
Jiménez, E., Fernández, L., Marín, M.L., Martín, R., Odriozola, J.M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., Rodríguez, J.M., 2005. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr. Microbiol. 51 (4), 270–274.
Jiménez, E., Marín, M.L., Martín, R., Odriozola, J.M., Olivares, M., Xaus, J., Fernández, L., Rodríguez, J.M., 2008. Is meconium from healthy newborns actually sterile? Res. Microbiol. 159 (3), 187–193.
Jodo, S., Hohlbaum, A.M., Xiao, S., Chan, D., Strehlow, D., Sherr, D.H., Marshak-Rothstein, A., Ju, S.T., 2000. CD95 (Fas) ligand-expressing vesicles display antibody-mediated, FcR-dependent enhancement of cytotoxicity. J. Immunol. 165 (10), 5487–5494.
Joffe, L.S., Nimrichter, L., Rodrigues, M.L., Del Poeta, M., 2016. Potential roles of fungal extracellular vesicles during infection. mSphere 1 (4) pii: e00099-16.
Johansson, M., Bromfield, J.J., Jasper, M.J., 2004. Robertson SA. Semen activates the female immune response during early pregnancy in mice. Immunology 112 (2), 290–300.
Juliano, P.B., Blotta, M.H., Altemani, A.M., 2006. ICAM-1 is overexpressed by villous trophoblasts in placentitis. Placenta 27 (6-7), 750–757.
Kadiu, I., Narayanasamy, P., Dash, P.K., Zhang, W., Gendelman, H.E., 2012. Biochemical and biologic characterization of exosomes and microvesicles as facilitators of HIV-1 infection in macrophages. J. Immunol. 189 (2), 744–754.
Kaminski, V.L., Ellwanger, J.H., Matte, M.C.C., Savaris, R.F., Vianna, P., Chies, J.A.B., 2018. IL-17 blood levels increase in healthy pregnancy but not in spontaneous abortion. Mol. Biol. Rep. 45 (5), 1565–1568.
Kaminski, V.L., Ellwanger, J.H., Sandrim, V., Pontillo, A., Chies, J.A.B., 2019. Influence of NKG2C gene deletion and CCR5Δ32 in Pre-eclampsia-Approaching the effect of innate immune gene variants in pregnancy. Int. J. Immunogenet. 46 (2), 82–87.
Karlsson, M., Lundin, S., Dahlgren, O., Kahu, H., Pettersson, I., Telemo, E., 2001. "Tolerosomes'' are produced by intestinal epithelial cells. Eur. J. Immunol. 31 (10), 2892-900.
Katz, J., Chegini, N., Shiverick, K.T., Lamont, R.J., 2009. Localization of P. gingivalis in preterm delivery placenta. J. Dent. Res. 88 (6), 575–578.
Kelly, R.W., Holland, P., Skibinski, G., Harrison, C., McMillan, L., Hargreave, T., James, K., 1991. Extracellular organelles (prostasomes) are immunosuppressive components of human semen. Clin. Exp. Immunol. 86 (3), 550–556.
Kim, D.K., Lee, J., Kim, S.R., Choi, D.S., Yoon, Y.J., Kim, J.H., Go, G., Nhung, D., Hong, K., Jang, S.C., Kim, S.H., Park, K.S., Kim, O.Y., Park, H.T., Seo, J.H., Aikawa, E., Baj-Krzyworzeka, M., van Balkom, B.W., Belting, M., Blanc, L., Bond, V., Bongiovanni, A., Borràs, F.E., Buée, L., Buzás, E.I., Cheng, L., Clayton, A., Cocucci, E., Dela Cruz, C.S., Desiderio, D.M., Di Vizio, D., Ekström, K., Falcon-Perez, J.M., Gardiner, C., Giebel, B., Greening, D.W., Gross, J.C., Gupta, D., Hendrix, A., Hill, A.F., Hill, M.M., Nolte-'t Hoen, E., Hwang, D.W., Inal, J., Jagannadham, M.V., Jayachandran, M., Jee, Y.K., Jørgensen, M., Kim, K.P., Kim, Y.K., Kislinger, T., Lässer, C., Lee, D.S., Lee, H., van Leeuwen, J., Lener, T., Liu, M.L., Lötvall, J., Marcilla, A., Mathivanan, S., Möller, A., Morhayim, J., Mullier, F., Nazarenko, I., Nieuwland, R., Nunes, D.N., Pang, K., Park, J., Patel, T., Pocsfalvi, G., Del Portillo, H., Putz, U., Ramirez, M.I., Rodrigues, M.L., Roh, T.Y., Royo, F., Sahoo, S., Schiffelers, R., Sharma, S., Siljander, P., Simpson, R.J., Soekmadji, C., Stahl, P., Stensballe, A., Stępień, E., Tahara, H., Trummer, A., Valadi, H., Vella, L.J., Wai, S.N., Witwer, K., Yáñez-Mó, M., Youn, H., Zeidler, R., Gho, Y.S., 2015. EVpedia: a community web portal for extracellular vesicles research. Bioinformatics 31 (6), 933–939.
Kim, M.J., Jung, B.K., Cho, J., Song, H., Pyo, K.H., Lee, J.M., Kim, M.K., Chai, J.Y., 2016. Exosomes secreted by Toxoplasma gondii-infected L6 cells: their effects on host cell proliferation and cell cycle changes. Korean J. Parasitol. 54 (2), 147–154.
Kim, Y.M., Romero, R., Chaiworapongsa, T., Kim, G.J., Kim, M.R., Kuivaniemi, H., Tromp, G., Espinoza, J., Bujold, E., Abrahams, V.M., Mor, G., 2004. Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. Am. J. Obstet. Gynecol. 191 (4), 1346–1355.
King, A., Allan, D.S., Bowen, M., Powis, S.J., Joseph, S., Verma, S., Hiby, S.E., McMichael, A.J., Loke, Y.W., Braud, V.M., 2000. HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. Eur. J. Immunol. 30 (6), 1623–1631.
Koh, Y.Q., Chan, H.W., Nitert, M.D., Vaswani, K., Mitchell, M.D., Rice, G.E., 2014. Differential response to lipopolysaccharide by JEG-3 and BeWo human choriocarcinoma cell lines. Eur. J. Obstet. Gynecol. Reprod. Biol. 175, 129–133.
Koi, H., Zhang, J., Parry, S., 2001. The mechanisms of placental viral infection. Ann. N. Y. Acad. Sci. 943, 148–156.
Krikun, G., Lockwood, C.J., Abrahams, V.M., Mor, G., Paidas, M., Guller, S., 2007. Expression of Toll-like receptors in the human decidua. Histol. Histopathol. 22, 847–854.
Kshirsagar, S.K., Alam, S.M., Jasti, S., Hodes, H., Nauser, T., Gilliam, M., Billstrand, C., Hunt, J.S., Petroff, M.G., 2012. Immunomodulatory molecules are released from the first trimester and term placenta via exosomes. Placenta 33 (12), 982–990.
Kudo, Y., Boyd, C.A., Spyropoulou, I., Redman, C.W., Takikawa, O., Katsuki, T., Hara, T., Ohama, K., Sargent, I.L., 2004. Indoleamine 2,3-dioxygenase: distribution and function in the developing human placenta. J. Reprod. Immunol. 61, 87–98.
Kumazaki, K., Nakayama, M., Yanagihara, I., Suehara, N., Wada, Y., 2004. Immunohistochemical distribution of Toll-like receptor 4 in term and preterm human placentas from normal and complicated pregnancy including chorioamnionitis. Hum. Pathol. 35 (1), 47–54.
Lai, A., Elfeky, O., Rice, G.E., Salomon, C., 2018. Optimized specific isolation of placenta-derived exosomes from maternal circulation. Methods Mol. Biol. 1710, 131–138.
Lash, G.E., Robson, S.C., Bulmer, J.N., 2010. Review: functional role of uterine natural killer (uNK) cells in human early pregnancy decidua. Placenta 31 (Suppl), S87–92.
LeMaoult, J., Krawice-Radanne, I., Dausset, J., Carosella, E.D., 2004. HLA-G1-expressing antigen-presenting cells induce immunosuppressive CD4+ T cells. Proc. Natl. Acad. Sci. U.S.A. 101 (18), 7064–7069.
Li, C., Houser, B.L., Nicotra, M.L., Strominger, J.L., 2009. HLA-G homodimer-induced cytokine secretion through HLA-G receptors on human decidual macrophages and natural killer cells. Proc. Natl. Acad. Sci. U.S.A. 106 (14), 5767–5772.
Li, J., Liu, K., Liu, Y., Xu, Y., Zhang, F., Yang, H., Liu, J., Pan, T., Chen, J., Wu, M., Zhou, X., Yuan, Z., 2013. Exosomes mediate the cell-to-cell transmission of IFN-α-induced antiviral activity. Nat. Immunol. 14 (8), 793–803.
Li, Y., Liu, Y., Xiu, F., Wang, J., Cong, H., He, S., Shi, Y., Wang, X., Li, X., Zhou, H., 2018. Characterization of exosomes derived from Toxoplasma gondii and their functions in modulating immune responses. Int. J. Nanomed. 13, 467–477.
Liu, S., Hossinger, A., Hofmann, J.P., Denner, P., Vorberg, I.M., 2016. Horizontal transmission of cytosolic Sup35 prions by extracellular vesicles. mBio 7 (4), e915–e916.
Liu, Z., Zhang, X., Yu, Q., He, J.J., 2014. Exosome-associated hepatitis C virus in cell cultures and patient plasma. Biochem. Biophys. Res. Commun. 455 (3-4), 218–222.
Lo Cicero, A., Stahl, P.D., Raposo, G., 2015. Extracellular vesicles shuffling intercellular messages: for good or for bad. Curr. Opin. Cell Biol. 35, 69–77.
Ma, Y., Krikun, G., Abrahams, V.M., Mor, G., Guller, S., 2007. Cell type-specific expression and function of toll-like receptors 2 and 4 in human placenta: implications in fetal infection. Placenta 28 (10), 1024–1031.
Madison, M.N., Roller, R.J., Okeoma, C.M., 2014. Human semen contains exosomes with potent anti- HIV-1 activity. Retrovirology 11, 102.
Maidji, E., McDonagh, S., Genbacev, O., Tabata, T., Pereira, L., 2006. Maternal antibodies enhance or prevent cytomegalovirus infection in the placenta by neonatal Fc receptor-mediated transcytosis. Am. J. Pathol. 168 (4), 1210–1226.
Maidji, E., Nigro, G., Tabata, T., McDonagh, S., Nozawa, N., Shiboski, S., Muci, S., Anceschi, M.M., Aziz, N., Adler, S.P., Pereira, L., 2010. Antibody treatment promotes compensation for human cytomegalovirus-induced pathogenesis and a hypoxia-like condition in placentas with congenital infection. Am. J. Pathol. 77 (3), 1298–1310.
Makrigiannakis, A., Vrekoussis, T., Zoumakis, E., Kalantaridou, S.N., Jeschke, U., 2017. The role of HCG in implantation: a mini-review of molecular and clinical evidence. Int. J. Mol. Sci. 18 (6), 1305.
Mantel, P.Y., Hjelmqvist, D., Walch, M., Kharoubi-Hess, S., Nilsson, S., Ravel, D., Ribeiro, M., Grüring, C., Ma, S., Padmanabhan, P., Trachtenberg, A., Ankarklev, J., Brancucci, N.M., Huttenhower, C., Duraisingh, M.T., Ghiran, I., Kuo, W.P., Filgueira, L., Martinelli, R., Marti, M., 2016. Infected erythrocyte-derived extracellular vesicles alter vascular function via regulatory Ago2-miRNA complexes in malaria. Nat. Commun. 7, 12727.
Martínez-García, E.A., Chávez-Robles, B., Sánchez-Hernández, P.E., Núñez-Atahualpa, L., Martín-Máquez, B.T., Muñoz-Gómez, A., González-López, L., Gámez-Nava, J.I., Salazar-Páramo, M., Dávalos-Rodríguez, I., Petri, M.H., Zúñiga-Tamayo, D., Vargas-Ramírez, R., Vázquez-Del Mercado, M., 2011. IL-17 increased in the third trimester in healthy women with term labor. Am. J. Reprod. Immunol. 65 (2), 99–103.

Martínez-Lorenzo, M.J., Anel, A., Gamen, S., Monleón, I., Lasierra, P., Larrad, L., Piñeiro, A., Alava, M.A., Naval, J., 1999. Activated human T cells release bioactive Fas ligand and APO2 ligand in microvesicles. J. Immunol. 163 (3), 1274–1281.
Martín, R., Langa, S., Reviriego, C., Jiménez, E., Marín, M.L., Olivares, M., Boza, J., Jiménez, J., Fernández, L., Xaus, J., Rodríguez, J.M., 2004. The commensal microflora of human milk: new perspectives for food bacteriotherapy and probiotics. Trends Food Sci. Technol. 15 (3–4), 121–127.
Martins, S.T., Kuczera, D., Lötvall, J., Bordignon, J., Alves, L.R., 2018. Characterization of dendritic cell-derived extracellular vesicles during dengue virus infection. Front. Microbiol. 9, 1792.
McDonagh, S., Maidji, E., Ma, W., Chang, H.T., Fisher, S., Pereira, L., 2004. Viral and bacterial pathogens at the maternal-fetal interface. J. Infect. Dis. 190 (4), 826–834.
Medzhitov, R., Janeway Jr., C.A., 1997. Innate immunity: the virtues of a nonclonal system of recognition. Cell 91 (3), 295–298.
Mengaud, J., Ohayon, H., Gounon, P., Mege, R.-M., Cossart, P., 1996. E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. Cell 84, 923–932.
Meuleman, T., Haasnoot, G.W., Jan Lith, J.M.M., Verduijn, W., Bloemenkamp, K.W.M., Claas, F.H.J., 2018. Paternal HLA-C is a risk factor in unexplained recurrent miscarriage. Am. J. Reprod. Immunol. 79.
Michita, R.T., Kaminski, V.L., Chies, J.A.B., 2018. Genetic variants in preeclampsia: lessons from studies in Latin-American populations. Front. Physiol. 9, 1771.
Michita, R.T., Zambra, F.M.B., Fraga, L.R., Sanseverino, M.T.V., Callegari-Jacques, S.M., Vianna, P., Chies, J.A.B., 2016. A tug-of-war between tolerance and rejection – new evidence for 3'UTR HLA-G haplotypes influence in recurrent pregnancy loss. Hum. Immunol. 77 (10), 892–897.
Mincheva-Nilsson, L., 2010. Placental exosome-mediated immune protection of the fetus: feeling groovy in a cloud of exosomes. Expert Rev. Obstet. Gynecol. 5 (5), 619–634.
Mincheva-Nilsson, L., Baranov, V., 2010. The role of placental exosomes in reproduction. Am. J. Reprod. Immunol. 63 (6), 520–533.
Mincheva-Nilsson, L., Baranov, V., Yeung, M.M., Hammarstrom, S., Hammarstrom, M.L., 1994. Immunomorphologic studies of human decidua-associated lymphoid cells in normal early pregnancy. J. Immunol. 152, 2020–2032.
Mincheva-Nilsson, L., Nagaeva, O., Chen, T., Stendahl, U., Antsiferova, J., Mogren, I., Hernestål, J., Baranov, V., 2006. Placenta-derived soluble MHC class I chain-related molecules down-regulate NKG2D receptor on peripheral blood mononuclear cells during human pregnancy: a possible novel immune escape mechanism for fetal survival. J. Immunol. 176 (6), 3585–3592.
Mincheva-Nilsson, L., Nagaeva, O., Sundqvist, K.G., Hammarström, M.L., Hammarström, S., Baranov, V., 2000. Gammadelta T cells of human early pregnancy decidua: evidence for cytotoxic potency. Int. Immunol. 12 (5), 585–596.
Mitchell, M.D., Peiris, H.N., Kobayashi, M., Koh, Y.Q., Duncombe, G., Illanes, S.E., Rice, G.E., Salomon, C., 2015. Placental exosomes in normal and complicated pregnancy. Am. J. Obstet. Gynecol. 213 (4 Suppl), S173–S181.
Moffett-King, A., 2002. Natural killer cells and pregnancy. Nat. Rev. Immunol. 2 (9), 656–663.
Monleón, I., Martínez-Lorenzo, M.J., Monteagudo, L., Lasierra, P., Taulés, M., Iturralde, M., Piñeiro, A., Larrad, L., Alava, M.A., Naval, J., Anel, A., 2001. Differential secretion of Fas ligand- or APO2 ligand/TNF-related apoptosis-inducing ligand-carrying microvesicles during activation-induced death of human T cells. J. Immunol. 167 (12), 6736–6744.
Mor, G., Cardenas, I., Abrahams, V., Guller, S., 2011. Inflammation and pregnancy: the role of the immune system at the implantation site. Ann. N. Y. Acad. Sci. 1221, 80–87.
Mor, G., Cardenas, I., 2010. The immune system in pregnancy: a unique complexity. Am. J. Reprod. Immunol. 63 (6), 425–433.
Mor, G., Gutierrez, L.S., Eliza, M., Kahyaoglu, F., Arici, A., 1998. Fas-fas ligand system-induced apoptosis in human placenta and gestational trophoblastic disease. Am. J. Reprod. Immunol. 40 (2), 89–94.
Mor, G., Straszewski, S., Kamsteeg, M., 2002. Role of the Fas/Fas ligand system in female reproductive organs: survival and apoptosis. Biochem. Pharmacol. 64 (9), 1305–1315.
Nagata, S., Golstein, P., 1995. The Fas death factor. Science 267 (5203), 1449–1456.
Nair, S., Salomon, C., 2018. Extracellular vesicles and their immunomodulatory functions in pregnancy. Semin. Immunopathol. 40 (5), 425–437.
Nancy, P., Tagliani, E., Tay, C.S., Asp, P., Levy, D.E., Erlebacher, A., 2012. Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. Science 336 (6086), 1317–1321.
Nguyen, D.G., Booth, A., Gould, S.J., Hildreth, J.E., 2003. Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. J. Biol. Chem. 278 (52), 52347–52354.
Noguer-Dance, M., Abu-Amero, S., Al-Khtib, M., Lefèvre, A., Coullin, P., Moore, G.E., Cavaillé, J., 2010. The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. Hum. Mol. Genet. 19 (18), 3566–3582.
Nour, A.M., Modis, Y., 2014. Endosomal vesicles as vehicles for viral genomes. Trends Cell Biol. 24 (8), 449–454.
Numasaki, M., Fukushi, J., Ono, M., Narula, S.K., Zavodny, P.J., Kudo, T., Robbins, P.D., Tahara, H., Lotze, M.T., 2003. Interleukin-17 promotes angiogenesis and tumor growth. Blood 101 (7), 2620–2627.
Ohshima, K., Nakashima, M., Sonoda, K., Kikuchi, M., Watanabe, T., 2001. Expression of RCAS1 and FasL in human trophoblasts and uterine glands during pregnancy: the possible role in immune privilege. Clin. Exp. Immunol. 123 (3), 481–486.
Onderdonk, A.B., Delaney, M.L., DuBois, A.M., Allred, E.N., Leviton, A., Extremely Low Gestational Age Newborns (ELGAN) Study Investigators., 2008a. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. Am. J. Obstet. Gynecol. 198 (1), 110.e1–110.e7.
Onderdonk, A.B., Hecht, J.L., McElrath, T.F., Delaney, M.L., Allred, E.N., Leviton, A., ELGAN Study Investigators, 2008b. Colonization of second-trimester placenta parenchyma. Am. J. Obstet. Gynecol. 199 (1), 52.e1-10.
Ouyang, Y., Bayer, A., Chu, T., Tyurin, V.A., Kagan, V.E., Morelli, A.E., Coyne, C.B., Sadovsky, Y., 2016. Isolation of human trophoblastic extracellular vesicles and characterization of their cargo and antiviral activity. Placenta 47, 86–95.
Oz, H.S., 2017. Fetomaternal and pediatric toxoplasmosis. J. Pediatr. Infect. Dis. 12 (4), 202–208.
Parnell, L.A., Briggs, C.M., Cao, B., Delannoy-Bruno, O., Schrieffer, A.E., Mysorekar, I.U., 2017. Microbial communities in placentas from term normal pregnancy exhibit spatially variable profiles. Sci. Rep. 7 (1), 11200.
Peres da Silva, R., Puccia, R., Rodrigues, M.L., Oliveira, D.L., Joffe, L.S., César, G.V., Nimrichter, L., Goldenberg, S., Alves, L.R., 2015. Extracellular vesicle-mediated export of fungal RNA. Sci. Rep. 5, 7763.
Persson, G., Melsted, W.N., Nilsson, L.L., Hviid, T.V.F., 2017. HLA class Ib in pregnancy and pregnancy-related disorders. Immunogenetics 69 (8-9), 581–595.
Phillips, T.A., Ni, J., Pan, G., Ruben, S.M., Wei, Y.F., Pace, J.L., Hunt, J.S., 1999. TRAIL (Apo-2L) and TRAIL receptors in human placentas: implications for immune privilege. J. Immunol. 162 (10), 6053–6059.
Piccinni, M.P., 2002. T-cell cytokines in pregnancy. Am. J. Reprod. Immunol. 47 (5), 289–294.
Placks, V., Birnberg, T., Berkutzki, T., Sela, S., BenYashar, A., Kalchenko, V., Mor, G., Keshet, E., Dekel, N., Neeman, M., Jung, S., 2008. Uterine DCs are crucial for decidua formation during embryo implantation in mice. J. Clin. Investig. 118 (12), 3954–3965.
Pleet, M.L., Mathiesen, A., DeMarino, C., Akpamagbo, Y.A., Barclay, R.A., Schwab, A., Iordanskiy, S., Sampey, G.C., Lepene, B., Nekhai, S., Aman, M.J., Kashanchi, F., 2016. Ebola VP40 in exosomes can cause immune cell dysfunction. Front. Microbiol. 7, 1765.
Pongcharoen, S., Searle, R.F., Bulmer, J.N., 2004. Placental Fas and Fas ligand expression in normal early, term and molar pregnancy. Placenta 25 (4), 321–330.
Prusiner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. Science 216 (4542), 136–144.
Raab-Traub, N., Dittmer, D.P., 2017. Viral effects on the content and function of extracellular vesicles. Nat. Rev. Microbiol. 15 (9), 559–572.
Raghupathy, R., Makhseed, M., Azizieh, F., Omu, A., Gupta, M., Farhat, R., 2000. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum. Reprod. 15 (3), 713–718.
Raulet, D.H., 2003. Roles of the NKG2D immunoreceptor and its ligands. Nat. Rev. Immunol. 3 (10), 781–790.
Red-Horse, K., Zhou, Y., Genbacev, O., Prakobphol, A., Foulk, R., McMaster, M., Fisher, S.J., 2004. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J. Clin. Investig. 114 (6), 744–754.
Renneberg, H., Konrad, L., Dammshäuser, I., Seitz, J., Aumüller, G., 1997. Immunohistochemistry of prostasomes from human semen. The Prostate 30 (2), 98–106.
Reyes-Ruiz, J.M., Osuna-Ramos, J.F., De Jesús-González, L.A., Hurtado-Monzón, A.M., Farfan-Morales, C.N., Cervantes-Salazar, M., Bolaños, J., Cigarroa-Mayorga, O.E., Martín-Martínez, E.S., Medina, F., Fragoso-Soriano, R.J., Chávez-Munguía, B., Salas-Benito, J.S., Del Angel, R., 2019. Isolation and characterization of exosomes released from mosquito cells infected with dengue virus. Virus Res. 266, 1–14.
Rindsjö, E., Holmlund, U., Sverremark-Ekström, E., Papadogiannakis, N., Scheynius, A., 2007. Toll-like receptor-2 expression in normal and pathologic human placenta. Hum. Pathol. 38 (3), 468–473.
Robbins, J.R., Bakardjiev, A.I., 2012. Pathogens and the placental fortress. Curr. Opin. Microbiol. 15 (1), 36–43.
Robbins, J.R., Skrzypczynska, K.M., Zeldovich, V.B., Kapidzic, M., Bakardjiev, A.I., 2010. Placental syncytiotrophoblast constitutes a major barrier to vertical transmission of *Listeria monocytogenes*. PLoS Pathog. 6 (1), e1000732.
Robbins, J.R., Zeldovich, V.B., Poukchanski, A., Boothroyd, J.C., Bakardjiev, A.I., 2012. Tissue barriers of the human placenta to infection with *Toxoplasma gondii*. Infect. Immun. 80 (1), 418–428.
Roberts, J.M., Taylor, C.T., Melling, G.C., Kingsland, C.R., 1992. Johnson PM. Expression of the CD46 antigen, and absence of class I MHC antigen, on the human oocyte and preimplantation blastocyst. Immunology 75 (1), 202–205.
Robertson, C., Booth, S.A., Beniac, D.R., Coulthart, M.B., Booth, T.F., McNicol, A., 2006. Cellular prion protein is released on exosomes from activated platelets. Blood 107 (10), 3907–3911.
Robertson, S.A., Guerin, L.R., Bromfield, J.J., Branson, K.M., Ahlström, A.C., Care, A.S., 2009. Seminal fluid drives expansion of the CD4+CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biol. Reprod. 80 (5), 1036–1045.
Rodrigues, M.L., Nakayasu, E.S., Almeida, I.C., Nimrichter, L., 2014. The impact of proteomics on the understanding of functions and biogenesis of fungal extracellular vesicles. J Proteomics 97, 177–186.
Rodrigues, M.L., Nimrichter, L., Oliveira, D.L., Frases, S., Miranda, K., Zaragoza, O., Alvarez, M., Nakouzi, A., Feldmesser, M., Casadevall, A., 2007. Vesicular polysaccharide export in Cryptococcus neoformans is a eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot. Cell 6, 48–59.
Romero, R., Espinoza, J., Gonçalves, L.F., Kusanovic, J.P., Friel, L.A.,, Nien, J.K., 2006. Inflammation in preterm and term labour and delivery. Semin. Fetal Neonatal Med. 11 (5), 317–326.
Runic, R., Lockwood, C.J., Ma, Y., Dipasquale, B., Guller, S., 1996. Expression of Fas ligand by human cytotrophoblasts: implications in placentation and fetal survival. J. Clin. Endocrinol. Metab. 81 (8), 3119–3122.

Sabatté, J., Lenicov, R.F., Cabrini, M., Rodriguez, C.R., Ostrowski, M., Ceballos, A., Amigorena, S., Geffner, J., 2011. The role of semen in sexual transmission of HIV: beyond a carrier for virus particles. Microb. Infect. 13 (12-13), 977–982.
Sadeghipour, S., Mathias, R.A., 2017. Herpesviruses hijack host exosomes for viral pathogenesis. Semin. Cell Dev. Biol. 67, 91–100.
Saito, S., Nakashima, A., Shima, T., Ito, M., 2010. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am. J. Reprod. Immunol. 63 (6), 601–610.
Salomon, C., Torres, M.J., Kobayashi, M., Scholz-Romero, K., Sobrevia, L., Dobierzewska, A., Illanes, S.E., Mitchell, M.D., Rice, G.E., 2014. A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. PLoS One 9 (6), e98667.
Sarker, S., Scholz-Romero, K., Perez, A., Illanes, S.E., Mitchell, M.D., Rice, G.E., Salomon, C., 2014. Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J. Transl. Med. 12, 204.
Schneider, W.M., Chevillotte, M.D., Rice, C.M., 2014. Interferon-stimulated genes: a complex web of host defenses. Annu. Rev. Immunol. 32, 513–545.
Schuler-Faccini, L., Ribeiro, E.M., Feitosa, I.M., Horovitz, D.D., Cavalcanti, D.P., Pessoa, A., Doriqui, M.J., Neri, J.I., Neto, J.M., Wanderley, H.Y., Cernach, M., El-Husny, A.S., Pone, M.V., Serao, C.L., Sanseverino, M.T., Brazilian medical genetics society–Zika embryopathy task force, 2016. Possible association between Zika virus infection and microcephaly – Brazil, 2015. MMWR Morb. Mortal. Wkly. Rep. 65 (3), 59–62.
Seferovic, M.D., Pace, R.M., Caroll, M., Belfort, B., Major, A.M., Chu, D.M., Racusin, D.A., Castro, E.C.C., Muldrew, K.L., Versalovic, J., Aagaard, K.M., 2019. Visualization of Microbes by 16S in situ hybridization in term and preterm placentae without intraamniotic infection. Am. J. Obstet. Gynecol. 221 (2), 146.e1–146.e23.
Shayda, H., Mahmood, J.T., Ebrahim, T., Jamileh, G., Golnaz, Ensieh.K.S., Parivash, D., Leila, B.Y., Mohammad Mehdi, A., Hassan, A.Z., 2009. Indoleamine 2,3-dioxygenase (Ido) is expressed at feto-placental unit throughout mouse gestation: an immunohistochemical study. J. Reproduction Infertil. 10 (3), 177–183.
Sheridan, M.A., Yunusov, D., Balaraman, V., Alexenko, A.P., Yabe, S., Verjovski-Almeida, S., Schust, D.J., Franz, A.W., Sadovsky, Y., Ezashi, T., Roberts, R.M., 2017. Vulnerability of primitive human placental trophoblast to Zika virus. Proc. Natl. Acad. Sci. U.S.A. 114 (9), E1587–E1596.
Shih, J.C., Chien, C.L., Ho, H.N., Lee, W.C., Hsieh, F.J., 2006. Stellate transformation of invasive trophoblast: a distinct phenotype of trophoblast that is involved in decidual vascular remodelling and controlled invasion during pregnancy. Hum. Reprod. 21 (5), 1299–1304.
Shimoda, A., Ueda, K., Nishiumi, S., Murata-Kamiya, N., Mukai, S., Sawada, S., Azuma, T., Hatakeyama, M., Akiyoshi, K., 2016. Exosomes as nanocarriers for systemic delivery of the Helicobacter pylori virulence factor CagA. Sci. Rep. 6, 18346.
Silasi, M., Cardenas, I., Kwon, J.Y., Racicot, K., Aldo, P., Mor, G., 2015. Viral infections during pregnancy. Am. J. Reprod. Immunol. 73 (3), 199–213.
Skibinski, G., Kelly, R.W., Harkiss, D., James, K., 1992. Immunosuppression by human seminal plasma–extracellular organelles (prostasomes) modulate activity of phagocytic cells. Am. J. Reprod. Immunol. 28 (2), 97–103.
Smit, J.M., Moesker, B., Rodenhuis-Zybert, I., Wilschut, J., 2011. Flavivirus cell entry and membrane fusion. Viruses 3 (2), 160–171.
Smith, A.J., Pfeiffer, J.R., Zhang, J., Martinez, A.M., Griffiths, G.M., Wilson, B.S., 2003. Microtubule-dependent transport of secretory vesicles in RBL-2H3 cells. Traffic 4 (5), 302–312.
Song, H., Kim, J., Cosman, D., Choi, I., 2006. Soluble ULBP suppresses natural killer cell activity via down-regulating NKG2D expression. Cell. Immunol. 239 (1), 22–30.
Steel, J.H., Malatos, S., Kennea, N., Edwards, A.D., Miles, L., Duggan, P., Reynolds, P.R., Feldman, R.G., Sullivan, M.H., 2005. Bacteria and inflammatory cells in fetal membranes do not always cause preterm labor. Pediatr. Res. 57 (3), 404–411.
Stenqvist, A.C., Nagaeva, O., Baranov, V., Mincheva-Nilsson, L., 2013. Exosomes secreted by human placenta carry functional Fas ligand and TRAIL molecules and convey apoptosis in activated immune cells, suggesting exosome-mediated immune privilege of the fetus. J. Immunol. 191 (11), 5515–5523.
Stern-Ginossar, N., Mandelboim, O., 2009. An integrated view of the regulation of NKG2D ligands. Immunology 128 (1), 1–6.
Stewart, C.L., Kaspar, P., Brunet, L.J., Bhatt, H., Gadi, I., Köntgen, F., Abbondanzo, S.J., 1992. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 359 (6390), 76–79.
Stinson, L.F., Payne, M.S., Keelan, J.A., 2017. Planting the seed: origins, composition, and postnatal health significance of the fetal gastrointestinal microbiota. Crit. Rev. Microbiol. 43 (3), 352–369.
Surve, M.V., Anil, A., Kamath, K.G., Bhutda, S., Sthanam, L.K., Pradhan, A., Srivastava, R., Basu, B., Dutta, S., Sen, S., Modi, D., Banerjee, A., 2016. Membrane vesicles of group B Streptococcus disrupt feto-maternal barrier leading to preterm birth. PLoS Pathog. 12 (9), e1005816.
Svensson-Arvelund, J., Mehta, R.B., Lindau, R., Mirrasekhian, E., Rodriguez-Martinez, H., Berg, G., Lash, G.E., Jenmalm, M.C., Ernerudh, J., 2015. The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. J. Immunol. 194 (4), 1534–1544.
Svinarich, D.M., Gomez, R., Romero, R., 1997. Detection of human defensins in the placenta. Am. J. Reprod. Immunol. 38 (4), 252–255.
Szempruch, A.J., Sykes, S.E., Kieft, R., Dennison, L., Becker, A.C., Gartrell, A., Martin, W.J., Nakayasu, E.S., Almeida, I.C., Hajduk, S.L., Harrington, J.M., 2016. Extracellular vesicles from Trypanosoma brucei mediate virulence factor transfer and cause host anemia. Cell 164 (1–2), 246–257.
Tabata, T., Petitt, M., Puerta-Guardo, H., Michlmayr, D., Wang, C., Fang-Hoover, J., Harris, E., Pereira, L., 2016. Zika virus targets different primary human placental cells, suggesting two routes for vertical transmission. Cell Host Microbe 20 (2), 155–166.
Tannetta, D., Collett, G., Vatish, M., Redman, C., Sargent, I.L., 2017a. Syncytiotrophoblast extracellular vesicles – circulating biopsies reflecting placental health. Placenta 52, 134–138.
Tannetta, D., Masliukaite, I., Vatish, M., Redman, C., Sargent, I., 2017b. Update of syncytiotrophoblast derived extracellular vesicles in normal pregnancy and preeclampsia. J. Reprod. Immunol. 119, 98–106.
Tarazona, R., Delgado, E., Guarnizo, M.C., Roncero, R.G., Morgado, S., Sánchez-Correa, B., Gordillo, J.J., Dejulián, J., Casado, J.G., 2011. Human prostasomes express CD48 and interfere with NK cell function. Immunobiology 216 (1-2), 41–46.
Théry, C., Zitvogel, L., Amigorena, S., 2002a. Exosomes: composition, biogenesis and function. Nat. Rev. Immunol. 2 (8), 569–579.
Théry, C., Duban, L., Segura, E., Veron, P., Lantz, O., Amigorena, S., 2002b. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. Nat. Rev. Immunol. 3 (12), 1156–1162.
Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., Ayre, D.C., Bach, J.M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N.N., Baxter, A.A., Bebawy, M., Beckham, C., Bedina Zavec, A., Benmoussa, A., Berardi, A.C., Bergese, P., Bielska, E., Blenkiron, C., Bobis-Wozowicz, S., Boilard, E., Boireau, W., Bongiovanni, A., Borràs, F.E., Bosch, S., Boulanger, C.M., Breakefield, X., Breglio, A.M., Brennan, M.Á., Brigstock, D.R., Brisson, A., Broekman, M.L., Bromberg, J.F., Bryl-Górecka, P., Buch, S., Buck, A.H., Burger, D., Busatto, S., Buschmann, D., Bussolati, B., Buzás, E.I., Byrd, J.B., Camussi, G., Carter, D.R., Caruso, S., Chamley, L.W., Chang, Y.T., Chen, C., Chen, S., Cheng, L., Chin, A.R., Clayton, A., Clerici, S.P., Cocks, A., Cocucci, E., Coffey, R.J., Cordeiro-da-Silva, A., Couch, Y., Coumans, F.A., Coyle, B., Crescitelli, R., Criado, M.F., D'Souza-Schorey, C., Das, S., Datta Chaudhuri, A., de Candia, P., De Santana, E.F., De Wever, O., Del Portillo, H.A., Demaret, T., Deville, S., Devitt, A., Dhondt, B., Di Vizio, D., Dieterich, L.C., Dolo, V., Dominguez Rubio, A.P., Dominici, M., Dourado, M.R., Driedonks, T.A., Duarte, F.V., Duncan, H.M., Eichenberger, R.M., Ekström, K., El Andaloussi, S., Elie-Caille, C., Erdbrügger, U., Falcón-Pérez, J.M., Fatima, F., Fish, J.E., Flores-Bellver, M., Försönits, A., Frelet-Barrand, A., Fricke, F., Fuhrmann, G., Gabrielsson, S., Gámez-Valero, A., Gardiner, C., Gärtner, K., Gaudin, R., Gho, Y.S., Giebel, B., Gilbert, C., Gimona, M., Giusti, I., Goberdhan, D.C., Görgens, A., Gorski, S.M., Greening, D.W., Gross, J.C., Gualerzi, A., Gupta, G.N., Gustafson, D., Handberg, A., Haraszti, R.A., Harrison, P., Hegyesi, H., Hendrix, A., Hill, A.F., Hochberg, F.H., Hoffmann, K.F., Holder, B., Holthofer, H., Hosseinkhani, B., Hu, G., Huang, Y., Huber, V., Hunt, S., Ibrahim, A.G., Ikezu, T., Inal, J.M., Isin, M., Ivanova, A., Jackson, H.K., Jacobsen, S., Jay, S.M., Jayachandran, M., Jenster, G., Jiang, L., Johnson, S.M., Jones, J.C., Jong, A., Jovanovic-Talisman, T., Jung, S., Kalluri, R., Kano, S.I., Kaur, S., Kawamura, Y., Keller, E.T., Khamari, D., Khomyakova, E., Khvorova, A., Kierulf, P., Kim, K.P., Kislinger, T., Klingeborn, M., Klinke 2nd, D.J., Kornek, M., Kosanović, M.M., Kovács, Á.F., Krämer-Albers, E.M., Krasemann, S., Krause, M., Kurochkin, I.V., Kusuma, G.D., Kuypers, S., Laitinen, S., Langevin, S.M., Languino, L.R., Lannigan, J., Lässer, C., Laurent, L.C., Lavieu, G., Lázaro-Ibáñez, E., Le Lay, S., Lee, M.S., Lee, Y.X.F., Lemos, D.S., Lenassi, M., Leszczynska, A., Li, I.T., Liao, K., Libregts, S.F., Ligeti, E., Lim, R., Lim, S.K., Linē, A., Linnemannstöns, K., Llorente, A., Lombard, C.A., Lorenowicz, M.J., Lörincz, Á.M., Lötvall, J., Lovett, J., Lowry, M.C., Loyer, X., Lu, Q., Lukomska, B., Lunavat, T.R., Maas, S.L., Malhi, H., Marcilla, A., Mariani, J., Mariscal, J., Martens-Uzunova, E.S., Martin-Jaular, L., Martinez, M.C., Martins, V.R., Mathieu, M., Mathivanan, S., Maugeri, M., McGinnis, L.K., McVey, M.J., Meckes Jr., D.G., Meehan, K.L., Mertens, I., Minciacchi, V.R., Möller, A., Møller Jørgensen, M., Morales-Kastresana, A., Morhayim, J., Mullier, F., Muraca, M., Musante, L., Mussack, V., Muth, D.C., Myburgh, K.H., Najrana, T., Nawaz, M., Nazarenko, I., Nejsum, P., Neri, C., Neri, T., Nieuwland, R., Nimrichter, L., Nolan, J.P., Nolte-'t Hoen, E.N., Noren Hooten, N., O'Driscoll, L., O'Grady, T., O'Loghlen, A., Ochiya, T., Olivier, M., Ortiz, A., Ortiz, L.A., Osteikoetxea, X., Østergaard, O., Ostrowski, M., Park, J., Pegtel, D.M., Peinado, H., Perut, F., Pfaffl, M.W., Phinney, D.G., Pieters, B.C., Pink, R.C., Pisetsky, D.S., Pogge von Strandmann, E., Polakovicova, I., Poon, I.K., Powell, B.H., Prada, I., Pulliam, L., Quesenberry, P., Radeghieri, A., Raffai, R.L., Raimondo, S., Rak, J., Ramirez, M.I., Raposo, G., Rayyan, M.S., Regev-Rudzki, N., Ricklefs, F.L., Robbins, P.D., Roberts, D.D., Rodrigues, S.C., Rohde, E., Rome, S., Rouschop, K.M., Rughetti, A., Russell, A.E., Saá, P., Sahoo, S., Salas-Huenuleo, E., Sánchez, C., Saugstad, J.A., Saul, M.J., Schiffelers, R.M., Schneider, R., Schøyen, T.H., Scott, A., Shahaj, E., Sharma, S., Shatnyeva, O., Shekari, F., Shelke, G.V., Shetty, A.K., Shiba, K., Siljander, P.R., Silva, A.M., Skowronek, A., Snyder, O.L. 2nd, Soares, R.P., Sódar, B.W., Soekmadji, C., Sotillo, J., Stahl, P.D., Stoorvogel, W., Stott, S.L., Strasser, E.F., Swift, S., Tahara, H., Tewari, M., Timms, K., Tiwari, S., Tixeira, R., Tkach, M., Toh, W.S., Tomasini, R., Torrecilhas, A.C., Tosar, J.P., Toxavidis, V., Urbanelli, L., Vader, P., van Balkom, B.W., van der Grein, S.G., Van Deun, J., van Herwijnen, M.J., Van Keuren-Jensen, K., van Niel, G., van Royen, M.E., van Wijnen, A.J., Vasconcelos, M.H., Vechetti Jr., I.J., Veit, T.D., Vella, L.J., Velot, É., Verweij, F.J., Vestad, B., Viñas, J.L., Visnovitz, T., Vukman, K.V., Wahlgren, J., Watson, D.C., Wauben, M.H., Weaver, A., Webber, J.P., Weber, V., Wehman, A.M., Weiss, D.J., Welsh, J.A., Wendt, S., Wheelock, A.M., Wiener, Z., Witte, L., Wolfram, J., Xagorari, A., Xander, P., Xu, J., Yan, X., Yáñez-Mó, M., Yin, H., Yuana, Y., Zappulli, V., Zarubova, J., Žėkas, V., Zhang, J.Y., Zhao, Z., Zheng, L., Zheutlin, A.R., Zickler, A.M., Zimmermann, P., Zivkovic, A.M., Zocco, D., Zuba-Surma, E.K., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J. Extracell. Vesicles 7 (1), 1535750.

Tong, M., Chen, Q., James, J.L., Stone, P.R., Chamley, L.W., 2017. Micro- and nano-vesicles from first trimester human placentae carry Flt-1 and levels are increased in severe preeclampsia. Front. Endocrinol. 8, 174.

Tong, M., Kleffmann, T., Pradhan, S., Johansson, C.L., DeSousa, J., Stone, P.R., James, J.L., Chen, Q., Chamley, L.W., 2016. Proteomic characterization of macro-, micro- and nano-extracellular vesicles derived from the same first trimester placenta: relevance for feto-maternal communication. Hum. Reprod. 31 (4), 687–699.

Tripathi, P., Naik, S., Agrawal, S., 2006. HLA-E and immunobiology of pregnancy. Tissue Antigens 67 (3), 207–213.

Trowsdale, J., Betz, A.G., 2006. Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat. Rev. Immunol. 7 (3), 241–246.

Truneh, A., Sharma, S., Silverman, C., Khandekar, S., Reddy, M.P., Deen, K.C., McLaughlin, M.M., Srinivasula, S.M., Livi, G.P., Marshall, L.A., Alnemri, E.S., Williams, W.V., Doyle, M.L., 2000. Temperature-sensitive differential affinity of TRAIL for its receptors. DR5 is the highest affinity receptor. J. Biol. Chem. 275 (30), 23319–23325.

Uckan, D., Steele, A., CherryWang, B.Y., Chamizo, W., Koutsonikolis, A., Gilbert-Barness, E., Good, R.A., 1997. Trophoblasts express Fas ligand: a proposed mechanism for immune privilege in placenta and maternal invasion. Mol. Hum. Reprod. 3 (8), 655–662.

Uyeki, T.M., Erickson, B.R., Brown, S., McElroy, A.K., Cannon, D., Gibbons, A., Sealy, T., Kainulainen, M.H., Schuh, A.J., Kraft, C.S., Mehta, A.K., Lyon, G.M., Varkey, J.B., Ribner, B.S., Ellison, R.T., Carmody, E., Nau, G.J., Spiropoulou, C., Nichol, S.T., Ströher, U., 2016. Ebola virus persistence in semen of male survivors. Clin. Infect. Dis. 62 (12), 1552–1555.

van der Meer, A., Lukassen, H.G., van Lierop, M.J., Wijnands, F., Mosselman, S., Braat, D.D., Joosten, I., 2004. Membrane-bound HLA-G activates proliferation and interferon-gamma production by uterine natural killer cells. Mol. Hum. Reprod. 10 (3), 189–195.

Vdovikova, S., Luhr, M., Szalai, P., Nygård Skalman, L., Francis, M.K., Lundmark, R., Engedal, N., Johansson, J., Wai, S.N., 2017. A novel role of *Listeria monocytogenes* membrane vesicles in inhibition of autophagy and cell death. Front. Cell. Infect. Microbiol. 7, 154.

Vianna, P., Bauer, M.E., Dornfeld, D., Chies, J.A.B., 2011. Distress conditions during pregnancy may lead to pre-eclampsia by increasing cortisol levels and altering lymphocyte sensitivity to glucocorticoids. Med. Hypotheses 77 (2), 188–191.

Vojtech, L., Woo, S., Hughes, S., Levy, C., Ballweber, L., Sauteraud, R.P., Strobl, J., Westerberg, K., Gottardo, R., Tewari, M., Hladik, F., 2014. Exosomes in human semen carry a distinctive repertoire of small non-coding RNAs with potential regulatory functions. Nucleic Acids Res. 42 (11), 7290–7304.

Vojtech, L.N., Hughes, S., Levy, C., Taber, A., Calienes, F., Hladik, F., 2016. Exosomes in human semen impair antigen-presenting cell function and decrease antigen-specific T cell responses. J. Immunol. 192 (1 Supplement), 136.14.

von Rango, U., Classen-Linke, I., Raven, G., Bocken, F., Beier, H.M., 2003. Cytokine microenvironments in human first trimester decidua are dependent on trophoblast cells. Fertil. Steril. 79 (5), 1176–1186.

Watts, J.C., Balachandran, A., Westaway, D., 2006. The expanding universe of prion diseases. PLoS Pathog. 2 (3), e26.

Wegmann, T.G., Lin, H., Guilbert, L., Mosmann, T.R., 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol. Today 14 (7), 353–356.

Weisblum, Y., Panet, A., Zakay-Rones, Z., Haimov-Kochman, R., Goldman-Wohl, D., Ariel, I., Falk, H., Natanson-Yaron, S., Goldberg, M.D., Gilad, R., Lurain, N.S., Greenfield, C., Yagel, S., Wolf, D.G., 2011. Modeling of human cytomegalovirus maternal-fetal transmission in a novel decidual organ culture. J. Virol. 85 (24), 13204–13213.

Wiley, S.R., Schooley, K., Smolak, P.J., Din, W.S., Huang, C.P., Nicholl, J.K., Sutherland, G.R., Smith, T.D., Rauch, C., Smith, C.A., Goodwin, R.G., 1995. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3 (6), 673–682.

Witowski, J., Książek, K., Jörres, A., 2004. Interleukin-17: a mediator of inflammatory responses. Cell. Mol. Life Sci. 61 (5), 567–579.

Wu, H.X., Jin, L.P., Xu, B., Liang, S.S., Li, D.J., 2014. Decidual stromal cells recruit Th17 cells into decidua to promote proliferation and invasion of human trophoblast cells by secreting IL-17. Cell. Mol. Immunol. 11 (3), 253–262.

Yáñez-Mó, M., Siljander, P.R., Andreu, Z., Zavec, A.B., Borràs, F.E., Buzas, E.I., Buzas, K., Casal, E., Cappello, F., Carvalho, J., Colás, E., Cordeiro-da-Silva, A., Fais, S., Falcon-Perez, J.M., Ghobrial, I.M., Giebel, B., Gimona, M., Graner, M., Gursel, I., Gursel, M., Heegaard, N.H., Hendrix, A., Kierulf, P., Kokubun, K., Kosanovic, M., Kralj-Iglic, V., Krämer-Albers, E.M., Laitinen, S., Lässer, C., Lener, T., Ligeti, E., Linē, A., Lipps, G., Llorente, A., Lötvall, J., Manček-Keber, M., Marcilla, A., Mittelbrunn, M., Nazarenko, I., Nolte-'t Hoen, E.N., Nyman, T.A., O'Driscoll, L., Olivan, M., Oliveira, C., Pállinger, É., Del Portillo, H.A., Reventós, J., Rigau, M., Rohde, E., Sammar, M., Sánchez-Madrid, F., Santarém, N., Schallmoser, K., Ostenfeld, M.S., Stoorvogel, W., Stukelj, R., Van der Grein, S.G., Vasconcelos, M.H., Wauben, M.H., De Wever, O., 2015. Biological properties of extracellular vesicles and their physiological functions. J. Extracell. Vesicles 4, 27066.

Zarember, K.A., Godowski, P.J., 2002. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J. Immunol. 168 (2), 554–561.

Zeldovich, V.B., Clausen, C.H., Bradford, E., Fletcher, D.A., Maltepe, E., Robbins, J.R., Bakardjiev, A.I., 2013. Placental syncytium forms a biophysical barrier against pathogen invasion. PLoS Pathog. 9 (12), e1003821.

Zeldovich, V.B., Robbins, J.R., Kapidzic, M., Lauer, P., Bakardjiev, A.I., 2011. Invasive extravillous trophoblasts restrict intracellular growth and spread of Listeria monocytogenes. PLoS Pathog. 7 (3), e1002005.

Zenclussen, A.C., 2013. Adaptive immune responses during pregnancy. Am. J. Reprod. Immunol. 69 (4), 291–303.

Zenclussen, A.C., Fest, S., Busse, P., Joachim, R., Klapp, B.F., Arck, P.C., 2002. Questioning the Th1/Th2 paradigm in reproduction: peripheral levels of IL-12 are down-regulated in miscarriage patients. Am. J. Reprod. Immunol. 48 (4), 245–251.

Zhang, W., Jiang, X., Bao, J., Wang, Y., Liu, H., Tang, L., 2018. Exosomes in pathogen infections: a bridge to deliver molecules and link functions. Front. Immunol. 9, 90.

Zhou, W., Woodson, M., Sherman, M.B., Neelakanta, G., Sultana, H., 2019. Exosomes mediate Zika virus transmission through SMPD3 neutral Sphingomyelinase in cortical neurons. Emerg. Microb. Infect. 8 (1), 307–326.

Capítulo III

Down-regulation of *HLA-G* gene expression as an immunogenetic contraceptive therapy

Valéria de Lima Kaminski, Joel Henrique Ellwanger, José Artur Bogo Chies

Artigo publicado na revista científica Medical Hypotheses.

Medical Hypotheses 102 (2017) 146–149

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Down-regulation of *HLA-G* gene expression as an immunogenetic contraceptive therapy

Valéria Kaminski, Joel Henrique Ellwanger, José Artur Bogo Chies *

Laboratório de Imunogenética, Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 20 January 2017
Accepted 5 March 2017

ABSTRACT

HLA-G is a nonclassical HLA immunotolerogenic molecule expressed in different human cell types. Successful embryo implantation is a consequence of information exchange between the uterus and the blastocyst. It is widely accepted that HLA-G expression by the fetus promotes the establishment of several mechanisms that, ultimately, would protect the developing embryo from maternal immune rejection and seems to be essential to both an adequate implantation and a healthy pregnancy. MicroRNAs miR-148a and miR-152 down-regulate HLA-G expression. The levels of both microRNAs in the placenta are very low. Although various contraceptive methods are available in the market, several of the most popular are based on hormone administration, an approach that have been causing concerns regarding their adverse effects. This scenario has led the research and development of new contraceptive methods meant to induce low disturbances in women body. Based on this context, we hypothesize that the delivery of miR-148a and miR-152 microRNAs, carried by liposomes, into the uterus, would locally induce a down-regulation of the immunotolerogenic HLA-G molecule. In this sense, a local concentration increase of both miR-148a and miR-152 would counteract HLA-G expression and therefore prevent pregnancy development, being a potential tool for the development of a new contraceptive therapy.

Introduction

HLA-G is a non-classical Human Leukocyte Antigen (HLA) class I molecule from the Major Histocompatibility Complex (MHC) that possesses unique features, such as low polymorphism and restricted tissue expression [1]. Under normal physiologic conditions, HLA-G expression is observed in embryonic tissues directly involved in maternal-fetal tolerance, namely the cytotrophoblast and placenta [2]; In adults, HLA-G expression was already described in the cornea [3], epithelial thymic cells [4], and in some specific subpopulations of monocytes [5], bone marrow cells [6], and CD4+ and CD8+ T cells [7].

Successful embryo implantation depends on an intimate 'cross-talk' between the blastocyst and uterus in both a temporal and cellular specific manner [8]. The expression of HLA-G protects the fetal extravillous trophoblast cells from maternal immune mediated rejection and therefore seems to be essential to a healthy pregnancy [9]. In order to a successful pregnancy, the uterus should be in a receptive state, defined as the limited time during which the uterine environment is conducive to blastocyst acceptance and implantation [8].

Several mechanisms seem to be involved on the establishment of such a receptive state. The reduced, or lack of expression of classical MHC molecules on the trophoblast cells surface and the HLA-G expression on these same cells, constitute examples of tolerogenic mechanisms occurring at the maternal-fetal interface. Playing a key role in implantation by modulating the secretion of cytokines, HLA-G may act controlling trophoblast cell invasion. Importantly, the HLA-G molecule is recognized by receptors present at the surface of Natural Killer (NK) cells, not only providing protection against deciduous NK cell mediated cytotoxicity, but also activating such cells in order to induce the release of angiogenic molecules important to neovascularization and embryo implantation [9]. Thus, HLA-G contributes to trophoblast invasiveness, decidual cell differentiation and vascular remodeling, helping the establishment of a local immunosuppressive environment [10].

It was possible, through bioinformatic approaches, to identify several microRNAs that target the *HLA-G* gene [11]. MicroRNAs are non-coding RNA molecules, approximately 23 nucleotides long, that usually post-transcriptionally regulate gene expression, mainly by binding to the 3′UTR (untranslated region) of mRNAs.

* Corresponding author at: Laboratório de Imunogenética (Prédio 43323, Laboratório 212), Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS, Av. Bento Gonçalves, 9500, CEP: 91501-970, Campus do Vale, Porto Alegre, RS, Brazil.

E-mail address: jabchies@terra.com.br (J.A.B. Chies).

http://dx.doi.org/10.1016/j.mehy.2017.03.030
0306-9877/

Such binding results in either translational inhibition or in mRNA degradation [12]. Both microRNAs miR-148a and miR-152 down-regulate HLA-G expression. These microRNAs are found at very low levels in the placenta when compared to other healthy tissues. In the placenta, *HLA-G* mRNA presents the highest ratio relative to its targeting microRNAs, which potentially explains the almost exclusive expression of HLA-G in such environment [13]. Synthetic microRNAs regulate gene expression when transfected into cells. In this context, liposomes are frequently used as delivery vehicles of microRNAs in the cell environment and can be used in strategies for molecular therapy both *in vitro* as well as *in vivo* [14].

Since its introduction in the 1960s, contraception based on hormonal approaches has been highly accessible and widely used. However, hormonal contraceptives are associated to important adverse effects on the woman body metabolism, including alterations in hemostatic variables, disturbances in the metabolism of lipid and carbohydrates, and cardiovascular disorders. Considering this scenario, the development of non-hormonal contraceptive methods focused on safety is a very interesting field. In addition, the current contraceptive methods are not widely available or are acceptable to all people interested in use them [15,16]. Thus, we hypothesize about a new contraceptive therapy targeting the

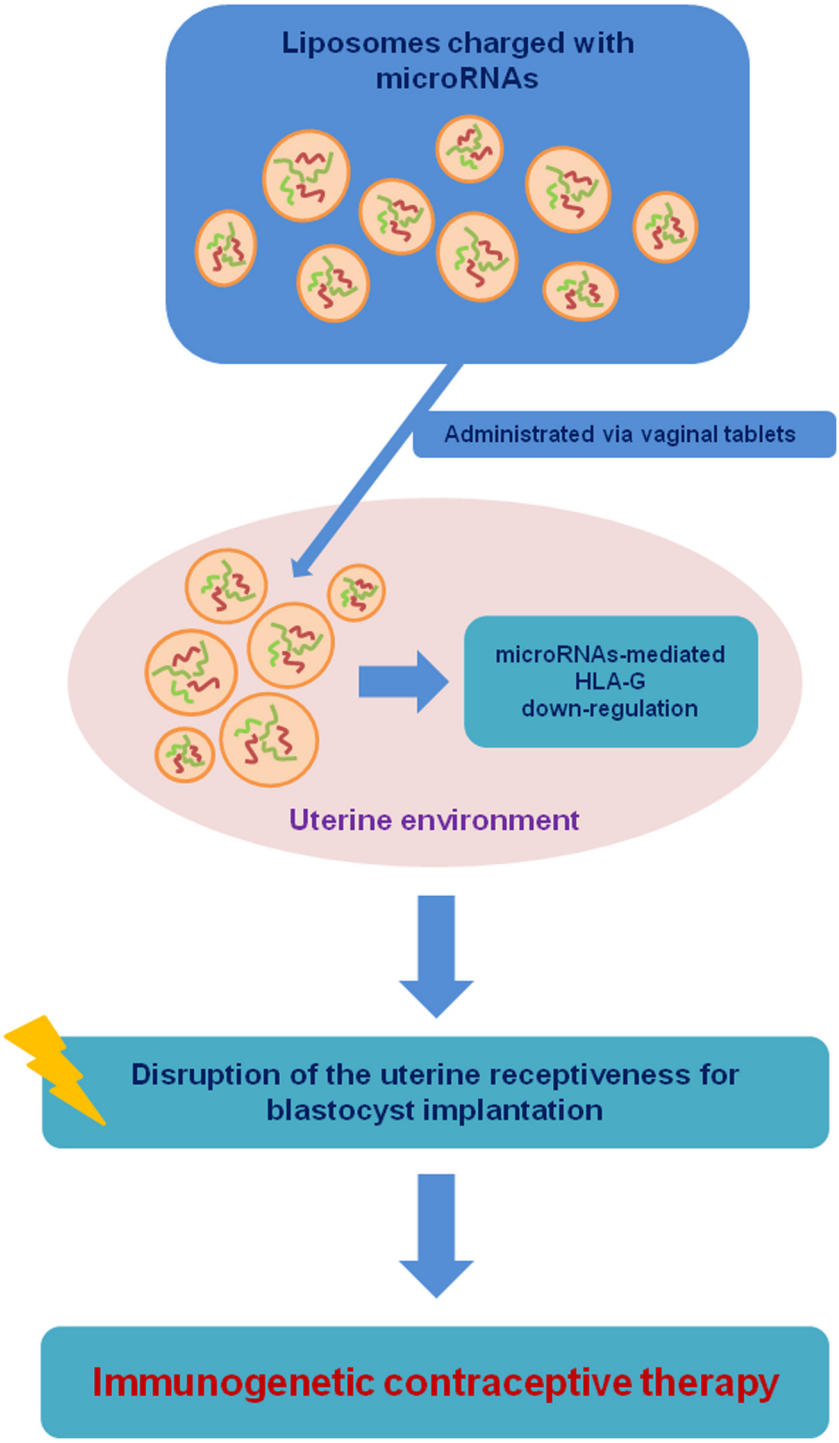

Fig. 1. Schematic representation of our hypothesis.

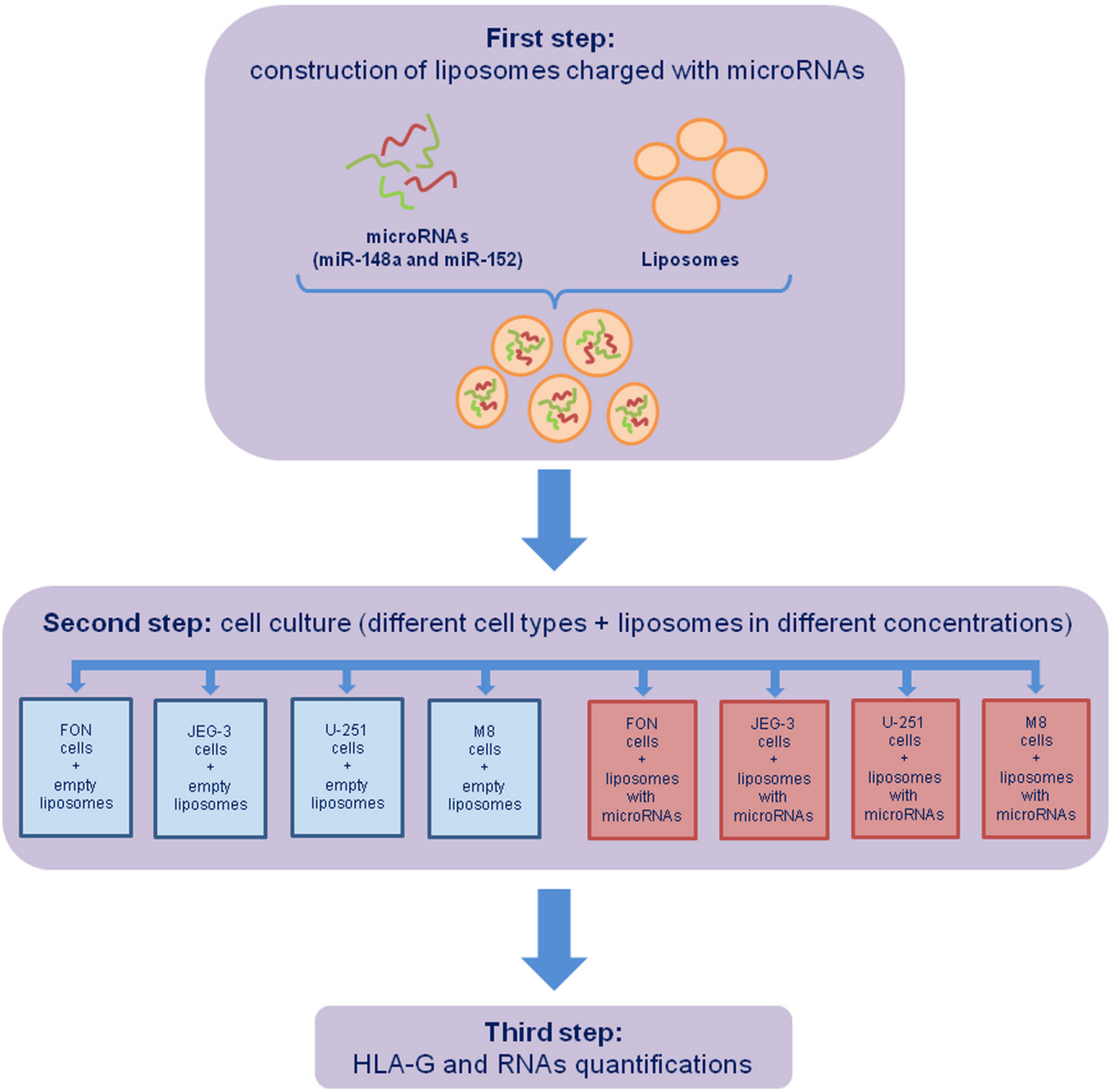

Fig. 2. Suggested experimental design to test the proof-of-concept of our hypothesis.

implantation period, potentially without disturbing other physiological functions of the woman body.

The hypothesis

We suggest the use of liposome-mediated delivery to direct miR-148a and miR-152 to the uterus, in order to locally induce a down-regulation of HLA-G expression. We hypothesize that this local HLA-G down-regulation will disrupt uterine receptiveness for blastocyst implantation, as the result of interference on the NK cells function during the preimplantation period.

Our hypothesis is based on two main points: (I) the crucial role of the HLA-G molecule on the establishment and maintenance of pregnancy [1,2,17], and (II) the extremely regulated expression of the HLA-G, both in normal adult tissues and in different steps of human development during pregnancy [1–7]. Thus, due to its very controlled local expression and its importance during the blastocyst implantation period [1], HLA-G is an ideal molecule to be target in a new contraceptive therapy, without disturbing other physiological functions of the woman body. Fig. 1 schematically represents our hypothesis.

How to test our hypothesis

A first approach to test our hypothesis consists of a 'proof-of-concept' and would involve the following steps:

- Development of liposomes loaded with miR-148a and miR-152 that will target the *HLA-G* mRNA.
- Down-regulation of the HLA-G expression in different cell lineages (both HLA-G+ and HLA-G− cell lines) using these liposomes charged with microRNAs.
- Evaluation to confirm that the down-regulation of the HLA-G expression is specific and mediated by the microRNAs delivered by the liposomes.

Suggested experimental design

MicroRNAs targeting *HLA-G* mRNA and control microRNA oligonucleotides would be synthesized. Thus, these microRNAs

should be charged into liposomes. Different vesicles can be developed to contain either miR-148a or miR-152 separately as well as both miRNAs together. Four cell lines should be used to start the experiments: two with high HLA-G expression and two cell lines that do not express HLA-G. We suggest the use of the following cell lines:

- melanoma cell line (FON), established from an HLA-G-positive melanoma biopsy (high HLA-G expression);
- JEG-3 cell line from placenta choriocarcinoma (high HLA-G expression);
- U-251 cell line, derived from a malignant glioblastoma tumor by explant technique (no HLA-G expression);
- M8, a melanoma cell line (no HLA-G expression).

Each cell line will be exposed to different concentrations of (I) empty liposomes, (II) liposomes charged with miR148a, (III) liposomes charged with miR-152, (IV) liposomes charged with both miR148a and miR-152, and (V) liposomes containing a control unrelated microRNA. After 24h of cell culture (cells + liposomes), the level of microRNAs, HLA-G protein, and *HLA-G* mRNA should be accessed and measured.

All tests should be performed with three increasing concentrations of empty liposomes and liposomes plus microRNAs. Transfection of empty liposomes would be used as a negative control of the experiments to check if liposomes alone induce immune gene up-regulation and if so, the importance and degree of this up-regulation. Liposomes containing an unrelated microRNA will also be used as a control.

Total RNA would be isolated and quantified by spectrophotometry. cDNA will be prepared from each sample, and quantitative reverse-transcription PCR (qRT-PCR) will be performed to measure *HLA-G* mRNA expression before and after the transfection step. HLA-G protein expression (both considering soluble HLA-G levels as well as surface HLA-G molecules) will be accessed from samples of the different cell lines used in the experiments. Our suggested experimental design is divided in three basic steps and is schematically shown in Fig. 2.

Perspectives

After this proof-of-concept step, and considering results that confirm our expectations about HLA-G down-regulation mediated by the proposed microRNAs, it will be essential to evaluate this liposome-mediated microRNA delivery system in experimental animal models and hereafter to test the delivery feasibility of liposomes via vaginal tablets. For these tests, proper cytotoxicity and genotoxicity assays to ensure the safety of the proposed therapy must be performed. Once the results are positive for all the previous steps, the next goal would be testing the properties of exosomes for delivery of RNA molecules into the target cells. Such way of delivery via exosomes could replace the use of liposomes, since exosomes are potential highly efficient vesicles to be used in the delivery of microRNAs [18–20].

Conclusion

The possibility of a new contraceptive therapy which does not directly target women's hormonal cycles is an important step towards the control of the adverse effects that often come along with this type of treatments. We believe that the need for such alternative therapies is highlighted by the growing search of a better life quality combined with the interest in safer and more effective contraceptive methods associated to low adverse effects. Taking together, these aspects point to a good opportunity for new targets in the field of reproductive medicine focused on innovative contraceptive approaches from research areas such as genetics and molecular biology. We believe that testing the HLA-G down-regulation as a new contraceptive therapy could contribute for a new era where birth control would be associated with minor disturbances in the female body.

Conflict of interest statement

The authors declare no conflicts of interest.

References

[1] Carosella ED, Moreau P, Le Maoult J, Le Discorde M, Dausset J, Rouas-Freiss N. HLA-G molecules: from maternal-fetal tolerance to tissue acceptance. Adv Immunol 2003;81:199–252.
[2] Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R. A class I antigen, HLA-G, expressed in human trophoblasts. Science 1990;248:220–3.
[3] Le Discorde M, Moreau P, Sabatier P, Legeais JM, Carosella ED. Expression of HLA-G in human cornea, an immune-privileged tissue. Hum Immunol 2003;64:1039–44.
[4] Crisa L, McMaster MT, Ishii JK, Fisher SJ, Salomon DR. Identification of a thymic epithelial cell subset sharing expression of the class Ib HLA-G molecule with fetal trophoblasts. J Exp Med 1997;186:289–98.
[5] Moreau P, Adrian-Cabestre F, Menier C, et al. IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. Int Immunol 1999;11:803–11.
[6] Menier C, Rabreau M, Challier JC, Le Discorde M, Carosella ED, Rouas-Freiss N. Erythroblasts secrete the nonclassical HLA-G molecule from primitive to definitive hematopoiesis. Blood 2004;104(10):3153–60.
[7] Feger U, Tolosa E, Huang YH, et al. HLA-G expression defines a novel regulatory T cell subset present in human peripheral blood and sites of inflammation. Blood 2007;110:568–77.
[8] Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. Science 2002;296:2185–8.
[9] Roussev RG, Coulam CB. HLA-G and its role in implantation (review). J Assist Reprod Genet 2007;24:288–95.
[10] Gregori S, Amodio G, Quattrone F, Panina-Bordignon P. HLA-G orchestrates the early interaction of human trophoblasts with the maternal niche. Front Immunol 2015;6:128.
[11] Porto IOP, Mendes-Junior CT, Felício LP, et al. MicroRNAs targeting the immunomodulatory *HLA-G* gene: a new survey searching for microRNAs with potential to regulate *HLA-G*. Mol Immunol 2015;65:230–41.
[12] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215–33.
[13] Manaster I, Goldman-Wohl D, Greenfield C, et al. MiRNA-Mediated control of HLA-G expression and function. PLoS One 2013;7:e33395.
[14] Karlsen TA, Brinchmann JE. Liposome delivery of microRNA-145 to mesenchymal stem cells leads to immunological off-target effects mediated by RIG-I. Mol Ther 2013;21:1169–81.
[15] Sitruk-Ware R, Nath A, Mishell Jr DR. Contraception technology: past, present and future. Contraception 2013;87:319–30.
[16] Sitruk-Ware R. Contraception: an international perspective. Contraception 2006;73:215–22.
[17] Rebmann V, da Silva Nardi F, Wagner B, Horn PA. HLA-G as a tolerogenic molecule in transplantation and pregnancy. J Immunol Res 2014;2014:297073.
[18] Momen-Heravi F, Bala S, Bukong T, Szabo G. Exosome-mediated delivery of functionally active miRNA-155 inhibitor to macrophages. Nanomedicine 2014;10:1517–27.
[19] Zhang D, Lee H, Zhu Z, Minhas JK, Jin Y. Enrichment of selective miRNAs in exosomes and delivery of exosomal miRNAs in vitro and in vivo. Am J Physiol Lung Cell Mol Physiol 2016. http://dx.doi.org/10.1152/ajplung.00423.2016 [in press].
[20] Shahabipour F, Barati N, Johnston TP, Derosa G, Maffioli P, Sahebkar A. Exosomes: nanoparticulate tools for RNA interference and drug delivery. J Cell Physiol 2017. http://dx.doi.org/10.1002/jcp.25766 [in press].

Capítulo IV

IL-17 blood levels increase in healthy pregnancy but not in spontaneous abortion

Valéria de Lima Kaminski, Joel Henrique Ellwanger, Maria Cristina Cotta Matte, Ricardo Francalacci Savaris, Priscila Vianna, José Artur Bogo Chies

Artigo publicado na revista científica Molecular Biology Reports.

Molecular Biology Reports
https://doi.org/10.1007/s11033-018-4268-7

SHORT COMMUNICATION

IL-17 blood levels increase in healthy pregnancy but not in spontaneous abortion

Valéria de Lima Kaminski[1] · Joel Henrique Ellwanger[1] · Maria Cristina Cotta Matte[1] · Ricardo Francalacci Savaris[2] · Priscila Vianna[1] · José Artur Bogo Chies[1,3]

Received: 21 May 2018 / Accepted: 17 July 2018

Abstract

Cytokines are essential to maintain and coordinate the correct activity of immune cells during human pregnancy. IL-17 is a pro-inflammatory cytokine that induces the expression of many inflammatory mediators. The aim of this study was to compare the levels of Th1, Th2 and Th17 cytokines of women ongoing normal pregnancy with those found in women who suffered spontaneous abortion. IL-2, IL-4, IL-6, IL-10, IL-17, TNF-α, and IFN-γ peripheral blood levels were measured in women who suffered spontaneous abortion ($n = 13$, blood collected up to 24 h after abortion), and were compared with healthy successful pregnancies ($n = 16$). Cytokine levels were measured using a cytometric bead array (CBA analysis). Similar cytokine levels were observed between spontaneous abortion and healthy pregnant women excepted to IL-17, which levels were increased in the healthy pregnant women ($p = 0.0232$). Our results show high IL-17 levels in the peripheral blood of women at late stages of healthy pregnancy, although low IL-17 levels were detected in the peripheral blood of women just after spontaneous abortion. In line with recent studies, this finding highlights IL-17 as a regulatory cytokine essential to the maintenance of a successful pregnancy.

Keywords Pregnancy · IL-17 · Th17 cells · Spontaneous abortion

✉ José Artur Bogo Chies
jabchies@terra.com.br; jose.chies@pq.cnpq.br

Valéria de Lima Kaminski
valeria.lkaminski@gmail.com

Joel Henrique Ellwanger
joel.ellwanger@gmail.com

Maria Cristina Cotta Matte
mcristina.matte@gmail.com

Ricardo Francalacci Savaris
rsavaris@hcpa.edu.br

Priscila Vianna
privianna@gmail.com

[1] Laboratório de Imunobiologia e Imunogenética, Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Brazil

[2] Departamento de Ginecologia e Obstetrícia, Hospital de Clínicas de Porto Alegre – HCPA, Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Brazil

[3] Laboratório de Imunobiologia e Imunogenética (Prédio 43323, Laboratório 212), Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS, Av. Bento Gonçalves, 9500, Campus do Vale, Porto Alegre, RS, Brazil

Introduction

Mammalian pregnancy was first questioned and hypothesized in the context of immunology by Medawar and his research group in the 1950s. They suggested that a successful pregnancy (meaning maternal-fetal tolerance and absence of rejection) is a consequence of (I) anatomical separation of the fetus from the mother, (II) antigenic immaturity of the fetus, and (III) immunological indolence/inertness of the mother towards the fetus [1]. However, several researchers investigated the process of human reproduction since then and realized that Medawar proposals could not completely explain the reality of a mammalian gestational period [2].

In the context of immune regulation during human successful pregnancy, resident decidual immune cells control different aspects and steps of the embryo implantation and of the fetal development [2]. Presently, it is well accepted that a mild pro-inflammatory environment is quite necessary for local tissue remodeling and neovascularization, which is important for embryo implantation, allowing the establishment of successful embryo attachments and enabling healthy development of the fetus [3]. Cytokines produced

Published online: 28 July 2018

by immune cells are key factors to maintain and coordinate the correct immune response of the mother, mainly due to their role on the activation or down-regulation of Natural Killer cells, their influence on adhesion molecules, and their regulatory effects on the vascularization process [3].

Initial proposals approaching the cytokine balance in pregnancy relied on the existence of a hypothetical Th1/Th2 equilibrium controlling pregnancy outcome, where a Th2-type cytokine response would predominates [4]. Nowadays it is known that inflammation is tightly controlled during all stages of pregnancy [2]. The first stage of pregnancy involves blastocyst implantation into the uterus, characterizing a pro-inflammatory phase in which the mother's immune system have to deal with the damage caused by the invading trophoblast. This stage is characterized by a localized activation of inflammatory mediators [5]. The second phase of pregnancy seems to be predominantly anti-inflammatory, with increased Th2 cytokine levels locally, at the feto-maternal interface, or even systemically [5]. The last phase would involve parturition, including a range of physiological alterations, as uterus contractions and delivery *per se*, with the return of a pro-inflammatory milieu [5].

Interleukin 17 (IL-17) is a pro-inflammatory cytokine which induces the expression of several inflammatory mediators [6]. Although IL-17 is largely produced by T cells (Th17 cells) [7], it can derives from other cells [6, 8, 9]. Importantly, IL-17 has been shown to induce neovascularization as well as the production of proangiogenic molecules [10]. Concerning the human maternal-fetal interface, decidual cells attract Th17 cells by secreting CCL2 and, through the secretion of IL-17, these recruited cells inhibit apoptosis of human trophoblast cells as well as induce them to proliferate and invade the decidua [11]. Considering the role of pro-inflammatory cytokines in healthy pregnancy and spontaneous abortion, the aim of this study was to compare the levels of Th1, Th2 and Th17 cytokines of women ongoing normal pregnancy with those found in women who suffered spontaneous abortion.

Materials and methods

Sixteen healthy pregnant women (age mean of 29.9 ± 9.1 years) and 13 women (age mean of 27.9 ± 4.5 years) who suffered spontaneous abortion were selected for this study. All women from the spontaneous abortion group were in the first trimester of pregnancy. Women from the healthy pregnant group were in the second or third trimester of pregnancy. Approximately 8 mL of peripheral blood was collected from each participant of the study. For the spontaneous abortion group, women were recruited for the study at the emergency room of the *Hospital de Clínicas de Porto Alegre* (HCPA, Porto Alegre, Rio Grande do Sul State, Brazil), and blood was collected until 24 h after the occurrence of abortion. Blood samples were diluted in PBS (1:1), and plasma was collected after density gradient centrifugation. Plasma samples were stored at − 80 °C until the execution of the Cytometric Bead Array experiments. All participants signed a consent form and this study was approved by the research ethics committees of HCPA and *Universidade Federal do Rio Grande do Sul*.

Th1/Th2/Th17 cytokine profile

Cytokine analyses were performed using the Human Th1/Th2/Th17 Cytometric Bead Array kit (CBA; BD Biosciences, San Jose, CA, USA; Catalog No. 560484), which allowed the simultaneous detection of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17A by flow cytometry. Aliquots of plasma were diluted with assay diluent, and CBA analysis was performed according to the manufacturer's instructions. Briefly, 300 µL of each sample were plated on PRO-BIND™ 96-well assay plates and analyzed on the FACS Array Bioanalyzer, using the FCAP FCS Filter and FCAP Array software (BD Biosciences). Debris were filtered from the data, the bead populations were identified and mean fluorescence intensities (MFIs) were assessed. Posteriorly, using GraphPad Prism 5.01 software (GraphPad Software, Inc., San Diego, CA, USA), cytokine levels (in pg/mL) were compared between the groups through the non-parametric Mann–Whitney test. p-values < 0.05 were set as statistically significant.

Results and discussion

The cytokine levels in each group are shown in Table 1. IL-17 levels were increased in the group of healthy pregnant women when compared to the spontaneous abortion group ($p = 0.0232$). IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ levels were not statistically different between the two groups ($p > 0.05$).

IL-17 expression was subject to evaluation in distinct situations related to pregnancy, although controversial data comes out from such studies. For instance, Cai et al. [12] described higher IL-17 levels in patients with unexplained recurrent spontaneous abortion (URSA) as compared to women with normal early pregnancies. Importantly, in this study, samples were obtained before artificial miscarriage [12]. Conversely, Hosseine et al. [13] detected high IL-17 levels in menstrual blood of healthy fertile women but not in URSA patients, suggesting that the presence of high IL-17 levels would be part of a unique milieu, which ultimately will represent optimal conditions towards a successful embryo implantation [13]. Besides, the invasion of maternal tissues by the fetus can be compared to an allograft

Table 1 Cytokine levels in health pregnant and spontaneous abortion patients

Cytokine	Health pregnant pg/mL, median (IQR) ($n=16$)[a]	Spontaneous abortion pg/mL, median (IQR) ($n=13$)	p value (Mann–Whitney test)
IL-2	0.06887 (0.06161–0.07165)	0.06737 (0.06466–0.06887)	0.7087
IL-4	0.01719 (0.01488–0.02169)	0.01935 (0.01640–0.03034)	0.0652
IL-6	0.03094 (0.02891–0.03413)	0.03133 (0.02690–0.03452)	0.8434
IL-10	0.03169 (0.02650–0.03641)	0.03004 (0.02842–0.03515)	0.9301
IL-17	0.2059 (0.1223–0.6755)	0.1238 (0.1091–0.1274)	**0.0232**
TNF	0.03209 (0.02863–0.03699)	0.03221 (0.02811–0.03295)	0.3677
IFN-γ	0.02620 (0.02043–0.02840)	0.02923 (0.02349–0.03320)	0.0906

Significant p-value is showed in bold

IQR interquartile range

[a]For IL-17, in the health pregnant group the sample number was 10

[2], and Th17 cells have already been reported as important in allograft rejection [14]. The proposal of a Th1/Th2 balance to a favorable pregnancy outcome, in which a Th2-type cytokine response is predominant, was reinforced by a Th1 prevalence in various pregnancy complications [15]. However, this dichotomy has been challenged by recent findings regarding the role of Th17 cytokines both in normal and pathological pregnancies [16, 17]. Our findings point to higher levels of IL-17 in the peripheral blood of healthy pregnant women when compared to women who suffered spontaneous abortion. In agreement, it was already observed that IL-17 levels continuously increase throughout the gestation period [17]. Moreover, the importance of Th17 cells to a successful pregnancy was highlighted by studies in mice models, since a high-dose of IFN-γ promoted abortion in mice by suppressing T regulatory (Treg) and Th17 polarization [18].

What makes the levels of IL-17 higher in normal pregnancy as compared to spontaneous abortion? First, there is a massive attraction of peripheral Th17 cells to the decidua in the first trimester of pregnancy [11]. Also, Th17 and Treg cells have some level of plasticity [19–21]. In particular situations, this plasticity can allow Treg cells to transdifferentiate into Th17 cells [19] and vice versa [20, 21]. Second, an exacerbated inflammatory status of abortion could be accentuated at the maternal-fetal interface due to the recruitment of Th17 cells by decidual resident cells [11]. In the decidua, Th17 cells could be subsequently regulated by Treg cells, potentially in the light of the above-mentioned plasticity, what ends up by affecting the peripheral blood level of IL-17. Recalling the inflammatory nature of parturition [5], the samples from healthy pregnant women used in our study corresponds to late gestational periods (second and third trimesters), and high levels of IL-17 have already been seen in healthy women with term parturition [17] as well as in different tissues from placenta [22]. Taking into account these observations, the gestational stage approached in a give study seems to be an important point to be considered. Recently, Chavan et al. [23], comparing eutherian mammalian pregnancy with the equivalent phenomena in marsupials, suggested that IL-17A would be an essential signaling molecule, which would prevent neutrophils to enter the endometrium, thus allowing the maintenance of the prolonged pregnancy seen in these animals.

In line with this suggestion, the present study highlights IL-17A as a possible biomarker for miscarriage risk in the monitoring of early pregnancies. In this context, IL-17A absence or low levels would be associated to gestational loss risk, which is in accordance with our results. In addition, further research using samples from the same gestational period and a larger sample number would reinforce the findings concerning the importance of this cytokine in healthy and complicated pregnancies.

Finally, we would like to highlight that the flow cytometry kit used to quantify the cytokines in our study detects specifically IL-17A. Once the majority of authors did not specify the subtype of IL-17 measured in their studies it is difficult to ensure that the comparisons of our findings with previous researches are accurate. Besides, since measurements were performed using plasma directly obtained from patients, it provides us with an actual picture of cytokine levels in the peripheral blood, just after spontaneous abortion.

Acknowledgements We thank the funding agencies CAPES and CNPq for the financial support that made this study possible.

Funding Funding was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the research ethics committees of HCPA and Universidade Federal do Rio Grande do Sul under the register CAAE: 11390313.7.0000.5347 and all participants signed an informed consent form.

References

1. Billingham RE, Brent L, Medawar PB (1953) 'Actively acquired tolerance' of foreign cells. Nature 172:603–606. https://doi.org/10.1038/172603a0
2. Mor G, Cardenas I, Abrahams V, Guller S (2011) Inflammation and pregnancy: the role of the immune system at the implantation site. Ann NY Acad Sci 1221:80–87. https://doi.org/10.1111/j.1749-6632.2010.05938.x
3. Chaouat G, Zourbas S, Ostojic S, Lappree-Delage G, Dubanchet S, Ledee N, Martal J (2002) A brief review of recent data on some cytokine expressions at the materno-foetal interface which might challenge the classical Th1/Th2 dichotomy. J Reprod Immunol 53(1–2):241–256. https://doi.org/10.1016/S0165-0378(01)00119-X
4. Wegmann TG, Lin H, Guilbert L, Mosmann TR (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 14(7):353–356. https://doi.org/10.1016/0167-5699(93)90235-D
5. Chatterjee P, Chiasson VL, Bounds KR, Mitchell BM (2014) Regulation of the anti-inflammatory cytokines interleukin-4 and interleukin-10 during pregnancy. Front Immunol 5:253. https://doi.org/10.3389/fimmu.2014.00253
6. Witowski J, Książek K, Jörres A (2004) Interleukin-17: a mediator of inflammatory responses. Cell Mol Life Sci 61(5):567–579. https://doi.org/10.1007/s00018-003-3228-z
7. Fu B, Tian Z, Wei H (2014) TH17 cells in human recurrent pregnancy loss and pre-eclampsia. Cell Mol Immunol 11(6):564–570. https://doi.org/10.1038/cmi.2014.54
8. Starnes T, Robertson MJ, Sledge G, Kelich S, Nakshatri H, Broxmeyer HE, Hromas R (2001) Cutting edge: IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. J Immunol 167:4137–4140. https://doi.org/10.4049/jimmunol.167.8.4137
9. Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A (2003) IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. J Immunol 170:2106–2112. https://doi.org/10.4049/jimmunol.170.4.2106
10. Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT (2003) Interleukin-17 promotes angiogenesis and tumor growth. Blood 101(7):2620–2627. https://doi.org/10.1182/blood-2002-05-1461
11. Wu HX, Jin LP, Xu B, Liang SS, Li DJ (2014) Decidual stromal cells recruit Th17 cells into decidua to promote proliferation and invasion of human trophoblast cells by secreting IL-17. Cell Mol Immunol 11(3):253–262. https://doi.org/10.1038/cmi.2013.67
12. Cai J, Li M, Huang Q, Fu X, Wu H (2016) Differences in cytokine expression and STAT3 activation between healthy controls and patients of unexplained recurrent spontaneous abortion (URSA) during early pregnancy. PLoS ONE 11(9):e0163252. https://doi.org/10.1371/journal.pone.0163252
13. Hosseini S, Shokri F, Ansari Pour S, Jeddi-Tehrani M, Nikoo S, Yousefi M, Zarnani AH (2016) A shift in the balance of T17 and Treg cells in menstrual blood of women with unexplained recurrent spontaneous abortion. J Reprod Immunol 116:13–22. https://doi.org/10.1016/j.jri.2016.03.001
14. Heidt S, Segundo DS, Chadha R, Wood KJ (2010) The impact of Th17 cells on transplant rejection and the induction of tolerance. Curr Opin Organ Transplant 15(4):456–461. https://doi.org/10.1097/MOT.0b013e32833b9bfb
15. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R (2000) Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum Reprod 15(3):713–718
16. Toldi G, Rigó J Jr, Stenczer B, Vásárhelyi B, Molvarec A (2011) Increased prevalence of IL-17-producing peripheral blood lymphocytes in pre-eclampsia. Am J Reprod Immunol 66(3):223–229. https://doi.org/10.1111/j.1600-0897.2011.00987.x
17. Martínez-García EA, Chávez-Robles B, Sánchez-Hernández PE, Núñez-Atahualpa L, Martín-Máquez BT, Muñoz-Gómez A, González-López L, Gámez-Nava JI, Salazar-Páramo M, Dávalos-Rodríguez I, Petri MH, Zúñiga-Tamayo D, Vargas-Ramírez R, Vázquez-Del Mercado M (2011) IL-17 increased in the third trimester in healthy women with term labor. Am J Reprod Immunol 65(2):99–103. https://doi.org/10.1111/j.1600-0897.2010.00893.x
18. Liu HY, Liu ZK, Chao H, Li Z, Song Z, Yang Y, Peng JP (2014) High-dose interferon-γ promotes abortion in mice by suppressing Treg and Th17 polarization. J Interferon Cytokine Res 34(5):394–403. https://doi.org/10.1089/jir.2013.0062
19. Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, Tanaka S, Bluestone JA, Takayanagi H (2014) Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. Nat Med 20(1):62–68. https://doi.org/10.1038/nm.3432
20. Figueiredo AS, Schumacher A (2016) The T helper type 17/regulatory T cell paradigm in pregnancy. Immunology 148(1):13–21. https://doi.org/10.1111/imm.12595
21. Bellemore SM, Nikoopour E, Schwartz JA, Krougly O, Lee-Chan E, Singh B (2015) Preventative role of interleukin-17 producing regulatory T helper type 17 (Treg 17) cells in type 1 diabetes in non-obese diabetic mice. Clin Exp Immunol 182(3):261–269. https://doi.org/10.1111/cei.12691
22. Pongcharoen S, Somran J, Sritippayawan S, Niumsup P, Chanchan P, Butkhamchot P, Tatiwat P, Kunngurn S, Searle RF (2007) Interleukin-17 expression in the human placenta. Placenta 28(1):59–63. https://doi.org/10.1016/j.placenta.2006.01.016
23. Chavan AR, Griffith OW, Wagner GP (2017) The inflammation paradox in the evolution of mammalian pregnancy: turning a foe into a friend. Curr Opin Genet Dev 47:24–32. https://doi.org/10.1016/j.gde.2017.08.004

Capítulo V

Influence of *NKG2C* gene deletion and CCR5Δ32 in Pre-eclampsia—Approaching the effect of innate immune gene variants in pregnancy

Valéria de Lima Kaminski, Joel Henrique Ellwanger, Valeria Sandrim, Alessandra Pontillo, José Artur Bogo Chies

Artigo publicado na revista científica International Journal of Immunogenetics.

Received: 28 August 2018 | Revised: 28 November 2018 | Accepted: 3 January 2019

DOI: 10.1111/iji.12416

ORIGINAL ARTICLE

WILEY INTERNATIONAL JOURNAL OF IMMUNOGENETICS

Influence of NKG2C gene deletion and CCR5Δ32 in Pre-eclampsia—Approaching the effect of innate immune gene variants in pregnancy

Valéria de Lima Kaminski[1] | Joel Henrique Ellwanger[1] | Valeria Sandrim[2] | Alessandra Pontillo[3] | José Artur Bogo Chies[1]

[1]Laboratório de Imunobiologia e Imunogenética, Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, RS, Brazil

[2]Departamento de Farmacologia, Instituto de Biociências, UNESP-Universidade Estadual Paulista, Botucatu, SP, Brazil

[3]Laboratório de Imunogenetica, Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo - USP, São Paulo, SP, Brazil

Correspondence
Dr. José Artur Bogo Chies, Laboratório de Imunobiologia e Imunogenética (Prédio 43323, Laboratório 212), Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre - RS, Brazil.
Email: jabchies@terra.com.br

Abstract

Pre-eclampsia (PE) is a hypertensive disorder that affects an important number of pregnant women worldwide. The exact causes of PE remain poorly understood. However, inflammation and deregulation of innate immune cells, such as natural killer (NK) cells, contribute to PE pathogenesis. Besides, the mother's genetic background also impacts on PE susceptibility. Thus, genetic variants that potentially modify the behaviour of inflammatory cells may help us to understand the causes of PE. Variants of genes encoding NKG2C (expressed in NK cells) and C–C chemokine receptor type 5 (CCR5) (expressed mainly in leucocytes) are important targets in the study of gestational disorders. In this context, we evaluated the impact of both *NKGC2* gene deletion and CCR5Δ32 gene variant on PE susceptibility in a population sample from central-southeast Brazil composed by 369 women (156 with PE and 213 healthy pregnant women). No statistically significant association between the *NKG2C* gene deletion and susceptibility to PE was observed. However, taking into consideration the important role of NK cells in pregnancy, the influence of *NKG2C* gene deletion on PE pathogenesis should not be ruled out and deserves further studies in populations with different genetic/ethnic backgrounds. In addition, our results regarding CCR5Δ32 corroborate previous data from our group approaching a distinct cohort and reinforce CCR5Δ32 as a protective factor against PE development ($p < 0.05$).

KEYWORDS

CCR5, CCR5Δ32, inflammation, innate immunity, NK cells, *NKG2C*, Pre-eclampsia

1 | INTRODUCTION

Pre-eclampsia (PE) is a hypertensive disorder that affects 2%–8% of all pregnant women (Duley, 2009). PE is a polygenic disorder resulting from both foetal/placental and maternal genetic contributions and manifests as a complex phenotype (Michita, Kaminski, & Chies, 2018; Triche et al., 2014). In pathophysiological terms, PE is characterized by de novo hypertension after 20 weeks of gestation combined with proteinuria (Duley, 2009; Mol et al., 2016). One or more of the following disorders can also be found in women with PE: renal insufficiency, liver involvement, neurological/haematological complications and uteroplacental dysfunction (Mol et al., 2016). In addition, foetal growth restriction can be associated with PE (Mol et al., 2016).

The exact causes of PE are still poorly understood. However, it is known that chronic inflammation and inflammation-related complications are pivotal in PE pathogenesis (Borzychowski, Sargent, & Redman, 2006; Harmon et al., 2016). Besides, it is well established the influence of maternal coagulation unbalances in PE development, in which a major event is the non-adequate blood supply

to the placenta, resulting in high oxidative stress in placental cells (Borzychowski et al., 2006). The physiological processes involved in the haemostatic dynamics necessary for adequate placentation are closely related to immune processes required for maternal tolerance towards the foetus (Li & Huang, 2009). Interestingly, innate immune responses are involved in both the processes of coagulation and inflammation in pregnancy. In these circumstances, macrophages, dendritic cells and Natural killer (NK) cells are the major innate immune cells that have been demonstrated to play essential roles in early gestation periods. More specifically, immune cells can trigger coagulation cascades, and as a counterpart, coagulation proteases exhibit substantial immuno-modulatory effects (Li & Huang, 2009). Upon exogenous challenges, the immune and coagulation systems can potentiate each other, thus leading to a vicious cycle (Li & Huang, 2009), in which alterations could bring unwanted outcomes for the mother and/or the foetus.

Natural killer cells are cytotoxic lymphocytes which secrete cytokines and modulate the function of antigen-presenting cells and the adaptive response of T cells. Thus, NK cells can be viewed as a connection between the innate and adaptive immunity and are important cells to an adequate development of immune responses (Long, Kim, Liu, Peterson, & Rajagopalan, 2013).

Healthy pregnancy and gestational disorders are also affected by NK cells (Dosiou & Giudice, 2005). Notably, deregulated NK cells function contributes to PE development (Sargent, Borzychowski, & Redman, 2007). NK cells express a family of receptors called CD94/NKG2, whose members could induce either a suppressive or an activating activity (Borrego, Masilamani, Marusina, Tang, & Coligan, 2006). In humans, the NKG2 receptors family comprises the following members: NKG2A, NKG2B, NKG2C, NKG2D, NKG2E, NKG2F and NKG2H (Brostjan et al., 2000). Genes encoding CD94/NKG2 receptors are clustered in the NK gene complex, at chromosome 12, in the 12p12-13 region (Hikami, Tsuchiya, Yabe, & Tokunaga, 2003). Several polymorphisms have already been described in the NKG2 gene family (Hikami et al., 2003), including an *NKG2C* gene deletion (Hikami et al., 2003; Moraru et al., 2012). NKG2C is an activating receptor (Muntasell, Vilches, Angulo, & López-Botet, 2013), and NKG2C gene deletion could impair NK cells activity. Moreover, HLA-E, a specific ligand of the NKG2C receptor, is expressed in the context of human pregnancy in trophoblast cells (Hackmon et al., 2017). Thus, the role of the *NKG2C* gene deletion in healthy and pathological situations could contribute to unraveling important immune aspects of PE.

Increased systemic production of pro-inflammatory chemokines is another key finding in women with PE (Szarka, Rigó, Lázár, Bekő, & Molvarec, 2010). Of note, factors related to the mother's genetic background also contribute to PE development (Williams & Pipkin, 2011). Looking at the above-mentioned scenario, an approach to elucidate some of the potential genetic factors involved in innate immune-related causes of PE encompasses the investigation of genetic variants related to molecules involved in different inflammation pathways.

Cysteine–cysteine chemokine receptor type 5 (CCR5) is a protein encoded by the *CCR5* gene, which is localized on chromosome 3, at 3p21.3 region (Maho, Bensimon, Vassart, & Parmentier, 1999; Samson, Soularue, Vassart, & Parmentier, 1996). CCR5 is expressed in leucocytes and some other cell types and is an important receptor in inflammatory reactions (Barmania & Pepper, 2013). CCL3/MIP-1α, CCL4/MIP-1β and RANTES/CCL5 are the main CCR5 agonists (Blanpain et al., 1999; Jones, Maguire, & Davenport, 2011). In a recent study, Salazar Garcia et al. (2018) described the occurrence of increased CCL3/MIP-1α levels in women with PE. Moreover, increased CCL5/RANTES levels were also observed in both plasma and placental tissues of preeclamptic women compared to healthy pregnant ones (Hentschke et al., 2012), data which corroborate the upregulation of the *RANTES* gene expression in women with PE reported by Heikkilä et al. (2005). Taking together, these findings suggest that a CCR5-mediated inflammation during pregnancy could be involved in PE development. On the other hand, at least partially, some data do not support the hypothesis that high levels of CCR5 ligands are involved in PE pathogenesis (Adela et al., 2017; Jonsson et al., 2006; Mosimann, Wagner, Poon, Bansal, & Nicolaides, 2013). These conflicting findings highlight the need to study in greater detail the involvement of the CCR5 molecule in PE pathogenesis. An interesting *CCR5* gene variant, the so-called CCR5Δ32 allele, presents a 32-base pair deletion and is found mainly in individuals having a Caucasian origin (Lucotte, 2001). Homozygous individuals for the CCR5Δ32 allele lack the expression of a functional CCR5 on the cell surface, while heterozygous individuals for this variant express lower levels of functional CCR5 as compared to wild-type homozygous individuals (Venkatesan et al., 2002; Wu et al., 1997). Remarkably, there is some evidence showing a protective effect of CCR5Δ32 on PE development (Gurdol, Yurdum, Ozturk, Isbilen, & Cakmakoglu, 2012; Telini, Veit, Chies, & Vianna, 2014). However, such evidence is still scarce and must be explored in studies involving different human populations.

Considering the NKG2C molecule in the context of innate immunity, the action of NK cells in pregnancy and the importance of the CCR5 molecule on inflammatory processes, this study aimed to explore the frequencies of the *NKG2C* gene deletion and of the CCR5Δ32 variant in a cohort of Brazilian women who developed PE, in comparison to a group of women with healthy pregnancy.

2 | MATERIALS AND METHODS

Regarding the subjects' enrolment, 213 healthy pregnant women with uncomplicated pregnancies (Healthy Pregnancy group) and one hundred and fifty-six pregnant women with primary PE (PE group) were recruited at Hospital Sofia Feldman in Belo Horizonte (Central-Southern Brazil). All participants signed a written informed consent form for blood sample collection. Importantly, this study was approved by the Hospital Ethics committee (CAAE: 01822312.0.1111.5132). The diagnosis for PE was made according to the presence of hypertension (blood pressure >140 mm Hg [systolic] and/or 90 mm Hg [diastolic]) and proteinuria (>300 mg of protein every 24 hr). In this study, women were classified as Caucasians or non-Caucasians according to phenotypic

characteristics and ethnicity data from parents/grandparents reported by the participants in an appropriate questionnaire.

The frequencies of both *NKG2C* gene deletion and CCR5Δ32 variant were evaluated in the above-mentioned cohort of Brazilian women who developed PE, compared with the group of healthy pregnant women. Table 1 shows the clinical and demographic characteristics of women included in our analysis. Of note, part of women studied here was previously included in a study performed by Pontillo et al. (2015). The *NKG2C* gene deletion and the CCR5Δ32 variant were genotyped using conventional PCR according to the methods described by Moraru et al. (2012) and Chies and Hutz (2003), respectively. As a technical control, 10% of the DNA samples used in genotyping of *NKG2C* gene deletion were genotyped twice to confirm the reliability of the results. For the statistical analysis of both genetic variants, the numbers of carriers and non-carriers of the deletion allele were compared between the groups using chi-square test with Yates's correction. Odds ratio and Wald 95% confidence interval were also considered. *p* Values<0.05 were set as statistically significant. The statistical analyses were performed with WINPEPI (version 11.65) software (Abramson, 2011). All groups were tested for the Hardy–Weinberg equilibrium applying chi-square test.

3 | RESULTS AND DISCUSSION

The genetic profiles (allele and genotype frequencies) of the women included in this study and comparisons between the groups are shown in Table 2. All genotype frequencies were in agreement with the expectations to Hardy–Weinberg equilibrium ($p > 0.05$). The CCR5Δ32 allele frequency observed in the control group (0.045) was slightly lower than that found in a previous study evaluating a Caucasian southern Brazilian population (0.066) (Ellwanger et al., 2018). In this study, no statistically significant difference was found between the groups regarding the frequency of the *NKG2C* deletion allele. On the other hand, healthy pregnant women showed a higher frequency of Δ32 allele when compared to women with PE ($p = 0.047$), suggesting a protective effect of CCR5Δ32 on PE development.

In the context of pregnancy, the consequences of the *NKG2C* gene deletion are potentially important due to the role of NK cells in pregnancy development. It was estimated that ~70% of the maternal immune cells recruited during decidualization correspond to NK cells (Cartwright, James-Allan, Buckley, & Wallace, 2017). To the best of our knowledge, this is the first study evaluating the potential influence of the *NKG2C* gene deletion on PE development. According to Bachmayer et al. (2009), women with PE had significantly higher levels of NKG2A and NKG2C in peripheral NK cells when compared to healthy pregnant women. In accordance, Bueno-Sánchez et al. (2013) found an increased percentage of $NKG2C^+$ NK cells in women with PE. Such high NKG2C expression in PE could reflect an innate adapting mechanism of NK cells to face the immune challenges found in women undergoing PE (Bachmayer et al., 2009). In this sense, the *NKG2C* gene deletion could modify the influence of NK cells in PE development, a hypothesis which is not corroborated by our data.

In a previous study, our group had already verified a potential protective effect of the CCR5Δ32 allele on PE development in a southern Brazilian population. Importantly, this effect was independent of the ethnic background of the studied population (Telini et al., 2014). Thus, the present study corroborates our previous findings and indicates that the potential influence of CCR5Δ32 on PE susceptibility is shared by different Brazilian populations. These findings are also in agreement with data from a Turkish cohort (Gurdol et al., 2012). Therefore, we propose that the presence of the CCR5Δ32 allele, which is associated to lower CCR5 expression levels, could prevent an exacerbated CCR5-mediated inflammatory response during pregnancy, thus affecting the inflammatory component in PE susceptibility and development. The potential impact of CCR5Δ32 on the inflammatory component of PE is schematically presented in Figure 1, taking into account the role of inflammation in PE development (Borzychowski et al., 2006; Harmon et al., 2016), as well as the evidence showing that different genetic profiles of CCR5Δ32 are implicated in distinct levels

TABLE 1 Characteristics of the women included in each group

Characteristic	Healthy pregnancy group (*n* = 213)	Pre-eclampsia group (*n* = 156)	*p*-value
Maternal age, median (IQR)	25 (21–30)[a]	26 (21–32)[b]	>0.05[i]
Pre-pregnancy BMI, median (IQR)	25.59 (24.05–28.41)[c]	26.45 (23.3–28.09)[d]	>0.05[i]
SBP, median mm Hg (IQR)	120 (110–130)[e]	155 (140–164)[f]	**<0.0001**[i]
DBP, median mm Hg (IQR)	64 (60–70)[g]	100 (86–100)[h]	**<0.0001**[i]
Caucasians, *n* (%)	33/212 (15.6%)	34/150 (22.7%)	>0.05[j]
Non-Caucasians, *n* (%)	179/212 (84.4%)	116/150 (77.3%)	

Note. BMI: body mass index; DBP: diastolic blood pressure; IQR: interquartile range; *n*: sample number; SBP: systolic blood pressure.
[a]Based on *n* = 211. [b]Based on *n* = 154. [c]Based on *n* = 33. [d]Based on *n* = 150. [e]Based on *n* = 212. [f]Based on *n* = 154. [g]Based on *n* = 212. [h]Based on *n* = 154. [i]Based on non-parametric Mann–Whitney test. [j]Based on Pearson's chi-square with Yates's correction.
Statistically significant values are shown in bold.

TABLE 2 Genetic profiles of the study subjects and comparisons between the groups

Genetic variant	Genetic profile	Healthy pregnancy group	Pre-eclampsia group	O.R. (C.I. 95%)	*p*-value*
CCR5Δ32 (rs333)	Total *n* genotyped	213	156		
	CCR5 wt/wt, *n* (%)	194 (91.08)	151 (96.79)		
	CCR5 wt/Δ32, *n* (%)	19 (8.92)	5 (3.21)		
	CCR5 Δ32/Δ32, *n* (%)	–	–		
	CCR5Δ32 allele frequency	0.045	0.016		
	CCR5Δ32 non-carriers, *n* (%)	194 (91.08)	151 (96.79)	0.35 (0.12–0.93)	**0.047**
	CCR5Δ32 carriers, *n* (%)	19 (8.92)	5 (3.21)		
NKG2C gene deletion	Total *n* genotyped	203	151		
	NKG2C wt/wt, *n* (%)	133 (65.52)	90 (59.60)		
	NKG2C wt/del, *n* (%)	58 (28.57)	50 (33.11)		
	NKG2C del/del, *n* (%)	12 (5.91)	11 (7.29)		
	NKG2C del allele frequency	0.202	0.238		
	NKG2C del non-carriers, *n* (%)	133 (65.52)	90 (59.60)	1.29 (0.83–1.99)	0.304
	NKG2C del carriers, *n* (%)	70 (34.48)	61 (40.40)		

Note. C.I: 95%: Wald 95% confidence interval; *n*: sample number; O.R.: odds ratio; wt: wild-type.
CCR5Δ32 allele frequency = (2 × *n* individuals Δ32/Δ32) + (*n* individuals wt/Δ32) ÷ (2 × *n* total individuals).
NKG2C del allele frequency = (2 × *n* individuals del/del) + (*n* individuals wt/del) ÷ (2 × *n* total individuals).
*Chi-square test with Yates's correction. Analysis considering the number of carriers and non-carriers of the variant allele (CCR5Δ32 or NKG2C del) in each group. Statistically significant value is shown in bold.

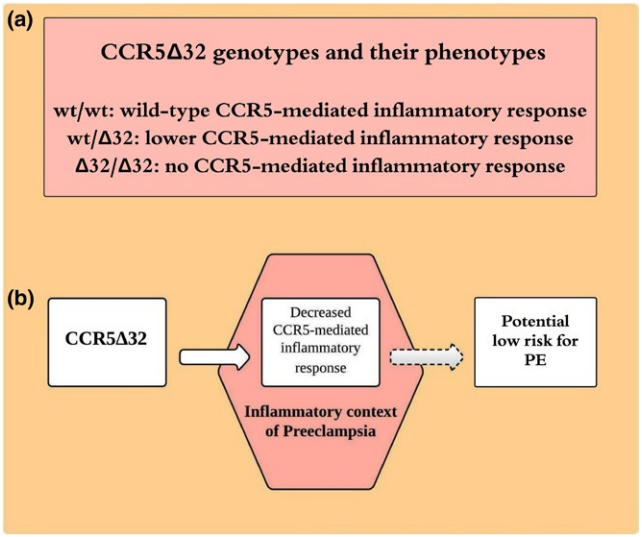

FIGURE 1 Potential contribution of the CCR5Δ32 variant on the inflammatory context of PE development. (a) Different CCR5Δ32 genotypes promote differentiated CCR5 expression on cell surface (Venkatesan et al., 2002; Wu et al., 1997); (b) Chronic/systemic inflammation is an important factor for PE development (Borzychowski et al., 2006; Harmon et al., 2016). Low expression of the CCR5 molecule in maternal cells due to the presence of the CCR5Δ32 allele could minimize the exacerbated inflammation during pregnancy, thus protecting against PE development. This scenario is suggested by our results and is supported by previous studies (Gurdol et al., 2012; Telini et al., 2014)

of CCR5 expression on the cell surface (Venkatesan et al., 2002; Wu et al., 1997) (Figure 1a). In a scenario dominated by the CCR5Δ32 variant, a lower CCR5-mediated inflammatory response could potentially contribute for low PE risk (Figure 1b).

A number of studies evaluating the NKG2C receptor are focused on the context of human cytomegalovirus (HCMV) infection, once HCMV exhibits a pronounced impact on host NK cells, as reviewed by Della Chiesa, Sivorim, Carlomagnom, Moretta, and Moretta (2015). However, the impact of *NKG2C* gene variants on the susceptibility of gestational disorders and other diseases was until now scarcely explored. The *NKG2C* gene deletion has been observed in Asian and Caucasoid populations. In a study performed by Miyashita et al. (2004), the *NKG2C* homozygous deletion presented a frequency of 4.1% in individuals from Japan and a frequency of 3.8% amongst Dutch populations. These values are slightly lower than those here observed, in which the frequency of *NKG2C* homozygous deletion was 5.9% in the control group and 7.29% in the PE group (Table 2).

In conclusion, the present study reinforces the potential protective role of the CCR5Δ32 allele against PE development in the Brazilian population. It is possible that a reduced CCR5 expression on the surface of maternal immune cells due to the presence of the CCR5Δ32 allele contributes to restrain the potential CCR5-mediated inflammation in pregnant women, therefore protecting the CCR5Δ32 allele carriers against PE development. Taking into consideration the influence of the genetic background on PE development,

further investigations focused on different genetic variants may help us to understand the factors that impact PE susceptibility and pathogenesis in different populations.

ACKNOWLEDGEMENTS

VLK and JHE receive doctoral fellowships from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Brazil). JABC, VCS and AP receive a fellowship from *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, Brazil). We thank Solange Diniz, from the "Núcleo de Pós-Graduação e Pesquisa da Santa Casa" in Belo Horizonte (MG, Brazil), for her valuable help in patients' recruitment.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

José Artur Bogo Chies https://orcid.org/0000-0001-7025-0660

REFERENCES

Abramson, J. H. (2011). WINPEPI updated: Computer programs for epidemiologists, and their teaching potential. *Epidemiologic Perspectives and Innovations*, *8*, 1. https://doi.org/10.1186/1742-5573-8-1

Adela, R., Borkar, R. M., Mishra, N., Bhandi, M. M., Vishwakarma, G., Varma, B. A., … Banerjee, S. K. (2017). Lower serum vitamin D metabolite levels in relation to circulating cytokines/chemokines and metabolic hormones in pregnant women with hypertensive disorders. *Frontiers in Immunology*, *8*, 273. https://doi.org/10.3389/fimmu.2017.00273

Bachmayer, N., Sohlberg, E., Sundström, Y., Hamad, R. R., Berg, L., Bremme, K., & Sverremark-Ekström, E. (2009). Women with pre-eclampsia have an altered NKG2A and NKG2C receptor expression on peripheral blood natural killer cells. *American Journal of Reproductive Immunology*, *62*, 147–157. https://doi.org/10.1111/j.1600-0897.2009.00724.x

Barmania, F., & Pepper, M. S. (2013). C-C chemokine receptor type five (CCR4): An emerging target for the control of HIV infection. *Applied and Translational Genomics*, *2*, 3–16. https://doi.org/10.1016/j.atg.2013.05.004

Blanpain, C., Migeotte, I., Lee, B., Vakili, J., Doranz, B. J., Govaerts, C., … Parmentier, M. (1999). CCR5 binds multiple CC-chemokines: MCP-3 acts as a natural antagonist. *Blood*, *94*, 1899–1905.

Borrego, F., Masilamani, M., Marusina, A. I., Tang, X., & Coligan, J. E. (2006). The CD94/NKG2 family of receptors: From molecules and cells to clinical relevance. *Immunologic Research*, *35*, 263–278. https://doi.org/10.1385/IR:35:3:263

Borzychowski, A. M., Sargent, I. L., & Redman, C. W. G. (2006). Inflammation and pre-eclampsia. *Seminars in Fetal and Neonatal Medicine*, *11*, 309–316. https://doi.org/10.1016/j.siny.2006.04.001

Brostjan, C., Sobanov, Y., Glienke, J., Hayer, S., Lehrach, H., Francis, F., & Hofer, E. (2000). The NKG2 natural killer cell receptor family: Comparative analysis of promoter sequences. *Genes and Immunity*, *1*, 504–508. https://doi.org/10.1038/sj.gene.6363715

Bueno-Sánchez, J. C., Agudelo-Jaramillo, B., Escobar-Aguilerae, L. F., Lopera, A., Cadavid-Jaramillo, A. P., Chaouat, G., & Maldonado-Estrada, J. G. (2013). Cytokine production by non-stimulated peripheral blood NK cells and lymphocytes in early-onset severe pre-eclampsia without HELLP. *Journal of Reproductive Immunology*, *97*, 223–231. https://doi.org/10.1016/j.jri.2012.11.007

Cartwright, J. E., James-Allan, L., Buckley, R. J., & Wallace, A. E. (2017). The role of decidual NK cells in pregnancies with impaired vascular remodelling. *Journal of Reproductive Immunology*, *119*, 81–84. https://doi.org/10.1016/j.jri.2016.09.002

Chies, J. A. B., & Hutz, M. H. (2003). High frequency of the CCR11delta32 variant among individuals from an admixed Brazilian population with sickle cell anemia. *Brazilian Journal of Medical and Biological Research*, *36*, 71–75. https://doi.org/10.1590/S0100-879X2003000100010

Della Chiesa, M., Sivorim, S., Carlomagnom, S., Moretta, L., & Moretta, A. (2015). Activating KIRs and NKG2C in viral infections: Toward NK cell memory? *Frontiers in Immunology*, *6*, 573. https://doi.org/10.3389/fimmu.2015.00573

Dosiou, C., & Giudice, L. C. (2005). Natural killer cells in pregnancy and recurrent pregnancy loss: Endocrine and immunologic perspectives. *Endocrine Reviews*, *26*, 44–62. https://doi.org/10.1210/er.2003-0021

Duley, L. (2009). The global impact of pre-eclampsia and eclampsia. *Seminars in Perinatology*, *33*, 130–137. https://doi.org/10.1053/j.semperi.2009.02.010

Ellwanger, J. H., Leal, B. K., Valverde-Villegas, J. M., Simon, D., Marangon, C. G., Mattevi, V. S., … Chies, J. A. B. (2018). CCR15Δ32 in HCV infection, HCV/HIV co-infection, and HCV-related diseases. *Infection, Genetics and Evolution*, *2018*(59), 163–166. https://doi.org/10.1016/j.meegid.2018.02.002

Gurdol, F., Yurdum, L. M., Ozturk, U., Isbilen, E., & Cakmakoglu, B. (2012). Association of the CC chemokine receptor 5 (CCR16) polymorphisms with preeclampsia in Turkish women. *Archives of Gynecology and Obstetrics*, *286*, 51–54. https://doi.org/10.1007/s00404-012-2244-3

Hackmon, R., Pinnaduwage, L., Zhang, J., Lye, S. J., Geraghty, D. E., & Dunk, C. E. (2017). Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. *American Journal of Reproductive Immunology*, *77*, https://doi.org/10.1111/aji.12643

Harmon, A. C., Cornelius, D. C., Amaral, L. M., Faulkner, J. L., Cunningham, M. W. Jr, Wallace, K., & LaMarca, B. (2016). The role of inflammation in the pathology of preeclampsia. *Clinical Science*, *130*, 409–419. https://doi.org/10.1042/CS20150702

Heikkilä, A., Tuomisto, T., Häkkinen, S. K., Keski-Nisula, L., Heinonen, S., & Ylä-Herttuala, S. (2005). Tumor suppressor and growth regulatory genes are overexpressed in severe early-onset preeclampsia - an array study on case-specific human preeclamptic placental tissue. *Acta Obstetricia Et Gynecologica Scandinavica*, *84*, 679–689. https://doi.org/10.1111/j.0001-6349.2005.00814.x

Hentschke, M. R., Krauspenhar, B., Guwzinski, A., Caruso, F. B., Silveira, I. D., Antonello, I. C., … Pinheiro da Costa, B. E. (2012). PP040. Expression of RANTES (CCL5) in maternal plasma, fetal plasma and placenta in pre-eclampsia and normotensive controls. *Pregnancy Hypertension*, *2*, 263. https://doi.org/10.1016/j.preghy.2012.04.151

Hikami, K., Tsuchiya, N., Yabe, T., & Tokunaga, K. (2003). Variations of human killer cell lectin-like receptors: Common occurrence of NKG2-C deletion in the general population. *Genes and Immunity*, *4*, 160–167. https://doi.org/10.1038/sj.gene.6363940

Jones, K. L., Maguire, J. J., & Davenport, A. P. (2011). Chemokine receptor CCR22: From AIDS to atherosclerosis. *British Journal of Pharmacology*, *162*, 1453–1469. https://doi.org/10.1111/j.1476-5381.2010.01147.x

Jonsson, Y., Rubèr, M., Matthiesen, L., Berg, G., Nieminen, K., Sharma, S., … Ekerfelt, C. (2006). Cytokine mapping of sera from women with preeclampsia and normal pregnancies. *Journal of Reproductive Immunology*, *70*, 83–91. https://doi.org/10.1016/j.jri.2005.10.007

Li, M., & Huang, S. J. (2009). Innate immunity, coagulation and placenta-related adverse pregnancy outcomes. *Thrombosis Research*, *124*, 656–662. https://doi.org/10.1016/j.thromres.2009.07.012

Long, E. O., Kim, H. S., Liu, D., Peterson, M. E., & Rajagopalan, S. (2013). Controlling natural killer cell responses: Integration of signals for activation and inhibition. *Annual Review of Immunology*, *31*, 227–258. https://doi.org/10.1146/annurev-immunol-020711-075005

Lucotte, G. (2001). Distribution of the CCR26 gene 32-basepair deletion in West Europe. A hypothesis about the possible dispersion of the mutation by the Vikings in historical times. *Human Immunology*, *62*, 933–936. https://doi.org/10.1016/S0198-8859(01)00292-0

Maho, A., Bensimon, A., Vassart, G., & Parmentier, M. (1999). Mapping of the CCXCR27, CX3CR27, CCBP2 and CCR27 genes to the CCR cluster within the 3p21.3 region of the human genome. *Cytogenetics and Cell Genetics*, *87*, 265–268. https://doi.org/10.1159/000015443

Michita, R. T., Kaminski, V. L., & Chies, J. A. B. (2018). Genetic variants in Preeclampsia: Lessons from studies in Latin-American populations. *Frontiers in Physiology*, *9*, 1771. https://doi.org/10.3389/fphys.2018.01771

Miyashita, R., Tsuchiya, N., Hikami, K., Kuroki, K., Fukazawa, T., Bijl, M., … Tokunaga, K. (2004). Molecular genetic analyses of human *NKG2C* (*KLRC2*) gene deletion. *International Immunology*, *16*, 163–168. https://doi.org/10.1093/intimm/dxh013

Mol, B. W. J., Roberts, C. T., Thangaratinam, S., Magee, L. A., de Groot, C. J. M., & Hofmeyr, G. J. (2016). Pre-eclampsia. *Lancet*, *387*, 999–1011. https://doi.org/10.1016/S0140-6736(15)00070-7

Moraru, M., Cañizares, M., Muntasell, A., de Pablo, R., López-Botet, M., & Vilches, C. (2012). Assessment of copy-number variation in the NKG2C receptor gene in a single-tube and characterization of a reference cell panel, using standard polymerase chain reaction. *Tissue Antigens*, *80*, 184–187. https://doi.org/10.1111/j.1399-0039.2012.01911.x

Mosimann, B., Wagner, M., Poon, L. C., Bansal, A. S., & Nicolaides, K. H. (2013). Maternal serum cytokines at 30–33 weeks in the prediction of preeclampsia. *Prenatal Diagnosis*, *33*, 823–830. https://doi.org/10.1002/pd.4129

Muntasell, A., Vilches, C., Angulo, A., & López-Botet, M. (2013). Adaptive reconfiguration of the human NK-cell compartment in response to cytomegalovirus: A different perspective of the host-pathogen interaction. *European Journal of Immunology*, *43*, 1133–1141. https://doi.org/10.1002/eji.201243117

Pontillo, A., Reis, E. C., Bricher, P. N., Vianna, P., Diniz, S., Fernandes, K. S., … Sandrim, V. (2015). *NLRP1* L155H polymorphism is a risk factor for preeclampsia development. *American Journal of Reproductive Immunology*, *73*, 577–581. https://doi.org/10.1111/aji.12353

Salazar Garcia, M. D., Mobley, Y., Henson, J., Davies, M., Skariah, A., Dambaeva, S., … Kwak-Kim, J. (2018). Early pregnancy immune biomarkers in peripheral blood may predict preeclampsia. *Journal of Reproductive Immunology*, *125*, 25–31. https://doi.org/10.1016/j.jri.2017.10.048

Samson, M., Soularue, P., Vassart, G., & Parmentier, M. (1996). The genes encoding the human CC-chemokine receptors CC-CKR1 to CC-CKR5 (CMKBR1-CMKBR5) are clustered in the p21.3-p24 region of chromosome 3. *Genomics*, *36*, 522–526. https://doi.org/10.1006/geno.1996.0498

Sargent, I. L., Borzychowski, A. M., & Redman, C. W. G. (2007). NK cells and pre-eclampsia. *Journal of Reproductive Immunology*, *76*, 40–44. https://doi.org/10.1016/j.jri.2007.03.009

Szarka, A., Rigó, J. Jr, Lázár, L., Bekő, G., & Molvarec, A. (2010). Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunology*, *11*, 59. https://doi.org/10.1186/1471-2172-11-59

Telini, B., Veit, T. D., Chies, J. A. B., & Vianna, P. (2014). The CCR39Δ32 polymorphism as a pre-eclampsia susceptibility marker: An evaluation in Brazilian women. *Archives of Gynecology and Obstetrics*, *290*, 1–3. https://doi.org/10.1007/s00404-014-3246-0

Triche, E. W., Uzun, A., DeWan, A. T., Kurihara, I., Liu, J., Occhiogrosso, R., … Padbury, J. F. (2014). Bioinformatic approach to the genetics of preeclampsia. *Obstetrics and Gynecology*, *123*(6), 1155–1161. https://doi.org/10.1097/AOG.0000000000000293

Venkatesan, S., Petrovic, A., Van Ryk, D. I., Locati, M., Weissman, D., & Murphy, P. M. (2002). Reduced cell surface expression of CCR41 in CCR41Δ32 heterozygotes is mediated by gene dosage, rather than by receptor sequestration. *Journal of Biological Chemistry*, *277*, 2287–2301. https://doi.org/10.1074/jbc.M108321200

Williams, P. J., & Pipkin, F. B. (2011). The genetics of pre-eclampsia and other hypertensive disorders of pregnancy. *Best Practice and Research Clinical Obstetrics and Gynaecology*, *25*, 405–417. https://doi.org/10.1016/j.bpobgyn.2011.02.007

Wu, L., Paxton, W. A., Kassam, N., Ruffing, N., Rottman, J. B., Sullivan, N., … Mackay, C. R. (1997). CCR43 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, . *Journal of Experimental Medicine*, *185*, 1681–1691. https://doi.org/10.1084/jem.185.9.1681

How to cite this article: Kaminski VL, Ellwanger JH, Sandrim V, Pontillo A, Chies JAB. Influence of NKG2C gene deletion and CCR5Δ32 in Pre-eclampsia—Approaching the effect of innate immune gene variants in pregnancy. *Int J Immunogenet*. 2019;00:1–6. https://doi.org/10.1111/iji.12416

Parte II

Capítulos VI, VII e VIII

Capítulo VI

Immunogenetic Factors in Autism Spectrum Disorder–Keeping Gene Variants on Stage

Valéria de Lima Kaminski, Guilherme Luís Tyska-Nunes, Andressa Gonçalves Rodrigues, Marina Ziliotto, Jaqueline Bohrer Schuch, Tatiana Roman, Marcelo Alves de Souza Bragatte, José Artur Bogo Chies

Manuscrito em preparação para submissão à revista Immunogenetics.

Capítulo VII

Inflammation and extracellular vesicles in Autism Spectrum Disorder

Valéria de Lima Kaminski, Rafael Tomoya Michita, Joel Henrique Ellwanger, Tiago Degani Veit, José Artur Bogo Chies

Manuscrito em preparação para submissão à revista Brain, Behavior, and Immunity.

Capítulo VIII

Association between *NKG2* gene variants and epilepsy in Autism Spectrum Disorder

Valéria de Lima Kaminski, Guilherme Luís Tyska-Nunes, Brenda Pedron Beltrame, Jaqueline Bohrer Schuch, Rudimar dos Santos Riesgo, Lavinia Schüler-Faccini, Tatiana Roman, José Artur Bogo Chies

Manuscrito em preparação.

Capítulo IX

Discussão geral e Conclusão

DISCUSSÃO GERAL E CONCLUSÃO

Este trabalho apresentou aspectos da imunologia da gestação humana e discutiu sobre a ligação de distúrbios gestacionais com o risco de desenvolvimento de Transtorno do Espectro Autista em crianças nascidas de mães que tiveram gestações com problemas relacionados à inflamação. Diante dos diferentes estudos revisados para comporem os trabalhos aqui apresentados, os dois tópicos a seguir norteiam o fechamento da presente tese.

Sobre os paradigmas imunológicos da gestação

Conforme resumido na Figura 1, a literatura sobre imunotolerância da mãe em relação ao feto é permeada de conceitos que direcionam o pensamento para uma visão simplista que não representa a dinâmica fisiológica de uma gestação. O equilíbrio imune estabelecido na interface materno-fetal vai muito além do "paradigma "Th1/Th2/Th17 e Treg" proposto por Saito et al. (2010). Além disso, nem sempre é possível acessar o *status* imune de uma gestação por medições de fatores presentes no plasma sanguíneo materno.

Porém, é importante ressaltar que, apesar de apresentarem limitações, os estudos que têm avaliado moléculas solúveis presentes na circulação das gestantes são de grande valia. Inclusive, são abordagens fundamentais para que se tenha uma visão mais detalhada da complexidade existente nas relações imunológicas entre a mãe e o feto em desenvolvimento. Como apresentado no Capítulo II, a descoberta do papel desempenhado por vesículas extracelulares na promoção de um ambiente imunossupressor em prol do feto agrega um grau de complexidade sem precedentes ao fenômeno imunológico da gestação. No mesmo capítulo e também na Tabela 1, diferentes moléculas do sistema imune que impactam no desfecho de uma gravidez foram apresentadas, e seus papéis na gestação, brevemente discutidos.

Dentre as novidades evolutivas dos mamíferos, destaca-se a placenta como um órgão transitório com importantes funções imunológicas. Nos humanos, além de constantemente balizar os possíveis ataques por linfócitos maternos ativados e de promover adequadas nutrição e oxigenação para o feto em desenvolvimento, a placenta apresenta características essenciais que evitam infecções no ambiente uterino. A dinâmica das respostas imunes que permeiam a gestação é enriquecida ao passo que analisamos, concomitantemente, as estratégias apresentadas pelos patógenos que, em última instância, podem burlar as defesas presentes na barreira placentária. Ainda, a placenta produz e secreta ativamente diferentes tipos de vesículas extracelulares, a ponto

de já ter sido proposto o estabelecimento de uma “nuvem de exossomos” na interface materno-fetal (Mincheva-Nilson, 2010; Mincheva-Nilsson e Baranov, 2010). No artigo que compõe o Capítulo II, também foram apresentadas as potenciais influências dessas vesículas em ambas infecções transplacentárias e sexualmente transmissíveis (Kaminski et al., 2019a).

Conforme discutido nos Capítulos II e VII, infecções durante a gestação podem apresentar riscos consideráveis para a mãe e para o feto. Além das questões envolvendo infecções, a placenta pode estar relacionada a outras intercorrências gestacionais, que também envolvem fatores imunológicos e oferecem risco para a gestante e para o feto. Nesse contexto, o Capítulo V abordou dois fatores genéticos com potencial de influenciar uma importante doença gestacional, a pré-eclâmpsia. Esse quadro clínico tem caráter multifatorial e acomete de 2 a 8% das mulheres gestantes e tem como principais diagnósticos a presença de hipertensão gestacional *de novo* e proteinúria (Michita et al., 2018). Por ser uma doença complexa, muitas variantes em diferentes genes vêm sendo investigadas no contexto da pré-eclâmpsia e, nesta tese, foram investigadas a deleção completa do gene *NKG2C* e a variante CCR5Δ32, presente no gene *CCR5*. A associação da variante CCR5Δ32 com pré-eclâmpsia foi corroborada, visto que um estudo prévio do Laboratório de Imunobiologia e Imunogenética da UFRGS já havia associado tal variante com menor incidência de pré-eclâmpsia em outro grupo amostral (Telini et al., 2014). Além disso, esse foi o primeiro estudo avaliando a deleção do gene *NKG2C* na população brasileira (Kaminski et al., 2019b).

Diferentes fatores imunológicos são investigados no contexto de problemas relacionados à gestação. Nesta tese, foi avaliado o perfil de citocinas de gestantes e de mulheres que sofreram aborto espontâneo e os níveis dessas moléculas foram comparados nos dois grupos. Conforme os resultados apresentados no Capítulo IV, a única diferença com significância estatística observada foi o aumento de Interleucina 17A (IL-17A) nas gestantes em comparação com os casos de aborto (Kaminski et al., 2018). Os resultados obtidos estão em acordo com dados prévios (Martínez-García et al., 2011) e vão ao encontro do já proposto papel da IL-17 como uma molécula atuante na manutenção de períodos gestacionais prolongados (Chavan et al., 2017). Estudos no contexto de perdas gestacionais são extremamente necessários, visto que cerca de 20% das gestações resultam em aborto espontâneo idiopático (Everett, 1997). Esse estudo foi motivado por evidências que indicam que fatores imunológicos podem influenciar nos casos de aborto espontâneo sem causas definidas (citadas em Kaminski et al., 2019a).

A molécula imunotolerogênica HLA-G possui grande impacto na gestação, e variantes no gene que a codifica têm sido alvo de investigação no contexto de aborto (Michita et al., 2016). Considerando esses aspectos, no Capítulo III é apresentada uma estratégia de contracepção baseada na diminuição da expressão do gene *HLA-G*. A contracepção seria efetivada com a metodologia

apresentada, com base no fato de que HLA-G atua na promoção de tolerância imunológica necessária ao estabelecimento de uma gestação. Além disso, essa molécula é importante tanto para a manutenção da tolerância quanto para a promoção da vascularização que garante a fixação do embrião na decídua.

Sobre sistema imune e Transtorno do Espectro Autista

A segunda parte deste trabalho abordou o papel de fatores imunogenéticos e da inflamação no Transtorno do Espectro Autista (TEA), caracterizado por dificuldades na comunicação e socialização e por comportamento restritivo e repetitivo (O'Hare, 2009). O Capítulo VI é composto por uma revisão de variantes em genes relacionados ao sistema imune que já foram estudados no TEA. Como já discutido, não se sabe a causa exata desse transtorno do desenvolvimento, apesar de já estar estabelecida a contribuição de fatores genéticos e ambientais de forma conjunta. Os genes abordados foram agrupados em três diferentes grupos (relacionados às respostas inflamatórias, relacionados ao MHC e relacionados ao imunometabolismo). Foi observado um maior volume de variantes investigadas e associadas a genes do imunometabolismo, sugerindo que as alterações imunogenéticas no contexto do TEA, embora com pequeno impacto, estão envolvidas em alterações constitutivas na fisiologia dos pacientes.

Nos Capítulos VI, VII e VIII também é extensamente discutido o papel da inflamação no TEA. Respostas inflamatórias alteradas são observadas em indivíduos diagnosticados e são potenciais contribuintes para as diferentes manifestações clínicas da doença (Masi et al., 2017; Bennabi et al., 2019). A ativação imune materna tem sido bastante discutida na literatura como um importante fator de risco para o TEA (Meltzer and Van de Water, 2017). No Capítulo VII, uma abordagem propondo a conexão entre ativação imune materna, TEA e vesículas extracelulares foi apresentada. As bases para tal proposta são a participação de vesículas extracelulares na gestação (tanto com ou sem intercorrências), a observação de perfis alterados dessas vesículas em pacientes com TEA (Tsilioni e Theoharides, 2018) e o potencial imunomodulador dessas vesículas (Kaminski et al., 2019a). É importante destacar que, apesar do provável papel de exposição pré-natal à inflamação como gatilho pro TEA, os fatores inflamatórios podem ser consequência, e não causa, da manifestação do TEA.

Na mesma linha do que foi dito anteriormente, o Capítulo VIII apresenta um estudo inspirado em um trabalho prévio que demonstrou expressão diferenciada do receptor NKG2C em células NKs de indivíduos adultos com TEA (Benabi et al., 2019). Assim, analisamos a deleção completa do gene *NKG2C* e SNPs nos genes *NKG2D* e *NKG2A* em indivíduos com TEA e seus respectivos pais biológicos. Os resultados preliminares desse estudo indicam associação da presença

da deleção do gene *NKG2C* e dois SNPs em *NKG2D* com um mesmo sintoma: epilepsia. Corroborando este estudo, atividade reduzida de células NKs em pacientes epiléticos já foi reportada (Wang et al., 1989). Ainda, aumento no número de células NKs foi reportado em medições realizadas até 24h após episódios de epilepsia (Bauer et al., 2008).

No contexto do TEA, estudos já demonstraram alterações relacionadas a células NKs em crianças diagnosticadas, onde a contagem dessas células na circulação de crianças com autismo de alto e baixo desempenho pode alcançar níveis até 40% maiores em comparação com crianças de desenvolvimento típico (Warren et al., 1987; Ashwood et al., 2011; Vojdani et al., 2008; Enstrom et al., 2009). Apesar do número elevado de células, sob estimulação *in vitro*, não observou-se a ativação esperada das NKs derivadas das crianças com TEA (López-Cacho et al., 2016). Diante de todos esses dados, pode-se sugerir que o número elevado de células NKs pode refletir sua baixa atividade, de forma que se estabelece um mecanismo compensatório. Por fim, destaca-se que o último capítulo desta tese agrega a esta discussão um dado importante: a presença da deleção de um gene que codifica um receptor ativatório de células NK está associada com a presença de epilepsia no quadro clínico do TEA.

REFERÊNCIAS BIBLIOGRÁFICAS

Abrahams BS e Geschwind DH (2008) Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 9(5):341-355. doi: 10.1038/nrg2346

APA - American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders, 5th edn. American Psychiatric Association: Washington, DC.

Ashwood P, Corbett BA, Kantor A, Schulman H, Van de Water J, Amaral DG (2011) In search of cellular immunophenotypes in the blood of children with autism. PLoS One. 6(5):e19299. doi: 10.1371/journal.pone.0019299

Bai D, Yip BHK, Windham GC, Sourander A, Francis R, Yoffe R, Glasson E, Mahjani B, Suominen A, Leonard H, Gissler M, Buxbaum JD, Wong K, Schendel D, Kodesh A, Breshnahan M, Levine SZ, Parner ET, Hansen SN, Hultman C, Reichenberg A e Sandin S (2019) Association of Genetic and Environmental Factors With Autism in a 5-Country Cohort. JAMA Psychiatry. doi: 10.1001/jamapsychiatry.2019.1411

Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, Kurzius-Spencer M, Zahorodny W., Rosenberg CR, White T, Durkin MS, Imm P, Nikolaou, L, Yeargin-Allsopp M, Lee LC, Harrington R, Lopez M, Fitzgerald RT, Hewitt A, Pettygrove S, Constantino JN, Vehorn A, Shenouda J, Hall-Lande J, Van Naarden Braun K, Dowling NF (2018). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. MMWR Surveill. Summ. 67, 1-23. doi: 10.15585/mmwr.ss6706a1

Bauer S, Köller M, Cepok S, Todorova-Rudolph A, Nowak M, Nockher WA, Lorenz R, Tackenberg B, Oertel WH, Rosenow F, Hemmer B e Hamer HM (2008) NK and CD4+ T cell changes in blood after seizures in temporal lobe epilepsy. Exp Neurol. 211(2):370-7. doi: 10.1016/j.expneurol.2008.01.017

Bauman MD, Iosif AM, Ashwood P, Braunschweig D, Lee A, Schumann CM, Van de Water J e Amaral DG (2013) Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey. Transl. Psychiatry. 3, e278. doi:10.1038/tp.2013.47

Bennabi M, Tarantino N, Gaman A, Scheid I, Krishnamoorthy R, Debré P, Bouleau A, Caralp M, Gueguen S, Le-Moal ML, Bouvard M, Amestoy A, Delorme R, Leboyer M, Tamouza R, Vieillard V (2019) Persistence of dysfunctional natural killer cells in adults with high-functioning autism spectrum disorders: stigma/consequence of unresolved early infectious events? Mol Autism. 10:22. doi: 10.1186/s13229-019-0269-1

Billingham RE, Brent L e Medawar PB (1953) 'Actively acquired tolerance' of foreign cells. Nature. 172 (4379), 603-6. doi: 10.1038/172603a0

Braude P, Bolton V e Moore S (1988) Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 332 (6163), 459-61. doi: 10.1038/332459a0

Braunschweig D, Golub MS, Koenig CM, Qi L, Pessah IN, Van de Water J e Berman RF (2012) Maternal autism-associated IgG antibodies delay development and produce anxiety in a mouse gestational transfer model. J. Neuroimmunol. 252, 56-65. doi: 10.1016/j.jneuroim.2012.08.002

Capmany G, Taylor A, Braude PR e Bolton VN (1996) The timing of pronuclear formation, DNA synthesis and cleavage in the human 1-cell embryo. Mol. Hum. Reprod. 2 (5), 299-306. doi: 10.1093/molehr/2.5.299

Chaouat G, Zourbas S, Ostojic S, Lappree-Delage G, Dubanchet S, Ledee N e Martal J (2002) A brief review of recent data on some cytokine expressions at the materno-foetal interface which might challenge the classical Th1/Th2 dichotomy. J. Reprod. Immunol. 53 (1-2), 241-56. doi: 10.1016/S0165-0378(01)00119-X

Chavan AR, Griffith OW e Wagner GP (2017) The inflammation paradox in the evolution of mammalian pregnancy: turning a foe into a friend. Curr. Opin. Genet. Dev. 47, 24-32. doi: 10.1016/j.gde.2017.08.004

Croen LA, Grether JK, Yoshida CK, Odouli R e Van de Water J (2005) Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Arch Pediatr Adolesc Med. 159(2):151-7. doi: 10.1001/archpedi.159.2.151

Dalton P, Deacon R, Blamire A, Pike M, McKinlay I, Stein J, Styles P e Vincent A (2003) Maternal neuronal antibodies associated with autism and a language disorder. Ann. Neurol. 53, 533-7. doi: 10.1002/ana.10557

Duffney LJ, Valdez P, Tremblay MW, Cao X, Montgomery S, McConkie-Rosell A e Jiang YH (2018) Epigenetics and autism spectrum disorder: A report of an autism case with mutation in H1 linker histone HIST1H1E and literature review. Am J Med Genet B Neuropsychiatr Genet. 177(4):426-433. doi: 10.1002/ajmg.b.32631

Ellwanger JH, Kaminski VL, Chies JA, (2019) What we say and what we mean when we say redundancy and robustness of the chemokine system - how CCR5 challenges these concepts. Immunol Cell Biol. doi: 10.1111/imcb.12291

Enstrom, A.M., Lit, L., Onore, C.E., Gregg, J.P., Hansen, R.L., Pessah, I.N., Hertz-Picciotto, I., Van de Water, J.A., Sharp, F.R., Ashwood, P., 2009. Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain Behav Immun. 23(1):124-33. doi: 10.1016/j.bbi.2008.08.001

Goines PE, Croen LA, Braunschweig D, Yoshida CK, Grether J, Hansen R, Kharrazi M, Ashwood P e Van de Water J (2011) Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Mol. Autism. 2, 13. doi: 10.1186/2040-2392-2-13

Gude NM, Roberts CT, Kalionis B e King RG (2004) Growth and function of the normal human placenta. Thromb. Res. 114 (5-6), 397-407. doi: 10.1016/j.thromres.2004.06.038

Hackmon R, Pinnaduwage L, Zhang J, Lye SJ, Geraghty DE, Dunk CE (2017). Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. Am. J. Reprod. Immunol. 77 (6). doi: 10.1111/aji.12643

Hedlund M, Stenqvist AC, Nagaeva O, Kjellberg L, Wulff M, Baranov V e Mincheva-Nilsson L (2009) Human placenta expresses and secretes NKG2D ligands via exosomes that down-modulate the cognate receptor expression: evidence for immunosuppressive function. J. Immunol. 183 (1), 340-51. doi: 10.4049/jimmunol.0803477

Hill JA, Polgar K e Anderson DJ (1995) T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. JAMA. 273 (24), 1933-6. doi: 10.1001/jama.1995.03520480053039

Ivanov HY, Stoyanova VK, Popov NT e Vachev TI (2015) Autism spectrum disorder – a complex gene disorder. Folia Medica 2015; 57(1): 19-28. doi: 10.1515/folmed-2015-0015

Jiang YH, Yuen RK, Jin X, Wang M, Chen N, Wu X, Ju J, Mei J, Shi Y, He M, Wang G, Liang J, Wang Z, Cao D, Carter MT, Chrysler C, Drmic IE, Howe JL, Lau L, Marshall CR, Merico D, Nalpathamkalam T, Thiruvahindrapuram B, Thompson A, Uddin M, Walker S, Luo J, Anagnostou E, Zwaigenbaum L, Ring RH, Wang J, Lajonchere C, Wang J, Shih A, Szatmari P, Yang H, Dawson G, Li Y e Scherer SW (2013) Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. Am J Hum Genet 93(2):249-263. doi: 10.1016/j.ajhg.2013.06.012

Kaminski VL, Ellwanger JH e Chies JAB (2019a) Extracellular vesicles in host-pathogen interactions and immune regulation — exosomes as emerging actors in the immunological theater of pregnancy. Heliyon 5, e02355. doi: 10.1016/j.heliyon.2019.e02355

Kaminski VL, Ellwanger JH, Matte MCC, Savaris RF, Vianna P e Chies JAB (2018) IL-17 blood levels increase in healthy pregnancy but not in spontaneous abortion. Mol. Biol. Rep. 45 (5), 1565-1568. doi: 10.1007/s11033-018-4268-7

Kaminski VL, Ellwanger JH, Sandrim V, Pontillo A e Chies JAB (2019b) Influence of NKG2C gene deletion and CCR5Δ32 in Pre-eclampsia-Approaching the effect of innate immune gene variants in pregnancy. Int J Immunogenet. 46(2):82-87. doi: 10.1111/iji.12416

Kanner L e Eisenberg L (1957) Early infantile autism, 1943-1955. Psychiatr Res Rep Am Psychiatr Assoc. (7):55-65. doi: 10.4159/harvard.9780674367012.c2

King A, Allan DS, Bowen M, Powis SJ, Joseph S, Verma S, Hiby SE, McMichael AJ, Loke YW, Braud VM (2000) HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. Eur. J. Immunol. 30(6):1623-31. doi: 10.1002/1521-4141(200006)30:6<1623::AID-IMMU1623>3.0.CO;2-M

Kraneveld AD, Szklany K, de Theije CG e Garssen J (2016) Gut-to-Brain Axis in Autism Spectrum Disorders: Central Role for the Microbiome. Int Rev Neurobiol. 131:263-287. doi: 10.1016/bs.irn.2016.09.001

Kshirsagar SK, Alam SM, Jasti S, Hodes H, Nauser T, Gilliam M, Billstrand C, Hunt JS e Petroff MG (2012) Immunomodulatory molecules are released from the first trimester and term placenta via exosomes. Placenta. 33 (12), 982-90. doi: 10.1016/j.placenta.2012.10.005

Lash GE, Robson SC e Bulmer JN (2010) Review: Functional role of uterine natural killer (uNK) cells in human early pregnancy decidua. Placenta. 31 Suppl, S87-92. doi: 10.1016/j.placenta.2009.12.022

Loke YJ, Hannan AJ e Craig JM (2015) The Role of Epigenetic Change in Autism Spectrum Disorders. Front Neurol. 6:107. doi: 10.3389/fneur.2015.00107

López-Cacho JM, Gallardo S, Posada M, Aguerri M, Calzada D, Mayayo T, Lahoz C, Cárdaba B, (2016) Characterization of immune cell phenotypes in adults with autism spectrum disorders. J Investig Med. 64(7):1179-85. doi: 10.1136/jim-2016-000070

Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, Park BY, Snyder NW, Schendel D, Volk H, Windham GC, Newschaffer C (2017) The Changing Epidemiology of Autism Spectrum Disorders. Annu. Rev. Public Health. 38:81–102. doi: 10.1146/annurev-publhealth-031816-044318

Maher GM, McCarthy FP, McCarthy CM, Kenny LC, Kearney PM, Khashan AS e O'Keeffe GW (2018) A perspective on pre-eclampsia and neurodevelopmental outcomes in the offspring: Does maternal inflammation play a role? Int J Dev Neurosci. S0736-5748(18)30269-7. doi: 10.1016/j.ijdevneu.2018.10.004

Makrigiannakis A, Vrekoussis T, Zoumakis E, Kalantaridou SN, Jeschke U (2017) The Role of HCG in Implantation: A Mini-Review of Molecular and Clinical Evidence. Int. J. Mol. Sci. 19;18(6). pii: E1305. doi: 10.3390/ijms18061305.

Martínez-García EA, Chávez-Robles B, Sánchez-Hernández PE, Núñez-Atahualpa L, Martín-Máquez BT, Muñoz-Gómez A, González-López L, Gámez-Nava JI, Salazar-Páramo M, Dávalos-Rodríguez I, Petri MH, Zúñiga-Tamayo D, Vargas-Ramírez R e Vázquez-Del Mercado M (2011) IL-17 increased in the third trimester in healthy women with term labor. Am. J. Reprod. Immunol. 65 (2), 99-103. doi: 10.1111/j.1600-0897.2010.00893.x

Masi A, Glozier N, Dale R e Guastella AJ (2017) The immune system, cytokines, and biomarkers in autism spectrum disorder. Neurosci. Bull. 33 (2).

Meltzer A e Van de Water J (2017) The Role of the Immune System in Autism Spectrum Disorder. Neuropsychopharmacology 42(1):284-298. https://doi.org/10.1038/npp.2016.158

Michaelson JJ, Shi Y, Gujral M, Zheng H, Malhotra D, Jin X, Jian M, Liu G, Greer D, Bhandari A, Wu W, Corominas R, Peoples A, Koren A, Gore A, Kang S, Lin GN, Estabillo J, Gadomski T, Singh B, Zhang K, Akshoomoff N, Corsello C, McCarroll S, Iakoucheva LM, Li Y, Wang J e Sebat J (2012) Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. Cell 151:1431-1442. https://doi.org/10.1016/j.cell.2012.11.019

Michita RT, Kaminski VL e Chies JAB (2018) Genetic Variants in Preeclampsia: Lessons From Studies in Latin-American Populations. Front. Physiol. 9:1771. doi: 10.3389/fphys.2018.01771

Michita RT, Zambra FMB, Fraga LR, Sanseverino MTV, Callegari-Jacques SM, Vianna P e Chies JAB (2016) A tug-of-war between tolerance and rejection - New evidence for 3'UTR HLA-G haplotypes influence in recurrent pregnancy loss. Hum. Immunol. 77 (10), 892-897. doi: 10.1016/j.humimm.2016.07.004

Mincheva-Nilsson L (2010) Placental exosome-mediated immune protection of the fetus: feeling groovy in a cloud of exosomes. Expert Rev. Obstet. Gynecol. 5(5):619–634.

Mincheva-Nilsson L e Baranov V (2010) The role of placental exosomes in reproduction. Am. J. Reprod. Immunol. 63 (6), 520-33. doi: 10.1111/j.1600-0897.2010.00822.x

Mincheva-Nilsson L, Baranov V, Yeung MM, Hammarstrom S e Hammarstrom ML (1994) Immunomorphologic studies of human decidua-associated lymphoid cells in normal early pregnancy. J. Immunol. 152, 2020-32

Moffett-King A (2002) Natural killer cells and pregnancy. Nat. Rev. Immunol. 2 (9), 656-63. doi: 10.1038/nri886

Mor G, Cardenas I, Abrahams V e Guller S (2011) Inflammation and pregnancy: the role of the immune system at the implantation site. Ann. N. Y. Acad. Sci. 1221, 80-7. doi: 10.1111/j.1749-6632.2010.05938.x

Muhle R, Trentacoste SV e Rapin I (2004) The genetics of autism. Pediatrics 113(5):e472-486. https://doi.org/10.1542/peds.113.5.e472

Nature. Immunogenetics. 2019. Disponível em: https://www.nature.com/subjects/immunogenetics. Acesso em: 31 de outubro de 2019.

Nicolini C e Fahnestock M (2018) The valproic acid-induced rodent model of autism. Exp Neurol. 299:217-227. doi: 10.1016/j.expneurol.2017.04.017

O'Hare A (2009) Autism spectrum disorder: diagnosis and management. Arch Dis Child Educ Pract Ed. 94(6):161-8. doi: 10.1136/adc.2008.150490

Piccinni MP (2002) T-cell cytokines in pregnancy. Am. J. Reprod. Immunol. 47 (5), 289-94. doi: 10.1034/j.1600-0897.2002.01104.x

Pongcharoen S, Searle RF e Bulmer JN (2004) Placental Fas and Fas ligand expression in normal early, term and molar pregnancy. Placenta. 25 (4), 321-30. doi: 10.1016/j.placenta.2003.08.020

Pongcharoen S, Somran J, Sritippayawan S, Niumsup P, Chanchan P, Butkhamchot P, Tatiwat P, Kunngurn S, Searle RF, (2007) Interleukin-17 expression in the human placenta. Placenta. 28(1):59-63. doi: 10.1016/j.placenta.2006.01.016

Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M e Farhat R (2000) Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum. Reprod. 15 (3), 713-8. doi: 10.1093/humrep/15.3.713

Saeedi S, Israel S, Nagy C, Turecki G, (2019) The emerging role of exosomes in mental disorders. Transl. Psychiatry. 9, 122. doi: 10.1038/s41398-019-0459-9

Saito S, Nakashima A, Shima T e Ito M (2010) Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am. J. Reprod. Immunol. 63 (6), 601-10. doi: 10.1111/j.1600-0897.2010.00852.x

Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A, Illanes SE, Mitchell MD e Rice GE (2014) A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. PLoS One. 9 (6), e98667. doi: 10.1371/journal.pone.0098667

Singer AB, Windham GC, Croen LA, Daniels JL, Lee BK, Qian Y, Schendel DE, Fallin MD, Burstyn I (2016) Maternal Exposure to Occupational Asthmagens During Pregnancy and Autism Spectrum Disorder in the Study to Explore Early Development. J. Autism Dev. Disord. 46(11):3458-3468.doi: 10.1007/s10803-016-2882-6

Singer HS, Morris C, Gause C, Pollard M, Zimmerman AW e Pletnikov M (2009) Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: A pregnant dam mouse model. J Neuroimmunol. 211(1-2):39-48

Stenqvist AC, Nagaeva O, Baranov V e Mincheva-Nilsson L (2013) Exosomes secreted by human placenta carry functional Fas ligand and TRAIL molecules and convey apoptosis in activated immune cells, suggesting exosome-mediated immune privilege of the fetus. J. Immunol. 191 (11), 5515-23. doi: 10.4049/jimmunol.1301885

Svensson-Arvelund J, Mehta RB, Lindau R, Mirrasekhian E, Rodriguez-Martinez H, Berg G, Lash GE, Jenmalm MC e Ernerudh J (2015) The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. J. Immunol. 194 (4), 1534-44. doi: 10.4049/jimmunol.1401536

Tannetta D, Collett G, Vatish M, Redman C e Sargent IL (2017a) Syncytiotrophoblast extracellular vesicles - Circulating biopsies reflecting placental health. Placenta. 52, 134-8. doi: 10.1016/j.placenta.2016.11.008

Tannetta D, Masliukaite I, Vatish M, Redman C e Sargent I (2017b) Update of syncytiotrophoblast derived extracellular vesicles in normal pregnancy and preeclampsia. J. Reprod. Immunol. 119, 98-106. doi: 10.1016/j.jri.2016.08.008

Théry C, Zitvogel L e Amigorena S (2002) Exosomes: composition, biogenesis and function. Nat. Rev. Immunol. 2 (8), 569-79. doi: 10.1038/nri855

Trowsdale J e Betz AG (2006) Mother's little helpers: mechanisms of maternal-fetal tolerance. Nat. Rev. Immunol. 7 (3), 241-6. doi: 10.1038/ni1317

Tsilioni, I., Theoharides, T.C., 2018. Extracellular vesicles are increased in the serum of children with autism spectrum disorder, contain mitochondrial DNA, and stimulate human microglia to secrete IL-1β. J. Neuroinflammation. 15, 239. doi: 10.1186/s12974-018-1275-5

van der Meer A, Lukassen HG, van Lierop MJ, Wijnands F, Mosselman S, Braat DD, Joosten I (2004) Membrane-bound HLA-G activates proliferation and
interferon-gamma production by uterine natural killer cells. Mol. Hum. Reprod. 10 (3), 189–195. doi: 10.1093/molehr/gah032

Vianna P, Bauer ME, Dornfeld D e Chies JAB (2011) Distress conditions during pregnancy may lead to pre-eclampsia by increasing cortisol levels and altering lymphocyte sensitivity to glucocorticoids. Med. Hypotheses. 77 (2), 188-91. doi: 10.1016/j.mehy.2011.04.007

Vojdani, A., Mumper, E., Granpeesheh, D., Mielke, L., Traver, D., Bock, K., Hirani, K., Neubrander, J., Woeller, K.N., O'Hara, N., Usman, A., Schneider, C., Hebroni, F., Berookhim, J., McCandless, J., 2008. Low natural killer cell cytotoxic activity in autism: the role of glutathione, IL-2 and IL-15. J Neuroimmunol. 205(1-2):148-54. doi: 10.1016/j.jneuroim.2008.09.005

Vorstman JAS, Parr JR, Moreno-De-Luca D, Anney RJL, Nurnberger JI Jr e Hallmayer JF (2017) Autism genetics: opportunities and challenges for clinical translation. Nat Rev Genet 18:362-376. https://doi.org/10.1038/nrg.2017.4

Wang XL, Shen GX, Sun B e Su N (1989) Studies on lymphocyte subpopulations and NK cell activities in epileptic patients. J Tongji Med Univ. 1989;9(1):25-8. doi: 10.1007/bf02933740

Warren, R.P., Foster, A., Margaretten, N.C., 1987. Reduced natural killer cell activity in autism. J Am Acad Child Adolesc Psychiatry. 26(3):333-5. doi: 10.1097/00004583-198705000-00008

Wegmann TG, Lin H, Guilbert L e Mosmann TR (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol. Today. 14 (7), 353-6. doi: 10.1016/0167-5699(93)90235-D

Wu HX, Jin LP, Xu B, Liang SS e Li DJ (2014) Decidual stromal cells recruit Th17 cells into decidua to promote proliferation and invasion of human trophoblast cells by secreting IL-17. Cell. Mol. Immunol. 11 (3), 253-62. doi: 10.1038/cmi.2013.67

Zenclussen AC (2013) Adaptive immune responses during pregnancy. Am. J. Reprod. Immunol. 69 (4), 291-303. doi: 10.1111/aji.12097

Zerbo O, Qian Y, Yoshida C, Fireman BH, Klein NP e Croen LA (2017) Association Between Influenza Infection and Vaccination During Pregnancy and Risk of Autism Spectrum Disorder. JAMA Pediatr. 171(1):e163609. doi: 10.1001/jamapediatrics.2016.3609

Zhang JM e An J (2007) Cytokines, inflammation, and pain. Int Anesthesiol Clin. 45(2):27-37. doi: 10.1097/AIA.0b013e318034194e