UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Instituto de Biociências

Programa de Pós-Graduação em Genética e Biologia Molecular

Quimiocinas e receptores de quimiocinas na infecção pelo HIV: genética e imunologia na modulação da resposta imune em pacientes HIV+ com diferentes perfis de progressão da doença e antes e após início dos antirretrovirais

Jacqueline María Valverde Villegas

Tese apresentada ao Curso de Pós-Graduação em Genética e Biologia Molecular, Instituto de Ciências Biológicas da UFRGS, como requisito parcial à obtenção do grau de Doutor em Ciências (Genética e Biologia Molecular).

Orientador: Prof. Dr. José Artur Bogo Chies.

Co-orientadora: Prof.ª Dr.ª Sabrina E. M. Almeida.

Porto Alegre, Fevereiro de 2017.

Este trabalho foi realizado no Laboratório de Imunogenética da Universidade Federal do Rio Grande do Sul e nas instalações do Centro de Desenvolvimento Científico e Tecnológico (CDCT) da Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS) com financiamento do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), da Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) e do Programa de Estudantes-Convênio de Pós-Graduação (PEC-PG).

A William Mario Valverde Villegas

Por inspirarme cada vez que espiaba tu cuarto y éste estaba lleno de libros y cuadernos con muchas anotaciones las cuales yo usé después cuando entré a la academia. Por inspirarme cada vez que te ponías tu mandil blanco, a veces solo por vanidad, porque tú eras así, vanidoso, y eso muchas veces resultaba gracioso y todos terminábamos riéndonos. Por inspirarme a ir atrás de lo que realmente a uno le gusta y disfruta hacer, tú dejaste los dientes y te fuiste atrás de las computadoras. Porque recuerdo perfectamente que cuando niña me llevabas de la mano al inicial y muchas veces, por ser el hermano mayor, hiciste cosas por tus hermanos menores. Por haber sido una persona sencilla, noble, generosa y muy alegre, aunque renegón, siempre terminabas cediendo solo para engreír a los demás. Por haber sido un padre amoroso con Luciano, siempre queriendo lo mejor para él y para tu familia. Por ser mi ángel de la guarda, con quién he conversado bastante en los últimos meses y siento que me escucha y me tranquiliza.

Amado hermano, te dedico ésta tesis, mi hija que acaba de nacer, porque estoy segura de lo orgulloso que hubieras estado de ella y además porque la hubieras disfrutado (aún sin entenderla completamente) tanto como yo. A propósito, nunca te dije que me encantaba tu letra y tu sencillez. Te amaré siempre!

"Feliz aquel que lleva consigo un ideal, un Dios interno, sea el ideal de la patria, el ideal de la ciencia o simplemente las virtudes del Evangelio".

Louis Pasteur

AGRADECIMENTOS

A Dios, porque muchas veces conversando con él, me he sentido liberada y en paz, de alguna forma creo que he sabido interpretar sus respuestas.

À população brasileira, porque através dos seus impostos consegui fazer este doutorado.

A todos os pacientes que contribuíram neste estudo, muitos deles aceitaram bem felizes de participar em nossos projetos.

Ao programa PEC-PG por ter me selecionado para receber a bolsa de doutorado, à CAPES por financiar minha bolsa e ao PPGBM da UFRGS por ser um dos melhores do Brasil. Muito obrigada por terem me dado essa oportunidade de contribuir mais um pouquinho no conhecimento sobre o HIV/Aids.

Ao meu orientador, Prof. José Artur Bogo Chies, "Zéca", por acreditar em mim desde o primeiro dia da nossa conversa. Obrigada chefe por me mostrar esse fantástico mundo da inmunogenética. É inevitável sair inspirado após cada aula ou palestra sua.

A minha co-orientadora, Prof[a]. Sabrina Esteves de Matos Almeida, por acreditar em mim e sempre apoiar em tudo aquilo que precisei para realizar este e outros trabalhos.

A Rúbia, porque sem você essa caminhada tivesse sido tudo muito, muito mais difícil. Pelo intensa que tu és em cada coisa que faz e esse completo comprometimento com teu trabalho, é um exemplo para mim.

Ao time da inmunogenética: Francis Maria, Joel, Valéria, Giovana, Maria Cristina, Rafael, Tiago, Lian, Bruno e Bruna. Pelos momentos dentro e fora do lab e por terem contribuído de alguma forma neste trabalho. Em especial ao Joelito e a Val por caminharem comigo nos últimos meses deste doutorado. E, a Fra, por ser essa pessoa amiga, sensível, alegre, brava, caprichosa e carinhosa. Obrigada por conquistar o coração do meu chiruço, teu eterno fã! E Vamos por mais aventuras nessa vida ne?!.

Ao time da Bioinformática: Gustavo e seus filhos: Martiela, Bragatte e Marcus, os irmãozinhos (em ordem de respeito, não de idade). Me encantou ter compartilhado com vocês esse último ano do doutorado nem que seja para rir da vida e de bobagens. Ao Dinler, antigo membro do lab, quem sempre torceu pelo sucesso desta minha louca aventura, ele é agora todo um sucesso nos EUA.

Ao grupo de HIV da FEPPS/CDCT: Sabrina, Rúbia, Karine, Dennis, Tiago e Hegger, pelos momentos muito produtivos de discussão e por ter sido um grupo que me inspirou a me encantar cada vez mais das nossas pesquisas no campo do HIV/AIDS.

Ao pessoal do serviço de infectologia do hospital Nossa Senhora da Conceição. Ao Dr. Breno e a Dra. Marineide pela parceria e constante apoio em nossos projetos. Kellin, Roberta e Vera, por nos providenciar acesso a todos os dados dos pacientes e pela ajuda constante na identificação deles durante as coletas. Às coletadoras, Salleti e Vera, pela amabilidade e sorriso que eu sempre encontrava nelas quando estava correndo nas entrevistas dos pacientes.

As pessoas que foram parte desta caminhada e contribuíram aos meus conhecimentos de citometria. A Priscila e Nadine, pessoas queridas e amigas que vou levar para sempre no meu coração, sempre dispostas em me ajudar com muito carinho. A Priscilla Costa da USP, quem na distancia me socorreu com as últimas análises.

As meninas da FEPPS/CDCT: Thaiane, Karine, Mayara. Pelos momentos na bancada e as discussões sobre o SNAPshot, sequenciamento, multiplex e a vida. Muito obrigada pelo carinho e a amizade!

A minhas "ICs", Karine e Glaucia, pela dedicação e comprometimento com seus trabalhos, e por terem me dado a oportunidade de repassar os conhecimentos teóricos e práticos. Tenho certeza que, independente das escolhas, vocês vão chegar longe.

A mi amada familia: mis padres, William y Maria Elena, por el amor infinito que me levanta cada mañana, por aguantar mi ausencia todos estos años y engreírme tanto, tanto, tanto cada vez que iba a visitarlos. A mis hermanos: William (Willy), Janet (Mayita) y Alex (Alechu), porque sé que ustedes estarán allí para mi bajo cualquier circunstancia (física y espiritualmente); y porque a pesar de la distancia estamos siempre unidos para superar juntos los malos y los mejores momentos de nuestras vidas. A mi bello ahijado Luciano y mi bella sobrina Ariana, personitas que alegran nuestros días sobre todo el corazón de mis papas. Los amo familia!!!

A Jorge Lucio, por el amor y apoyo incondicional durante todo este tiempo, siempre socorriéndome con los bancos de datos, figuras de los papers y la tesis. Muchas gracias amor, eres lo máximo!

A Omaira, mi amiga colombiana, por los momentos (y comidas) compartidos desde que nos conocemos en 2011 y por haber cuidado tan bien de mi Gringa. Gracias!!!

A Tássia, Diny, Lia, e Ana Karine, pelas gordices a cada encontro, pelas longas conversas, pelos desabafos, pelas aventuras e pela amizade, adoro vocês suas lindas!

A mis mejores amigas de la vida: Mariela, Claudia y Lizeth. Porque a pesar de la distancia nuestra amistad se solidifica cada vez más y porque cada una continua con la misma esencia. Gracias amigas por estar siempre pendiente de mí y hacerme barritas dándome ánimos desde la tribuna mientras yo estaba corriendo.

ABREVIATURAS, SIGLAS E SÍMBOLOS

AIDS	Acquired Immunodeficiency Syndrome (Síndrome da Imunodeficiência Adquirida)
APC	Antigen Presenting Cell (Célula apresentadora de antígeno)
ARV	Antiretroviral (Antirretroviral)
AZT	Azidothymidine (Azidotimidina)
CCL2	Chemokine (C-C motif) ligand 2 (Quimiocina CC 2)
CCL2	Chemokine (C-C motif) ligand 3 (Quimiocina CC 3)
CCL5	Chemokine (C-C motif) ligand 5 (Quimiocina CC 5)
CCL17	Chemokine (C-C motif) ligand 17 (Quimiocina CC 17)
CCL20	Chemokine (C-C motif) ligand 20 (Quimiocina CC 20)
CCL22	Chemokine (C-C motif) ligand 22 (Quimiocina CC 22)
CCR4	C-C chemokine receptor type 4 (Receptor de quimiocinas tipo CC 4)
CCR5	C-C chemokine receptor type 5 (Receptor de quimiocinas tipo CC 5)
CCR6	C-C chemokine receptor type 6 (Receptor de quimiocinas tipo CC 6)
CD4	Cluster of Differentiation 4 (Grupo de diferenciação 4)
CD8	Cluster of Differentiation 8 (Grupo de diferenciação 8)
sCD14	soluble Cluster of Differentiation 14 (Grupo de diferenciação solúvel 14)
CD25	Cluster of Differentiation 25 (Grupo de diferenciação 25)
CD38	Cluster of Differentiation 38 (Grupo de diferenciação 38)
CD69	Cluster of Differentiation 69 (Grupo de diferenciação 69)
cDNA	complementary Desoxirybonucleic Acid (Ácido desoxirribonucléico complementar)

CXCL10	C-X-C motif chemokine 10 Quimiocina CXC 10
CXCL9	C-X-C motif chemokine 9 Quimiocina CXC 9
CXCR3	C-X-C chemokine receptor type 3 (Receptor de quimiocinas tipo CXC 3)
CXCR4	C-X-C chemokine receptor type 4 (Receptor de quimiocinas tipo CXC 4)
DC	Dendrític Cell (Célula dentrítica)
DC-SING	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (Molécula de adesão intracelular 3 não-integrina especifica de Célula dendrítica)
DNA	Desoxirybonucleic Acid (Ácido desoxirribonucléico)
DST	Doença sexualmente transmissível
EC	Elite controller (Controlador de elite)
GALT	Gut-associated lymphoid tissue (Tecido linfóide associado ao intestino)
GWAS	Genome-wide association study Estudo de Associação Genômica
HAART	Highly Active Anti-Retroviral Therapy (Tearapia Antrirretroviral de Alta Potência)
HBV	Hepatitis B Virus (Vírus da hepatite B)
HCV	Hepatitis C Virus (Vírus da hepatite C)
HIV	Human Immunodeficiency Vírus (Vírus da Imunodeficiência Humana)
HLA	Human Leukocyte Antigen (Antígeno Leucocitário Humano)
HESN	HIV exposed seronegative (Exposto não infectado ao HIV)
IFN-γ	Interferon-γ (Interferon-γ)
IL-1	Interleukin-1 (Interleucina-1) (Interleucina-1 Beta)
IL-2	Interleukin-2 (Interleucina-2)
IL-4	Interleukin-4

	(Interleucina-4)
IL-5	Interleukin-5
	(Interleucina-5)
IL-6	Interleukin-6
	(Interleucina-6)
IL-7	Interleukin-7
	(Interleucina-7)
IL-10	Interleukin-10
	(Interleucina-10)
IL-12	Interleukin-12
	(Interleucina-12)
IL-13	Interleukin-13
	(Interleucina-13)
IL-15	Interleukin-15
	(Interleucina-15)
IL-18	Interleukin-18
	(Interleucina-18)
IL-21	Interleukin-21
	(Interleucina-21)
IP-10	Interferon gamma-induced protein 10
	Proteína induzida por IFN - 10
LPS	Lipopolysaccharides
	(Lipopolissacarídeo)
LTNPs	Long-Term NonProgressors
	(Não progressores de longo prazo)
NF-κβ	Nuclear Factor kappa βeta
	(Fator Nuclear kappa beta)
NK	Natural killer
	(Exterminadora natural)
NKT	Natural killer T
	(Exterminadora natural T)
NRTI	Nucleoside Reverse Transcriptase Inhibitor
	(Inibidor Não Análogo de Nucleotídeo da Transcriptase Reversa)
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
	(Inibidor Análogo de Nucleotídeo da Transcriptase Reversa)
MDR	Multifactor dimensionality reduction
	(Redução Multifatorial de Dimensionalidade)
MHC I	Major Histocompatibility Complex class I
	(Complexo principal de histocompatibilidade classe I)
MHC II	Major Histocompatibility Complex class II
	(Complexo principal de histocompatibilidade classe II)
PAMPs	Pathogen-Associated Molecular Patterns

	(Padrões moleculares associados a patógenos)
PD-1	Programmed cell death protein 1
	(Proteína de morte celular programada 1)
PI	Protease Inhibitor
	Inibidor de Protease
PrEP	Pre-exposure prophylaxis
	(Profilaxia Pré-Exposição)
PRRs	Pattern recognition receptors
	(Receptores de reconhecimento de padrões)
RNA	Rybonucleic Acid
	(Ácido ribonucléico)
RP	Rapid progressor
	(progressor rápido)
SIV	Simian Immunodeficiency Virus
	Vírus da Imunodeficiência Simia
SNP	Single Nucleotide Polymorphism
	(Polimorfismo de base única)
SP	Slow progressor
	(progressor lento)
SUS	Sistema Único de Saúde
TCR	T cell receptor
	(Receptor de Células T)
Tfh	T follicular helper
	(T auxiliar folicular)
TGF-β	Transforming growth factor beta
	(Fator de Transformação do Crescimento beta)
Th	T helper
	(T auxiliar)
Tγδ	T gamma delta
	(T gamma delta)
TLR	Toll-Like Receptor
	(Receptores do tipo Toll)
TNF-α	Tumor Necrosis Factor-α
	(Fator de Necrose Tumoral)
Treg	T regulatory
	(T regulatória)
UNAIDS	United Nations Programme on HIV/AIDS
	(Programa das Nações Unidas para HIV/AIDS)

RESUMO

Fatores genéticos e imunológicos influenciam as diferentes respostas observadas entre indivíduos, desde a exposição ao HIV até o desenvolvimento da aids. As quimiocinas e seus receptores atuam na comunicação entre o sistema imune inato e adaptativo após o estabelecimento da infecção. A variabilidade genética dessas moléculas pode ser essencial para a resposta imune, principalmente se essas moléculas agem na fase inicial da infecção, fase esta que definirá o transcurso da doença. Além disso, a investigação da expressão de quimiocinas nos diferentes estágios clínicos da infecção e sua modulação após iniciado o tratamento com ARVs (antirretrovirais) é importante na busca de biomarcadores. Neste estudo, exploramos o papel de quinze polimorfismos candidatos em genes de receptores de quimiocinas e seus ligantes na susceptibilidade e na progressão à aids. Além disso, foram quantificados os níveis plasmáticos de seis quimiocinas em progressores extremos nos diferentes estágios clínicos da infecção e avaliamos o impacto da terapia como moduladora da resposta imune. Os resultados nos mostram que os polimorfismos rs56061981 no *CXCL10* (CT/TT, OR: 1,819, IC 95% 1,074-3,081, *P*=0,026) e rs3091250 no *CCR3* (TT, OR: 2,147, IC 95% 1,076-4,287, *P*=0,030) influenciam na susceptibilidade à infecção pelo HIV. Nas análises de interação gene-gene realizadas por redução multifatorial de dimensionalidade (MDR), observou-se que o rs56061981 no *CXCL10* e rs4359426 no *CCL22*, juntos predizem 57% da susceptibilidade à infecção pelo HIV (*P*=0,008). Ademais, observou-se que os polimorfismos rs13034664 no *CCL20* (CC, OR: 0,214, IC 95% 0,063-0,730, *P*=0,014) e rs4359426 no *CCL22* (CA/AA, OR: 2,685, IC 95% 1,128-6,392, *P*=0,026) foram associados com a progressão rápida à aids. Com relação aos níveis plasmáticos, o CXCL10 estava significativamente aumentado nos progressores rápidos ($P_{corrigido(c)}$=0,003) e lentos ($P_c \leq 0,0001$) pré-aids quando comparado com os controles saudáveis. Neste contexto, sugere-se o CXCL10 como biomarcador em indivíduos crônicos HIV+. Quando avaliadas as subpopulações celulares T auxiliares em HIV+ sob ARV, se observou uma frequência aumentada de linfócitos $TCD4^+$ ativados nos progressores rápidos (1,3% vs. 13,6%, P_c=0,008) e nos progressores lentos (1,3% vs. 5,4%, P_c=0,044) quando comparados com os controles saudáveis. Já a frequência de linfócitos T $CD8^+$ ativados foi mais alta nos progressores rápidos quando comparados com os controles saudáveis (0,32% vs. 8,7%; P_c=0,001). A frequência de células Th2 estava diminuída nos progressores rápidos (P_c=0,027) e, nos progressores lentos, as células Th1 estavam com frequência diminuída, enquanto que a frequência das Th17 estava aumentada quando comparados com os controles saudáveis

(P_c=0,007 e P_c=0,042, respectivamente), se observando um desequilíbrio de subconjuntos celulares T $CD4^+$ nos progressores extremos sob ARV.

ABSTRACT

From HIV exposition to AIDS disease different responses against HIV infection are influenced by immunological and genetic host factors. Chemokines and their receptors link the innate and adaptive system after the establishment of HIV infection. Genetic diversity of these molecules is crucial to the immune response, since they have a pivotal role in the early infection, clinical stage which predicts the disease progression. Furthermore, to investigate the expression of chemokines in different clinical stages of HIV infection and their modulation before and after initiated ART (antiretroviral therapy) is important to identify biomarkers of progression. In this study, we explored the role of 15 candidate polymorphisms in chemokine receptor and chemokine genes on susceptibility to HIV infection and progression to AIDS. Also, plasma levels of six chemokines were quantified in extreme progressors in different clinical stages of infection and the impact of ART, as a modulator of the immune response, was evaluated. The *CXCL10* rs56061981 (CT/TT, OR: 1.819, CI 95% 1.074-3.081, P=0.026) and *CCR3* rs3091250 (TT, OR: 2.147, CI 95% 1.076-4.287, P=0.030) variants were associated with susceptibility to HIV infection. Also, in the MDR (Multifactor Dimensionality Reduction) analyses, the best model to predict the susceptibility to HIV infection was composed by *CXCL10* rs56061981 and *CCL22* rs4359426 with 57% of accuracy (P=0.008). In analysis of disease progression, *CCL20* rs13034664 (CC, OR: 0.214, CI 95% 0.063-0.730, P=0.014) and *CCL22* rs4359426 (CA/AA, OR: 2.685, CI 95% 1.128-6.392, P=0.026) variants were associated with rapid progression to AIDS. Regarding plasma levels, CXCL10 levels were higher in rapid progressors (RPs) ($P_{\text{corrected}(c)}$=0.003) and slow progressors (SPs) ($P_c \leq 0.0001$) in pre-AIDS when compared to healthy controls and this molecule was suggested as a potential biomarker of disease progression. Furthermore, frequencies of activated $CD4^+$ T-cell were higher in SPs (1.3% vs. 5.4%, P_c=0.044) and RPs (1.3% vs. 13.6%, P_c=0.008) under ART when compared with healthy controls. On the other hand, frequencies of activated $CD8^+$ T cell were elevated in RPs (0.32% vs. 8.7%, P_c=0.001) under ART when compared with controls. Th2 cell frequency was lower in RPs under ART (P_c=0.027) when compared with controls, and Th1 cell frequency was lower (P_c=0.007) and Th17 cells were higher (P_c=0.042) in SPs under ART when compared with healthy controls.

SUMÁRIO

Capítulo 1

1 Introdução

1.1 HIV/AIDS

1.1.1 A infecção pelo vírus da imunodeficiência humana

O Vírus da Imunodeficiência Humana (HIV) é um lentivírus da família *Retroviridae* e é o agente etiológico da síndrome da imunodeficiência adquirida (aids) em humanos (Levy 1993). As vias de exposição ao HIV podem envolver a transfusão de sangue infectado, via intravenosa no caso de usuários de drogas injetáveis ou acidentes, transmissão materno-fetal (durante o parto ou amamentação) e a via sexual, sendo esta última a principal via de transmissão do HIV (Galvin and Cohen 2004). A transmissão do HIV é influenciada pela quantidade de partículas virais presentes no fluído corporal infectado e pelo número de exposições de um indivíduo a esse fluído (Levy 2010). O estabelecimento da infecção pelo HIV na primeira exposição geralmente não é fácil, dependendo principalmente de três fatores: *i)* fatores virais (virulência e infecciosidade), *ii)* fatores do hospedeiro (imunológicos e genéticos) e *iii)* fatores ambientais (sociais, culturais e políticos) (Levy 2010).

Após uma exposição sexual, através do trato genital ou da mucosa anal, o vírus atravessa a barreira epitelial (esse evento torna-se mais fácil se a mucosa genital estiver fisicamente danificada ou se o organismo apresentar coinfecções) (Galvin and Cohen 2004). Uma vez estabelecida a infecção, as células dendríticas (DCs) presentes na mucosa serão as principais responsáveis pela disseminação do vírus aos linfonodos, onde ocorre a infecção dos linfócitos T $CD4^+$, alvos celulares do HIV e fundamentais para a coordenação das defesas do organismo (Wu and KewalRamani 2006). O vírus, então, liga-se ao receptor do tipo lectina (DC-SING, L-SIGN e Siglec-1) presente na membrana celular da

DC e é internalizado mantendo-se intacto até a passagem para um linfócito T $CD4^+$, ocorrendo então infecção das células T $CD4^+$ (Izquierdo-Useros et al. 2012).

1.1.2 Epidemiologia do HIV/AIDS

Segundo o último relato global da UNAIDS/2016, existem aproximadamente 36,7 milhões de pessoas vivendo com o HIV no mundo. Em 2015 foram registradas ~2,1 milhões novas infecções e ~1.1 milhões de mortes por causa da aids. Considerando-se todas as regiões do mundo, a maioria dos indivíduos que vivem com HIV (97%) reside em países de baixa e média renda, especialmente na África subsaariana (Global report UNAIDS, 2016).

No Brasil, nos últimos dez anos a taxa de detecção de aids encontra-se estabilizada com uma média de 20,7 casos/100 mil habitantes. Porem, ela está concentrada em algumas regiões do país. No ano de 2015, foram notificados 32.321 casos de infecção pelo HIV, sendo 2.988 casos na região Norte (9,2%), 6.435 casos na região Nordeste (19,9%), 13.059 na região Sudeste (40,4%), 7.265 na região Sul (22,5%) e 2.574 na região Centro-Oeste (8,0%) (Ministério da Saúde, 2016). O ranking das Unidades da Federação referente às taxas de detecção de aids mostrou que os estados do Rio Grande do Sul e de Santa Catarina apresentaram as maiores taxas, com valores de 34,7 e 31,9 casos/100 mil habitantes, respectivamente. Entre as capitais, Porto Alegre apresentou taxa de 74,0 casos/100 mil habitantes em 2015, valor correspondente ao dobro da taxa do Rio Grande do Sul e a quase quatro vezes a taxa nacional (Ministério da Saúde, 2016). Devido a esse cenário epidemiológico, a capital gaúcha é considerada um *hotspot* da infecção pelo HIV. A fim de impactar a situação das regiões *hotspot* o Ministério da Saúde propôs ações de prevenção e estratégias para controle da epidemia. (Ministério da Saúde, 2016).

O Brasil tem adotado as metas 90-90-90 propostas pela UNAIDS, cujos objetivos são garantir que até 2020: 90% das pessoas vivendo com HIV estejam diagnosticadas e cientes de seu estado sorológico positivo, 90% das pessoas diagnosticadas soropositivas estejam em tratamento e 90% das pessoas em tratamento antirretroviral tenham carga viral suprimida (Ministério da Saúde, 2016). Para alcançar tais metas o país tem tomado algumas medidas. O autoteste vem sendo objeto de um estudo-piloto, e pode ser feito pela própria pessoa sem a necessidade de ajuda de um profissional ou técnico. Também estão em andamento estudos para avaliar o impacto do uso de antirretrovirais como Profilaxia

Pré-Exposição (PrEP) para homens que fazem sexo com homens e mulheres transsexuais. Apesar das grandes discussões sobre a implementação de PrEP no Brasil, as políticas públicas de saúde sobre o acesso universal da terapia antirretroviral podem ser grandes facilitadoras para a implementação da PrEP devido a existência de um alto número de serviços de prevenção e cuidados já disponíveis para estas populações consideradas de risco. Mais detalhes sobre as políticas públicas da terapia antirretroviral no Brasil serão abordados no capítulo 5 desta tese.

1.1.3 Fases clínicas da infecção pelo HIV

1.1.3.1 Fases aguda e primária

A fase aguda corresponde ao período entre 1-4 semanas após a transmissão do vírus e antes da detecção dos anticorpos específicos contra o vírus. O período logo depois da infecção e a primeira detecção do RNA do HIV no sangue periférico geralmente corresponde a uma subfase da fase aguda, que é chamada de fase eclipse que dura ~10 dias. Após a fase eclipse, a carga viral no plasma aumenta até alcançar um pico máximo após 21-28 dias da infecção seguido pelo declínio do número de linfócitos T $CD4^+$ de memória predominantemente da mucosa, lugar onde se localizam um grande número de células. Aproximadamente 80% das células T $CD4^+$ localizadas na submucosa intestinal (*Gut Associated Lymphoid Tissue* – GALT) são depletadas nas primeiras três semanas da infecção (Brenchley et al. 2004). A ativação das células do sistema imune inato, células B e T, é uma característica marcante da fase aguda a qual persiste variavelmente durante a fase crônica (McMichael et al. 2010). Ao mesmo tempo em que se aumenta a viremia, uma tempestade de citocinas e quimiocinas é observada nesta fase, a qual ao invés de controlar a infecção pode estimular a replicação viral desencadeando uma imunopatologia (Figura 1).

O termo “infecção primária” ou inicial pelo HIV refere-se aos primeiros 3-6 meses subsequentes ao período da infecção aguda, após a formação de anticorpos específicos (soroconversão) contra o vírus, iniciando entre 3-4 semanas após a infecção (Levy 2010) (Figura 1). Essa fase primária da infecção também é caracterizada pela infecção de vários tipos de células, tais como monócitos, macrófagos, células microgliais e linfócitos T $CD4^+$;

por uma perda persistente de células T $CD4^+$, uma grande quantidade de imunoglobulinas, presença de marcadores de ativação em subpopulações de linfócitos e, pelo aumento de linfócitos T $CD8^+$ (Levy 2010). Nesta fase há expressão de uma grande quantidade de citocinas/quimiocinas pró-inflamatórias, alguns estudos sugerem que isso poderia direcionar o aumento das células T $CD8^+$ (Graziosi *et al.* 1996).

Indivíduos na fase aguda ou primária podem desenvolver sintomas que são similares aos da Síndrome Retroviral Aguda (SRA), os quais incluem influenza febril, fadiga, garganta inflamada, suor noturno, gânglios aumentados nos quais a erupção cutânea ocorre frequentemente e, em alguns casos, linfoadenopatia e exantema (Kahn and Walker 1998). Alguns estudos têm observado que indivíduos com doença primária (doença febril de início agudo) em associação com a soroconversão (*seroconversion illnes*) apresentam um risco aumentado de desenvolver uma progressão rápida a aids, quando comparados com os que não apresentam essas sintomatologias (Pedersen *et al*. 1989; Schechter *et al*. 1990).

1.1.3.2 Fase crônica

A fase crônica da doença está caracterizada por uma acelerada renovação celular, ocorrendo o aumento do número de células T $CD4^+$ circulantes até níveis relativamente normais, paralelamente ocorre a diminuição da viremia em níveis variáveis (*set-points virais*) e o estabelecimento de uma infecção assintomática por um longo período de tempo (fase de latência clínica) (Figura 1). Embora a carga viral plasmática apresente uma redução significativa após a fase de infecção aguda, uma acentuada ativação imune persiste no estágio crônico da doença. Um maior número de células T $CD4^+$ expressa marcadores de ativação celular o que as caracteriza como células de memória diferenciadas para uma determinada subpopulação celular (Grossman *et al*. 2006). Nessa fase também há níveis aumentados de citocinas/quimiocinas pró-inflamatórias e elevação das concentrações de proteína C reativa. As células B são policlonalmente ativadas com uma consequente hipergamaglobulinemia. Ademais, a ativação e número de células *Natural Killers* (NK) são incrementados e o número de DCs decresce no sangue periférico. Danos imunológicos no trato gastrointestinal levam ao rompimento da barreira da mucosa permitindo a translocação microbiana, com entrada de bactérias potencialmente perigosas, como aquelas

contendo LPS (lipopolissacarídeo), já correlacionadas com a ativação imune persistente (Brenchley *et al.* 2004). Além disso, danos no tecido linfoide são observados: disfunção tímica, fibrose dependente do fator de transformação do crescimento beta e alterações na arquitetura do folículo linfóide. Tais danos podem ser revertidos progressivamente com o início do tratamento (Hardy *et al*. 2004). O período de progressão desta fase crônica é variável entre os indivíduos, sendo que os indivíduos HIV^+ podem progredir de uma forma típica, rápida ou lenta para a fase de aids (Figura 2), o que vai depender tanto de fatores imunológicos e genéticos do hospedeiro, como de fatores virais (Langford *et al.* 2007). Os indivíduos com uma progressão diferencial extrema têm sido alvo de estudos para uma melhor compreensão do papel genético e imunológico na progressão para aids.

1.1.3.2.1 Progressão típica

Os pacientes infectados pelo HIV-1 que seguem o curso normal da infecção são conhecidos como progressores crônicos ou típicos. Esses indivíduos representam entre 70% e 80% da população soropositiva e desenvolvem uma infecção sintomática entre o 3^o e o 10^o ano após a soroconversão (Langford *et al*. 2007). Eles iniciam o tratamento antirretroviral quando as células T $CD4^+$ declinam drasticamente (<200 células/mm^3), ocorre um aumento da carga viral e infecções oportunistas aparecem. A progressão da doença é retardada por meio de terapia, o que induz uma restauração da contagem total de células T $CD4^+$ no sangue periférico, e uma redução significativa da carga viral, resposta essa que varia para cada indivíduo.

1.1.3.2.2 Progressão rápida

Os indivíduos que desenvolvem uma progressão rápida para a aids apresentam um rápido declínio da taxa de células T $CD4^+$ e a ocorrência de eventos relacionados com a aids poucos anos após a infecção. Segundo estimativas, cerca de 15% dos pacientes HIV-positivos são progressores rápidos (Langford *et al*. 2007). Os critérios para classificar os progressores rápidos são variados, mas a maioria dos estudos tem coincidido em observar a queda dos linfócitos T $CD4^+$ em 200-300 células/mm^3 dentro de um período de até 3 anos após a soroconversão, sendo recomendado o início da terapia antirretroviral neste período (Gurdasani *et al*. 2014). Uma ativação imune indireta, causada

pela translocação microbiana, e perda de linfócitos T $CD4^+$ associados à submucosa intestinal tem sido observada em progressores rápidos (Marchetti *et al*. 2011). Ademais, um estudo observou que a combinação de febre com erupção cutânea não é só uma sintomatologia clínica da fase aguda/primária, senão também está correlacionada com uma progressão rápida (Pedersen *et al*. 1989; Schechter *et al.* 1990; Keet *et al*. 1993).

1.1.3.2.3 Progressão lenta

A progressão lenta para a aids é caracterizada pela manutenção estável da taxa de células T $CD4^+$ em ausência da terapia e sem sintomas por um período de tempo de entre 7 e 10 anos ou mais. Esses indivíduos representam uma pequena porcentagem da população HIV+ (em geral 5-8%). Devido a diferenças encontradas entre esses indivíduos, geralmente, eles são classificados em subgrupos: os não-progressores de longo prazo (*long term non-progressors*, LTNPs), os quais permanecem assintomáticos por mais de 10 anos com >500 células T $CD4^+$ e mantêm a carga viral entre 5000-15000 cópias/mL de RNA viral no curso da infecção (a maioria tem ≤ 10 000 cópias/mL de RNA viral em ausência da terapia ao longo do tempo) (Hunt 2009); os progressores lentos (*slow progressors*, SPs), ao serem comparados com os LTNPs, mantêm uma carga viral mais elevada e episódios com <500 células T $CD4^+$, assintomáticos por mais de 8 anos sem terapia (Poropatich and Sullivan 2011). Apesar desses indivíduos progredirem de uma forma mais estável que os progressores rápidos, a presença da carga viral parece deixar o indivíduo com uma ativação imune persistente. Assim, um estudo observou que a proporção de diferentes células ativadas de progressores lentos foi similar quando comparada com progressores rápidos (Goicoechea *et al*. 2009).

1.1.3.2.4 Controladores de Elite

Estudos têm observado que esse grupo de indivíduos representa ≤ 1% dos HIV soropositivos e apesar de não haver consenso em sua definição, geralmente estão divididos em dois grupos: os controladores avirêmicos, os quais se caracterizam por apresentar cargas virais indetectáveis (abaixo de 50 cópias/mL de RNA viral) e níveis de células T $CD4^+$ > 500 em ausência de terapia por ao menos 1 ano de acompanhamento; e os controladores virêmicos que apresentam episódios de carga viral entre 50-2000 copias/mL e células T $CD4^+$ < 350/mm^3 e progridem mais rapidamente à aids quando

comparados com aqueles controladores que mantêm níveis mais elevados de células T $CD4^+/mm^3$ (Okulicz *et al*. 2009).

Estudos observaram que fatores genéticos são importantes para a caracterização deste grupo (Migueles *et al*. 2000; Han *et al*. 2008). Porém, os fatores imunológicos ainda são objeto de estudo para uma melhor compreensão do controle natural à infecção pelo HIV. Apesar dos controladores avirêmicos apresentarem carga viral indetectável, foi observada uma elevada ativação celular de células T $CD4^+$ e $CD8^+$ quando comparados estes pacientes com os controles saudáveis soronegativos, e uma elevada ativação de linfócitos T $CD8^+$ quando comparados com indivíduos HIV+ em tratamento. Ademais, esses controladores apresentaram altos níveis de LPS no plasma o qual foi associado com ativação celular, sugerindo que a translocação microbiana pode ser causa ou consequência da ativação imunológica (Hunt *et al*. 2008). Contudo, outro estudo observou que controladores avirêmicos apresentaram uma manutenção da integridade da mucosa intestinal quando comparados com pacientes HIV+ (com alta carga viral e linfócitos T $CD4^+$ em declínio). Esse estudo observou ainda, baixos níveis de expressão em genes que regulam a ativação imune, migração celular e resposta inflamatória intestinal nos controladores avirêmicos (Sankaran *et al*. 2005). De acordo com esses resultados, mais recentemente foi observada uma expressão diminuída de marcadores de ativação celular e de exaustão celular em controladores avirêmicos (com nível estável de $CD4^+$) quando comparados com controladores avirêmicos (com baixa frequência de $CD4^+$), e essa expressão foi similar aos HIV soronegativos (Bansal *et al*. 2015).

1.1.3.3 Aids

Uma ativação imune persistente na fase crônica, quando descontrolada, leva ao esgotamento das células T $CD4^+$ e ao aumento de carga viral, desencadeando uma alta supressão do sistema imune. Esses eventos deixam o indivíduo imunodeficiente, ou seja, incapaz de lutar contra outros agentes infecciosos ou células tumorais (e.g. tuberculose ou pneumonia ou câncer) levando à morte se o tratamento não é iniciado. Essa fase sintomática da doença é denominada de síndrome da imunodeficiência adquirida (aids) (An and Winkler 2010).

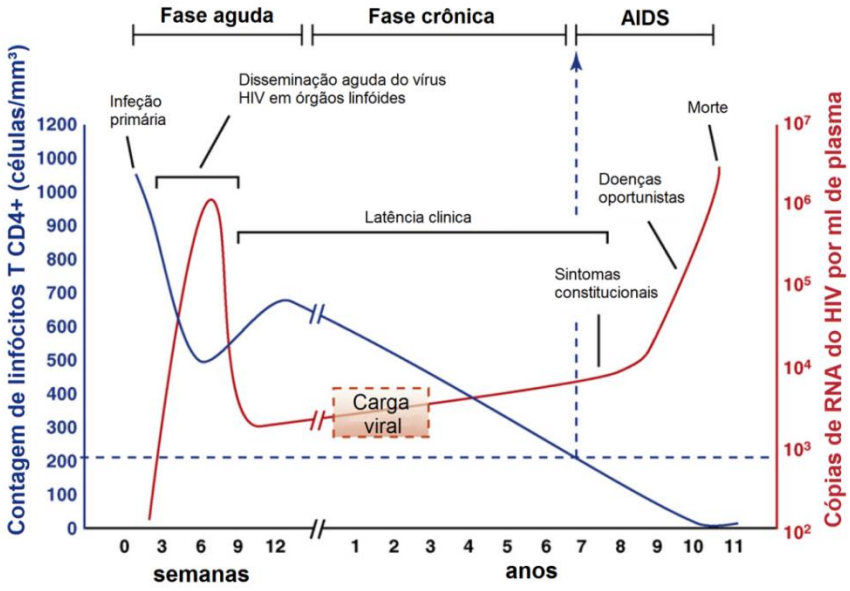


Figura 1. Fases clínicas da infecção pelo HIV e progressão para a aids (Adaptado de An &Winkler, 2010).

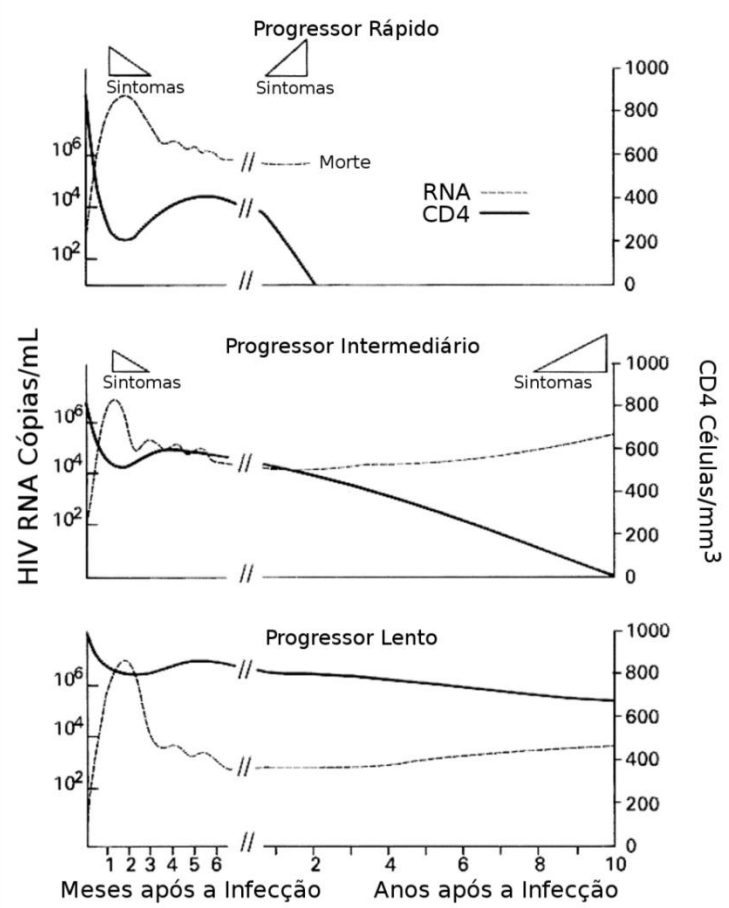


Figura 2. Diferentes tipos de progressão da aids (Adaptado de Langford SE *et al*., 2007)

1.2 Imunopatogênese do HIV/Aids

1.2.1 Sistema imune inato e adaptativo

Frente à infecção pelo HIV a primeira resposta do sistema imunológico é a indução da fase aguda. Os TLRs (*Toll like-receptors*) do hospedeiro têm um papel importante nesta resposta, pois reconhecem PAMPs (*Pathogen-associated molecular patterns*) associados ao vírus. Após essa sinalização de reconhecimento as células iniciam uma resposta de defesa antiviral e são produzidas citocinas e quimiocinas pró-inflamatórias (e.g., IP-10/CXCL10, IFN-I e III) as quais recrutam e ativam células do sistema imune inato [e.g., células dendríticas, macrófagos, neutrófilos, células NK, células NK-T (linfócitos NK), células T γδ (*gamma delta* T *cells*)] (Altfeld and Gale Jr 2015). As quimiocinas induzem a migração dessas células para uma área de infecção ou inflamação, colaborando com a maturação de células dendríticas (DCs) que atuam como células apresentadoras de antígenos (APCs) ao sistema imunológico adaptativo. Como o HIV não é eliminado na fase aguda dá-se passo para a fase crônica da infecção, a qual envolve o recrutamento, migração e ativação de leucócitos do sistema imune adaptativo (e.g., linfócitos T CD4+ e CD8+) (Abbas AK, Litchman AH 2012). As citocinas quando ligadas a seus receptores estimulam a proliferação e diferenciação das células T e ativam outras subpopulações celulares. As quimiocinas, por sua vez, quando ligadas a seus receptores, além de também ativar as células, são as principais responsáveis pelo recrutamento e migração de leucócitos aos locais de infecção induzindo a ativação de linfócitos e monócitos os quais vão produzir mais citocinas e quimiocinas (Levy 2010).

A resposta imune adaptativa inicial à infecção por HIV é caracterizada pela expansão dos linfócitos T $CD8^+$ citotóxicos (CTLs) específicos para peptídeos de HIV. Estes CTLs controlam a infecção na fase aguda, mas terminam sendo ineficazes devido ao surgimento de mutantes de escape viral (Levy 2010). Os linfócitos T $CD4^+$ também respondem ao vírus e podem contribuir para o controle viral de diversas maneiras, tais como a ativação das células B, e também estão fortemente envolvidos na diferenciação das células T $CD8^+$. Quando as células $CD4^+$ virgens (*naïve*) entram em contato com os seus antígenos específicos e recebem sinais co-estimuladores, elas se tornam ativas,

secretam citocinas, proliferam e se diferenciam, resultando na formação de clones de células T $CD4^+$ efetoras específicas para um determinado antígeno do HIV (Abbas AK, Litchman AH 2012).

O grau de replicação e disseminação do vírus nos indivíduos soropositivos dependerá basicamente do local da infecção (e.g. mucosa) e da resposta do sistema imunológico tanto inato quanto adaptativo. As respostas imunes inata e adaptativa se complementam a fim de controlar eficazmente a infecção pelo HIV-1, e os mediadores que conectam ambas as respostas são citocinas e quimiocinas (Figura 3) (Esche *et al*. 2005). A existência de indivíduos altamente expostos ao HIV-1 e não infectados, pode ser a prova de que fatores do sistema imune, tais como características genéticas, são capazes de neutralizar a infecção, mas esses mecanismos de proteção permanecem pouco entendidos, sua melhor compreensão está restrita a um grupo específico de indivíduos (e.g. profissionais do sexo, homens que fazem sexo com homens, etc) e elas são de difícil manejo.

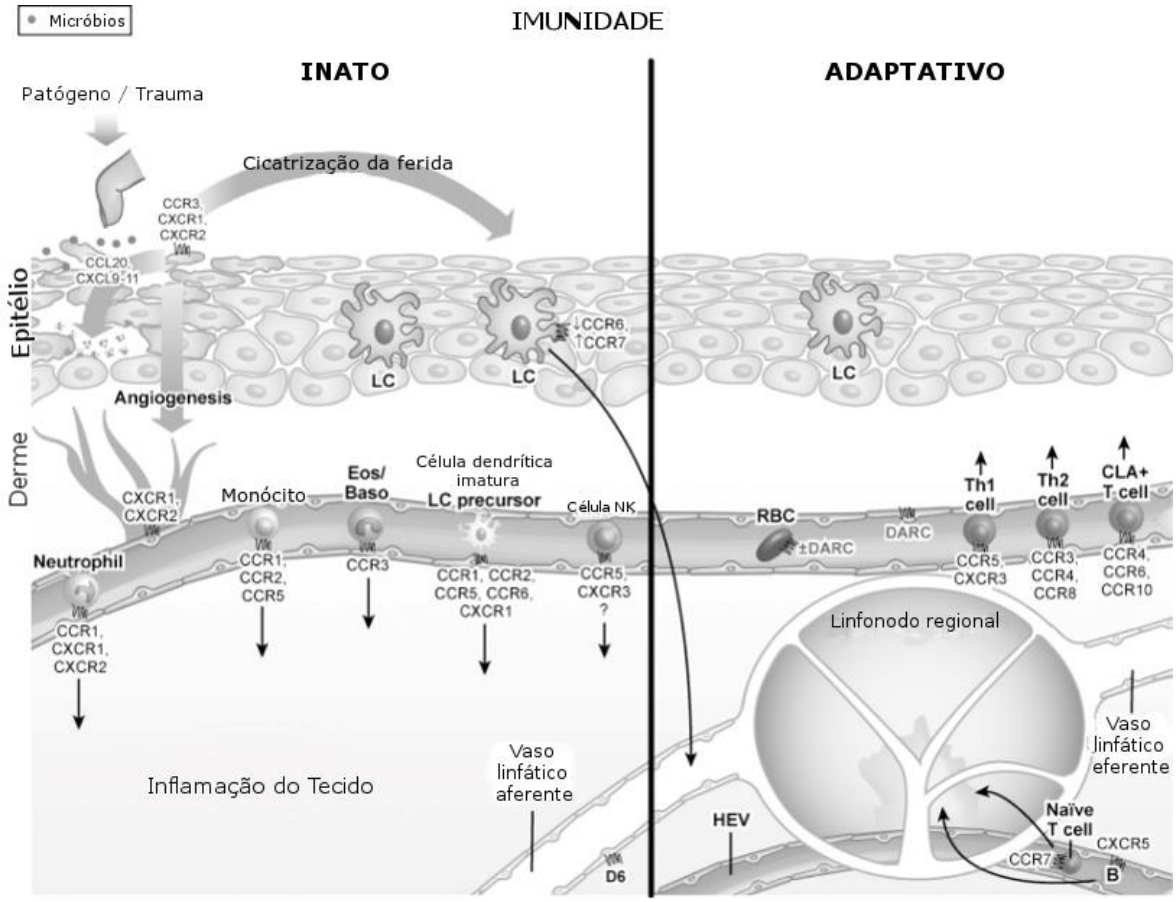


Figura 3. O papel de quimiocinas e receptores de quimiocinas na comunicação entre o sistema imune inato e adaptativo (Adaptado de Esche *et al*., 2005).

1.2.2 Ativação do sistema imune

O *set-point* da ativação imunológica é estabelecido na fase aguda da infeção pelo HIV. Assim, o nível da ativação na primeira fase da infecção vai determinar a progressão da doença (Deeks 2004). Na fase clínica da aids foi observado que os linfócitos T $CD8^+$ expressam níveis aumentados de CD38, HLA-DR (marcadores de ativação celular) e PD-1 (marcador de exaustão imune), indicando desregulações funcionais nestas células, associadas com a progressão da doença (Giorgi *et al*. 1993; Day *et al*. 2006). Assim, já foi observado que a ativação imune tem sido significativamente associada com a destruição do sistema imune e uma progressão

rápida para a aids (Hunt 2012). Por outro lado, como já citado anteriormente, os controladores de elite avirêmicos apresentaram uma expressão diminuída de CD38/HLA-DR e de PD-1 quando comparados com controladores avirêmicos com baixos níveis de células $CD4^+$ e, essa expressão foi similar aos controles saudáveis (Bansal *et al*. 2015).

Porém, ainda pouco se sabe sobre a relação da ativação imune e a susceptibilidade à infecção pelo HIV. Algumas hipóteses foram propostas para entender essa relação. Como já está bem estabelecido que o HIV se replica preferencialmente nos linfócitos T $CD4^+$ ativados, acredita-se que uma "quiesciência imunológica" poderia proteger o indivíduo da infecção pelo HIV, limitando a disponibilidade das células alvo à infecção (Card *et al*. 2013). Assim, estudos avaliaram fatores de ativação celular e supressão da resposta imune. Como já citado, a fase aguda/primária da infecção tem um papel importante na ativação imune, ocorrendo um dano massivo da mucosa intestinal levando à translocação microbiana. Essa translocação microbiana pode ser quantificada pelos níveis plasmáticos de LPS e da proteína solúvel sCD14 (liberada pelos monócitos e macrófagos após estímulo pelo LPS). O LPS finalmente é reconhecido pelo TLR4 levando a uma ativação imune sistêmica e inflamação (Shan and Siliciano 2014). Por outro lado, as células T regulatórias (Tregs), que são supressoras da ativação celular, podem reverter esta ativação inibindo a proliferação e função de algumas subpopulações celulares como Th1, Th2, Th17 e células T $CD8^+$ (Campbell and Koch 2011).

Contudo, o papel da ativação imune na susceptibilidade à infecção pelo HIV é controverso, devido aos resultados opostos de estudos que analisaram indivíduos expostos não infectados (HESN, *HIV exposed seronegative*,). Observou-se que os HESN apresentaram um fenótipo "imunoquiescente" associado a baixos níveis de linfócitos T ativados, redução da proliferação de linfócitos T $CD4^+$ e $CD8^+$ e diminuição da secreção de citocinas pró-inflamatórias (McLaren *et al*. 2010; Card *et al*. 2013). Já outro estudo observou uma reduzida frequência de células expressando o marcador de ativação celular CD69 e uma alta proporção de Tregs em HESN (mulheres comerciantes do sexo) quando comparado com controles HIV soronegativos (Card *et al.* 2009). Contrários a esses resultados, outros estudos observaram que os HESN (neste caso composto por casais sorodiscordantes) tinham um perfil mais similar aos HIV soropositivos quando comparados com HIV soronegativos (Suy *et al*.

2007; Saulle *et al*. 2016). Ainda neste estudo, avaliou-se a correlação entre a ativação celular e translocação microbiana e observou-se que os níveis plasmáticos de LPS e sCD14 foram similares entre os HESN e os HIV soronegativos (Saulle *et al*. 2016).

1.2.3 Diferenciação das células T CD4^{+}: Th1, Th2, Th17 e Tregs

Após a apresentação de antígenos e ativação da célula T, as células T CD4+ têm a capacidade de se diferenciar em células T de memória e logo em células T efetoras específicas para atuar em diferentes tipos de respostas direcionadas por patógenos ou estímulos externos (Zinkernagel 2000). As subpopulações celulares melhor caracterizadas são: Th1, Th2, Th17 e T regulatórias (Tregs). Porém, novas subpopulações celulares foram descobertas tais como as Th9, Th22 e T foliculares e estão sendo cada vez mais estudadas para entendermos seu papel na regulação do sistema imune frente aos patógenos. Os principais fatores que definem os tipos diferenciados das células são as citocinas e quimiocinas que elas produzem, os receptores de quimiocinas que expressam na superfície, os fatores de transcrição e alterações epigenéticas nos *loci* dos genes das citocinas (Valverde-Villegas *et al*. 2015).

As células Th2 secretam IL-4, IL-5, IL-10 e IL-13 e estão envolvidas em respostas dominadas pela IgE, eosinófilos e basófilos. Essas células expressam predominantemente o receptor de quimiocinas CCR3 na sua superfície (receptor para eotaxin 1, 2 e 3, RANTES/CCL5, MCP-1/CCL2, MIP-1α/CCL3 e MIP1-β/4 (Sallusto *et al*. 1997), porém já foi observado que CCR4 (receptor para TARC/CCL17 e MDC/CCL22) e CCR8 (receptor para CCL17, CCL4, CCL1) também são altamente expressos nas Th2 (Sallusto *et al*. 1998b). Essas células estão altamente presentes em respostas alérgicas e nos sítios de infecção principalmente por helmintos. As células Th1 expressam predominantemente o CXCR3 (receptor para CXCL10/IP-10, CXCL9/Mig e CXCL11/I-TAC) e produzem IFN-γ, TNF-α, sendo que essas citocinas e quimiocinas têm um papel importante na resposta imune contra patógenos intracelulares, virais e bacterianos (Sallusto *et al*. 2000); é por isso que as Th1 tendem a ser abundantes nos sítios de infecção. No contexto do HIV, tem

sido bastante discutida a teoria de que o *switch* Th1→Th2 estaria associado à progressão para a aids. Estudos observaram um aumento das citocinas do perfil Th2 e uma diminuição das citocinas do perfil Th1 em pacientes HIV+ progredindo para a aids (Becker 2004).

As Th17 e Tregs, apesar de compartilharem características similares e vias de diferenciação interconectadas, apresentam papéis opostos na resposta imune contra doenças infecciosas e autoimunes. Enquanto que as células Th17 promovem uma resposta inflamatória, as Tregs são encarregadas de controlar a ativação imune e a expansão de células T efetoras auto reativas, além de manter a tolerância ao reconhecimento do "próprio" pelo sistema imune (Noack and Miossec 2014). As células Th17 compõem uma linhagem induzida por IL-17 e expressam predominantemente o CCR6, que se liga à CCL20 e beta-defensinas. A migração de células Th17 aos locais de inflamação/infecção é dependente de CCR6-CCL20. Essas células são comumente observadas na mucosa da lâmina própria intestinal e em GALT e por isso, aliado ao fato deste ser o local de preferência para a replicação do HIV, são as células mais permissíveis à infecção pelo HIV (Gosselin *et al.* 2010). Por outro lado, a capacidade supressora das Tregs é influenciada por IL-2, IL-10, TGF-β e IL-35. Elas também expressam o CCR6 na sua superfície e já foi sugerido que as Th17 atraem Tregs $CCR6^+$ aos locais de inflamação/infecção via CCL20 (Yamazaki *et al*. 2008). Devido ao papel oposto das Th17 e das Tregs, vários estudos têm avaliado a proporção dessas células para verificar se um desequilíbrio entre essas duas subpopulações celulares está associado com susceptibilidade ou progressão para a aids. Assim, foi observado que indivíduos HIV+ sem tratamento apresentaram um desequilíbrio Th17/Tregs ao longo de um ano. Em contrapartida, nos controladores de elite essa proporção se manteve similar quando comparados com os HIV soronegativos (Li *et al*. 2011) [para mais detalhes do papel das células Th17 e Tregs na infecção pelo HIV e na progressão da doença, pode ser vista no artigo de revisão no anexo A: *New insights about Treg and Th17 cells in HIV infection and disease progression* (Valverde-Villegas *et al*. 2015)].

1.2.4 Quimiocinas e Receptores de quimiocinas

Os receptores de quimiocinas pertencem à superfamília de receptores transmembrana acoplados à proteína (G) que se ligam à guanina trifosfato (GTP) (GPCR), e atravessam sete vezes a membrana. Diferentes combinações de mais de 17 receptores de quimiocinas distintos são expressas em diversos tipos de leucócitos, resultando em padrões distintos de migração das células (Epstein and Luster 1998). Existem 10 receptores diferentes para as quimiocinas CC (denominados CCR1 a CCR10), seis receptores para as quimiocinas CXC (denominados CXCR1 a CXCR6) e um receptor para CX_3CL1 (denominado CX_3CR1). Esses receptores são expressos em todos os leucócitos, dentre os quais as células T exibem o maior número e a maior diversidade (Abbas AK, Litchman AH 2012).

As quimiocinas são parte de uma família de citocinas e a função que melhor as descreve é a quimiotaxia, já que quando ligadas ao seu receptor levam ao recrutamento e migração das células do sangue periférico para locais de infecção ou inflamação no organismo. Algumas quimiocinas são produzidas pelos leucócitos e por outras células em resposta a estímulos externos e estão envolvidas em reações inflamatórias e ativação imune, enquanto outras são produzidas de modo constitutivo nos tecidos e desempenham um papel importante na organização destes (Proudfoot 2002). Além de sua função quimiotáctica, as quimiocinas também podem induzir adesão a células endoteliais ativadas mediada por integrinas, podem agir como estimulantes ou inibidores da proliferação de células mieloides progenitoras, podem agir como angiogênicas e angiostáticas e podem inibir o crescimento tumoral (Mackay 2001).

Quando as células se diferenciam, elas adquirem novos padrões de migração. Por um lado, as células efetoras podem migrar rumo a tecidos periféricos onde, após o reconhecimento de antígenos, disparam uma resposta inflamatória, muitas vezes desencadeando reações alérgicas (Zinkernagel 2000). Já por outro lado, as células T de memória mantêm a capacidade de migrar para os tecidos periféricos, mas também podem migrar para os gânglios linfáticos, onde podem ser estimuladas para gerar novas células T efetoras (Sallusto *et al.* 1998a). Contudo, observamos que receptores de quimiocinas e seus ligantes têm um papel relevante na diferenciação, recrutamento,

migração e ativação das células como resposta frente ao patógeno ou estímulos externos.

1.2.4.1 Receptores de quimiocinas e seus ligantes na infecção pelo HIV

Para penetrar na célula o HIV-1 necessita interagir com o CD4, o principal receptor, e um receptor secundário, ambos geralmente essenciais para o vírus infectar as células de forma eficiente (Clapham and McKnight 2002). O receptor secundário é, em geral, um receptor de quimiocina, como o CCR5. O CCR5 é o co-receptor predominantemente utilizado *in vivo* pelo HIV-1. Mutações no gene *Env* (codificante das proteínas do envelope do vírus) podem direcionar mudanças na proteína Env as quais induziriam uma maior afinidade com o receptor CXCR4. Assim, foi observado que cepas R5 (que utilizam CCR5) predominam durante a infecção primária e a fase assintomática, enquanto que o surgimento de cepas X4 (que utilizam CXCR4) ou R5X4 (que utilizam CCR5 e CXCR4) foi associado com a diminuição de células T CD4 e progressão da doença desde um estágio crônico ao avançado (Connor *et al*. 1997). Visto que esses receptores de quimiocinas se ligam aos seus ligantes (e.g. CCR5 liga-se a CCL3, CCL4, CCL5 e CXCR4 liga-se a CXCL12) foi sugerido que essa ligação é capaz de inibir a via de entrada do HIV na célula, o que poderia ocorrer por três mecanismos: *i)* após essa ligação receptor-ligante o envelope do vírus fica impedido de interagir com o receptor, *ii)* as quimiocinas induzem endocitose do receptor, limitando a disponibilidade do receptor ao vírus, *iii)* as quimiocinas podem também induzir dimerização do receptor, também inibindo a interação vírus-receptor (Amara *et al*. 1997; Signoret *et al.* 1998; Vila-Coro *et al.* 2000). De acordo com isso, estudos observaram o papel das quimiocinas CCL5, CCL3, CCL4 como potentes inibidores a infecção as células T $CD4^+$ pelo HIV (Cocchi *et al.* 1995) e CXCL12 bloqueou a infecção de células por cepas X4 (Arenzana-Seisdedos 2015).

Em relação aos chamados receptores alternativos, um trabalho observou que o CCR8 foi utilizado como co-receptor por uma proporção significativa de isolados primários de HIV-1 e HIV-2 e pode, de fato, ser relevante como receptor alternativo, pelo menos sob determinadas condições e subpopulações de células T específicas (Jinno

et al. 1998; Cilliers *et al.* 2005; Calado *et al.* 2010). Já os receptores GPR1 (*G protein-coupled receptor 1*) e FPRL1 (*formylpeptide receptor homolog-1*) também foram propostos como coreceptores alternativos predominantes (Shimizu *et al.* 2010) (Shimizu *et al.* 2009). Outro estudo observou que CCR3 poderia ser usado como um co-receptor substituto pelo subtipo B do HIV-1 enquanto que o receptor FPRL1 pode ser usado como co-receptor substituto pelos subtipos A e C (Björndal *et al.* 1997; Aasa-Chapman *et al.* 2006; Nedellec *et al.* 2009).

1.2.4.2 Heterogeneidade funcional de células T $CD4^+$ na infecção pelo HIV

A identificação de vários marcadores de superfície em células Th17 de humanos, tais como IL-23R, CCR6, CCR4, CXCR3 e β7 integrina pode auxiliar a compreensão da dinâmica da diferenciação de células T $CD4^+$ em indivíduos infectados pelo HIV (Brenchley *et al.* 2008; Gosselin *et al.* 2010). Subtipos de células T $CD4^+$ primárias que expressaram CCR4, CXCR3, CCR6 mostraram comportamento distinto frente à infecção pelo HIV-1 e isto revela uma heterogeneidade das células T $CD4^+$ frente ao vírus. Os subtipos de células T $CCR4^+CCR6^+$ (perfil Th17) e $CXCR3^+CCR6^+$ (perfil Th1Th17) foram caracterizados como altamente permissivos à replicação de HIV-1 *in vitro* (Gosselin *et al.* 2010). As células T $CCR4^+CCR6^+$ e $CXCR3^+CCR6^+$ têm potencial para serem recrutadas para a mucosa intestinal e vaginal, e também para o cérebro, através de um mecanismo CCR6-CCL20 dependente, podendo contribuir de forma significativa para a disseminação do HIV-1 e sua persistência, atraindo outras células T $CCR6^+$ para sítios de replicação viral *in vivo* (Wang *et al.* 2009) (Figura 2).

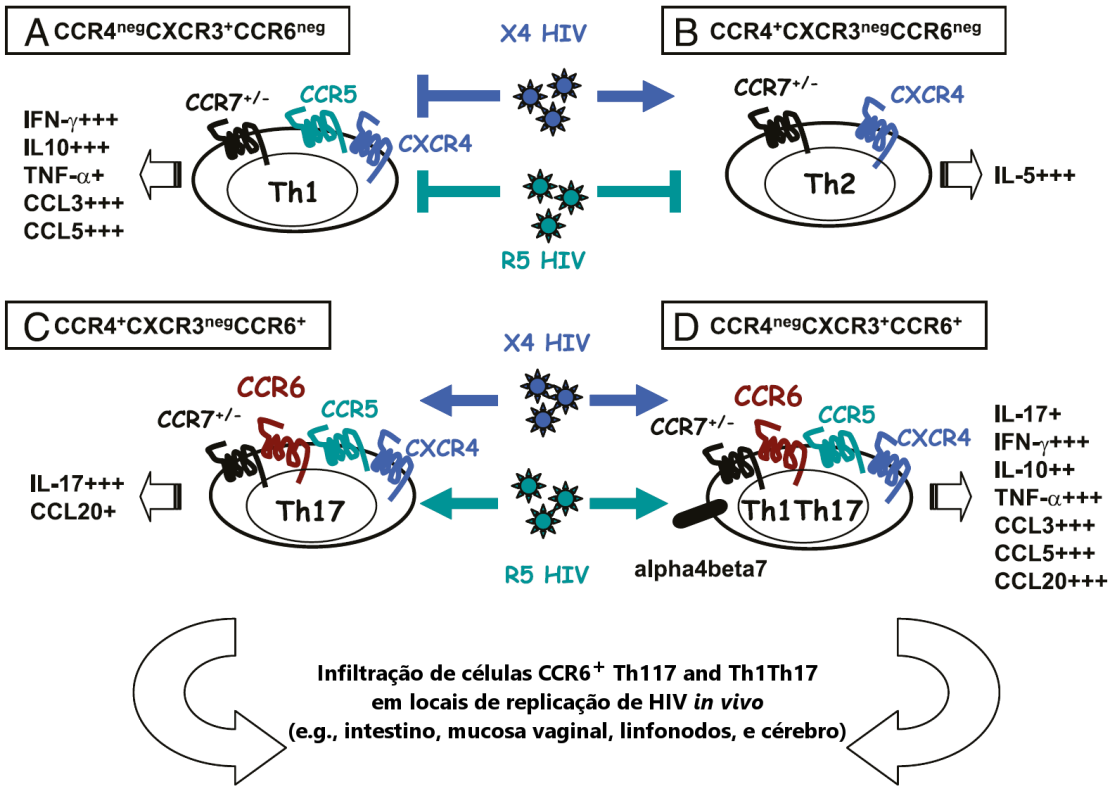


Figura 4. Características de subpopulações de células T CD4$^+$ que comprometem o *homing* e a permissividade ao HIV (adaptado de Gosselin A *et al.*, 2010).

1.2.4.3 Migração dos linfócitos T CD4$^+$ na infecção pelo HIV

Recentes avanços em imunologia vincularam o comprometimento da linhagem e a especificidade antigênica de subtipos de células T CD4$^+$ de memória à expressão de receptores de quimiocinas, tais como: CCR4 para a migração dentro do tecido da pele, CCR6 para migração no intestino, cérebro e outros tecidos e CXCR3 para migração dentro de sítios inflamatórios incluindo o intestino. Mais recentemente foi observado que subpopulações de células T CD4$^+$ são caracterizadas pela combinação da expressão desses receptores. Por exemplo, a subpopulação de células T CD4$^+$ com o fenótipo CCR4$^+$CCR6$^+$ produziu IL-17 e expressou o fator de transcrição RORγτ (perfil Th17).

Já as células T $CD4^+$ com o fenótipo $CXCR3^+CCR6^+$ produziram IL-17 e IFN-γ e expressaram os fatores de transcrição RORγτ e T-bet (perfil Th1Th17) (Acosta-Rodriguez *et al*., 2007).

Como mencionado anteriormente, as células dendríticas têm um papel chave na fase inicial de uma infecção e a expressão de receptores de quimiocinas nas células dendríticas mieloides (mDCs) e plasmocitoides (pDCs) é crítica para a migração diante desta resposta inflamatória/infecciosa. Os receptores CCR6 e CCR7 dirigem a migração das DCs desde a superfície da mucosa para os linfonodos, enquanto que o CXCR3 direciona a migração para o fígado, por exemplo, durante a infecção por hepatite C (Penna *et al*. 2002). No contexto do HIV, após o estabelecimento da infecção, o dano normalmente ocorre na mucosa consequentemente iniciando uma resposta imune inata. Dentro de 24 horas depois da infecção na mucosa, o epitélio produz a quimiocina CCL20 (MIP-3α), e mediante o eixo CCR6-CCL20 recruta células dendríticas plasmocitoides (pDCs). Estas últimas, uma vez recrutadas vão produzir IFN-α, IFN-β, MIP-1α (CCL3) e MIP-1β (CCL4) nos primeiros dias da infecção (Li *et al*. 2009) (Figura 4). Ademais, foi observado que IFN-α e IL-15 são as primeiras citocinas com altos níveis plasmáticos incrementados durante os cinco primeiros dias após detecção da viremia, seguidas pelo CXCL10, TNF-α e MCP-1 e logo IL-6, IL-8 e IL18 (Stacey *et al*. 2009). CCL17 e CCL24 são quimiocinas mais marcadamente associadas à inflamação alérgica, elas se ligam com alta afinidade ao CCR3, receptor que é seletivamente expresso nas células Th2, e vão dirigir seu *homing* rumo a sítios específicos de inflamação.

Contudo, observamos que o papel de receptores de quimiocinas e seus ligantes é bastante complexo, pois se por um lado agem no recrutamento de células e migração das mesmas, levando o vírus aos locais de inflamação/infecção, ativando células e consequentemente induzindo a replicação do vírus, por outro, essa ligação quimiocina-receptor pode inibir a entrada do vírus na célula. Além de mecanismos celulares, fatores moleculares e muito provavelmente também genéticos vão influenciar nos níveis de expressão de receptores e seus ligantes afetando a susceptibilidade à infecção pelo HIV e a progressão da doença.

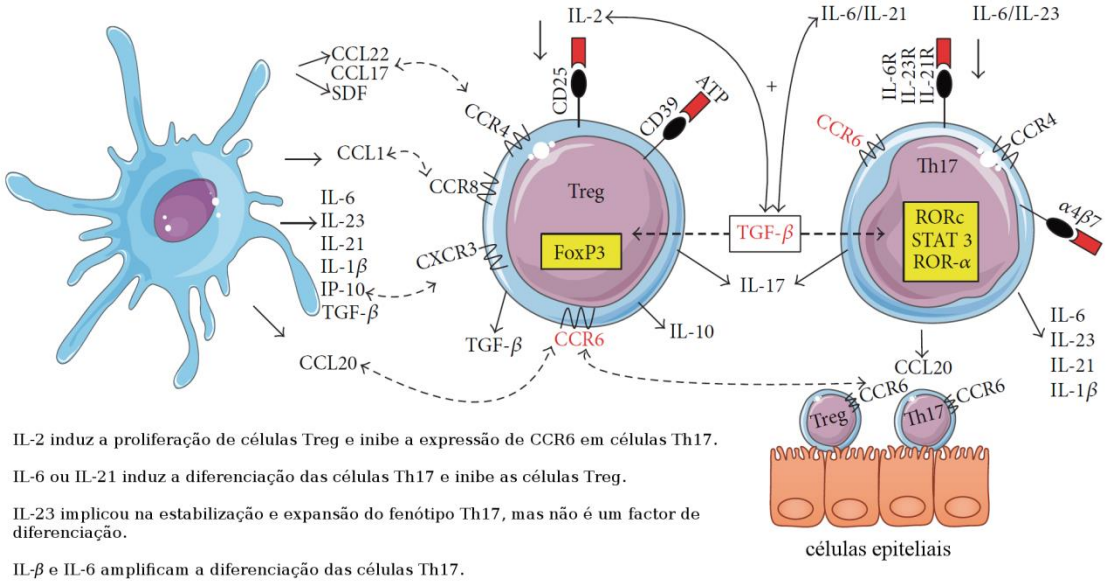


Figura 5. Migração dos linfócitos Th17 e Tregs rumo a sítios alvo de infecção pela interação de receptores de quimiocinas e quimiocinas, em colaboração com as células dendríticas (Adaptado de Valverde-Villegas JM *et al*., 2015).

1.2.4.4 Polimorfismos genéticos de receptores de quimiocinas e quimiocinas

Diversos estudos têm observado que fatores genéticos do hospedeiro têm um papel chave para predizer a susceptibilidade à infecção pelo HIV e a progressão para a aids. Em 1996, um dos primeiros polimorfismos que foi associado com proteção à infecção pelo HIV e progressão lenta à aids foi a deleção de 32pb do *CCR5* (CCR5Δ32) (Dean *et al*. 1996). Esse polimorfismo introduz um *códon de terminação* prematuro no *CCR5* gerando uma proteína truncada, a qual não é mais expressa na superfície da célula reduzindo as chances de entrada do vírus. Porém, foram reportados alguns casos de indivíduos heterozigotos ao CCR5Δ32 que não progrediram de uma forma lenta como era de esperar, salientando-se que isso ocorria pela existência de cepas virais que também utilizam o CXCR4 para entrar nas células (Dean *et al*. 1996). Todavia, foi observada uma baixa frequência do alelo Δ32 em populações afrodescendentes, onde há

uma maior incidência de casos de infecção pelo HIV. Todos esses eventos levaram os pesquisadores a buscar outras variantes genéticas que pudessem estar influenciando a susceptibilidade à infecção pelo HIV, o controle da infecção e as diferentes formas de progressão da doença. O papel de outros polimorfismos nos genes *CCR5*, *CXCR4*, *CCR2*, em genes de outros receptores e também em genes de quimiocinas foi investigado.

Assim, polimorfismos no *CCR2* (V64I e G190A), *CCR3* (Y17Y) e *CCR8* (A27G) (os quais codificam receptores que são usados como moléculas coreceptoras pelo HIV), foram associados com a progressão à aids (Smith M.W *et al*., 1997) (An *et al.* 2011). Polimorfismos em genes que codificam CCL3, CCL4 (ligantes do CCR5) e CCL18 (ligante do CCR8) foram associados à susceptibilidade à infecção pelo HIV e à progressão para a aids (Modi *et al.* 2006). Foi observado também que alguns polimorfismos no promotor do *CCL5* (RANTES) aumentam os níveis de CCL5, influenciando a progressão para a aids (McDermott *et al*. 2000). Variantes nos receptores *CXCR6* e *CX_3CR1* foram analisadas em relação à resposta à terapia e foi observada associação do alelo *CXCR6*-3K com menor supressão viral, enquanto que os alelos *CX3CR1*-280M e *CX3CR1*-249I foram associados com uma melhor resposta imunológica, em termos de uma rápida elevação do número de células T CD4+ na infecção pelo HIV (Passam *et al*. 2007). Outro estudo avaliou a influência de 3 variantes genéticas das quimiocinas *CCL2(MCP-1)*, *CCL7(MCP-3)* e *CCL11(eotaxin)*, ligantes dos receptores CCR2 e CCR3. Foi observado que esses polimorfismos, em quase completo desequilíbrio de ligação, formam um haplótipo o qual foi mais frequente em indivíduos expostos não infectados quando comparado com pacientes HIV soropositivos, sugerindo que esse haplótipo esteja associado com proteção à infecção pelo HIV (Modi *et al*. 2003). Ainda há poucos estudos avaliando o papel da diversidade genética de receptores de quimiocinas e seus ligantes, que participam potencialmente na migração, recrutamento, diferenciação e ativação celular na resposta imune frente ao HIV, e, nenhum estudo avaliando aquelas quimiocinas que potencialmente participam após o estabelecimento da infecção pelo HIV, tais como o CXCL10 e o CCL20 e seus receptores. Além disso, mais estudos sobre a variabilidade genética de quimiocinas e receptores de quimiocinas implicadas na resposta Th1 e Th2 frente ao HIV, deve nos

proporcionar mais dados para uma melhor compreensão do papel Th1:Th2 na patogênese do HIV.

Contudo, estudos de replicação em diferentes populações humanas mostram resultados diversos sugerindo que fatores étnicos devem ser considerados nas análises e que novas metodologias estatísticas avaliando interações genéticas e abordagens de biologia de sistemas devem ser consideradas para uma melhor compreensão da influência do papel genético de receptores de quimiocinas e seus ligantes em conjunto. Por outro lado, em contextos de doenças autoimunes e em câncer, o papel genético de quimiocinas e seus receptores já é objeto de grande interesse na busca de marcadores genéticos para uso de agonistas e antagonistas dessas proteínas na modulação da resposta imune (Proudfoot 2002).

2 Tratamento antirretroviral

2.1 Mudanças de políticas públicas de saúde no Brasil: O antes e o depois

Em novembro de 1996 o governo Brasileiro aprovou a Lei n° 9.313, a qual garante o acesso gratuito e universal à terapia antirretroviral altamente ativa, ou HAART (*Highly Active Antiretroviral Therapy*), que inclui a combinação de três ou mais fármacos de duas classes distintas: inibidores da protease e inibidores da transcriptase reversa, que agem em diferentes etapas da replicação do HIV. Até 1996, as recomendações baseavam-se na monoterapia inicial com zidovudina (AZT). Os antirretrovirais (ARV) eram então distribuídos a qualquer brasileiro portador do HIV e vivendo com aids mediante o Sistema Único de Saúde (SUS)(BRASIL 1996). Na época, o Programa Nacional de DST/Aids (atual Departamento de DST, Aids e Hepatites Virais) do Ministério da Saúde formou o Grupo Assessor para Terapia Antiretroviral de Adultos e Adolescentes, cuja tarefa era identificar as melhores estratégias para o tratamento contra o HIV (Ministerio da Saúde, 1997). Reavaliou-se também o momento adequado para iniciar o tratamento e a importância de exames de quantificação de linfócitos T $CD4^+$ e da carga viral como marcadores laboratoriais para determinar o grau da imunodeficiência e a progressão da doença. Em 1997 essa portaria entrou em vigor junto com o consenso sobre as recomendações para o início de ARV (Tabela 1) e a entrega dos fármacos foi acompanhada por uma rede nacional de laboratórios que fornecia os testes de quantificação de linfócitos T $CD4^+$ e, logo depois, em 1998, os testes de quantificação da carga viral. Já em 2002 alguns laboratórios do país implementaram o teste de genotipagem para mutações de resistência do vírus no caso de falha virológica. No início do tratamento foram recomendadas as combinações de dois inibidores de transcriptase reversa análogos de nucleosídeos (ITRN) combinados a um inibidor de transcriptase reversa não-análogo de nucleosídeo (ITRNN) ou a um inibidor da protease (IP) (Bartlett *et al*. 2006).

Tabela 1. Recomendações para o início da terapia de acordo com o consenso em 1997.

Situação clínica	Contagem de $CD4^+$ (células/mm^3)	Carga viral (cópias/mL)	Recomendação
Assintomático	≥ 500	1. ≥30.000 2. ≥10.000<30.000 3. <10.000 4. Carga viral não disponível	1. Tratar 2. Considerar tratamento 3. Não tratar 4. Não tratar
Assintomático	≥ 350 < 500	1. Carga viral não disponível 2. < 5000 3. ≥ 5000	1. Tratar ou monitorar 2. Tratar ou monitorar 3. Tratar
Assintomático	≥200 < 350	Independentemente da carga viral	Tratar
Assintomático	< 200	Independentemente da carga viral	Iniciar terapia e profilaxia para infecções oportunistas[a]
Sintomático	Independentemente da contagem das células $CD4^+$	Independentemente da carga viral	Iniciar terapia e profilaxia para infecções oportunistas[a]

[a] Pneumonia por *P. carinii* e toxoplasmose.

Adaptado de Ministério da Saúde, Brasil (1997).

Após a implementação destas políticas públicas de saúde, estudos abordando centros de referência de diferentes regiões do Brasil avaliaram o impacto do tratamento antirretroviral no tempo de sobrevivência dos pacientes após o diagnóstico da aids, comparando os episódios da doença antes da terapia e o impacto da distribuição universal e gratuita (Chequer *et al*. 1992; Marins *et al*. 2003). Um estudo realizado por Marins *et al*., (2003), observou um aumento significativo do tempo de sobrevivência nos pacientes adultos diagnosticados com a aids em 1995 e 1996 comparado com os diagnosticados em 1980 (início da epidemia). Ainda, os diagnosticados em 1996 apresentaram uma mediana de sobrevivência três vezes maior que os diagnosticados em 1995 e tal aumento coincidiu com a disponibilidade da terapia antirretroviral altamente ativa (Marins *et al*. 2003). Estudos subsequentes observaram também o impacto da terapia no tempo de sobrevivência dos pacientes HIV+ (Gadelha *et al*. 2002; Signorini *et al*. 2005) corroborando os achados de Marins *et al*. (2003).

Já em 2011, um interessante estudo com 1763 indivíduos de uma amostragem composta por casais sorodiscordantes, comparou a taxa de transmissão do HIV em indivíduos que iniciaram ARV após duas medidas consecutivas abaixo de 250 células T $CD4^+$ vs. aqueles que iniciaram ARV com 350-550 células T $CD4^+$ (Cohen *et al*. 2011). Os resultados desse estudo mostraram que os indivíduos que iniciaram ARV com 350-550

células T CD4+ tiveram o risco de transmissão do HIV reduzido em 96% e junto com isso os eventos clínicos da doença também foram diminuídos (Cohen *et al*. 2011). Adicionalmente Silva *et al.* (2012) mostraram evidências de que, mesmo em indivíduos assintomáticos com níveis >500 de CD4+, a replicação viral e a ativação imune crônica estão associadas ao desenvolvimento de doenças não necessariamente relacionadas à infecção pelo HIV, por exemplo, doenças cardiovasculares, tromboembólicas, câncer, entre outras (Hunt 2012). Tais evidências científicas deram suporte para o surgimento de iniciativas para mudar as políticas públicas de saúde sobre as recomendações do início/introdução de ARV. Desta forma, em 2013 novas recomendações foram dadas pelo Ministério de Saúde.

Tabela 2. Recomendações para início da terapia antirretroviral no Brasil a partir de 2013.

Todas as pessoas vivendo com HIV/Aids, independentemente da contagem de células T $CD4^+$: Estimular início imediato de ARV, na perspectiva de redução da transmissibilidade do HIV, considerando a motivação do paciente	
Sintomáticos (incluindo tuberculose ativa), independentemente da contagem de T $CD4^+$: Iniciar ARV	
Assintomáticos:	
$CD4^+ \leq 500$ células/mm^3	Iniciar ARV
$CD4^+ > 500$ células/mm^3	Iniciar ARV na coinfecção HIV-HBV com indicação de tratamento para hepatite B Considerar TARV nas seguintes situações: a) neoplasias não definidoras de aids com indicação de quimioterapia ou radioterapia b) doença cardiovascular estabelecida ou risco cardiovascular elevado (acima de 20%, segundo escore de Framingham) c) coinfecção HIV-HCV d) carga viral do HIV acima de 100.000 cópias/mL
Sem contagem de $CD4^+$ disponível	Na impossibilidade de se obter contagem de $CD4^+$, não se deve adiar o início do tratamento.
Gestantes: Iniciar ARV	

Adaptado de Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em Adultos. Ministério da Saúde, Brasília 2013.

2.2 Imunodinâmica após início do tratamento

A pesar de que já é bastante conhecido os efeitos colaterais dos ARVs assim como a geração de resistência viral, é importante relatar o impacto dos ARVs na regulação da resposta imune. Diversos estudos têm avaliado o impacto da terapia após o início do tratamento, primeiramente as abordagens investigaram a recuperação de linfócitos T $CD4^+$ e a ativação do sistema imune. Nos últimos anos tem-se dado mais atenção aos seguintes pontos: a) ao impacto do nível de linfócitos T $CD4^+$ no momento do início do tratamento, o qual influenciaria a normalização de níveis de citocinas e quimiocinas; b) à modulação de subpopulações celulares que são alvos do vírus no início da infecção; c) à ativação do sistema imune; e d) à proporção de células CD4:CD8.

Os resultados destes estudos têm observado uma persistente ativação imune e inflamação apesar do indivíduo estar com viremia suprimida pelo uso regular de ARV. Além disso, um cenário negativo é observado: pessoas sob ARV por um longo período de tempo continuam a ter maior mortalidade e morbidades crônicas, normalmente associadas ao envelhecimento do organismo, mais significativas do que a população em geral (Hunt 2012). Alguns estudos recentes têm apontado que o controle da ativação imune pode depender da fase clínica da infecção na qual é iniciado o tratamento. Assim, marcadores solúveis de ativação de monócitos permaneceram anormalmente persistentes quando se inicia ARV na fase crônica, porém, esses níveis foram normalizados quando ARV foi iniciada na fase aguda (Burdo *et al*. 2011).

Estudos já observaram que uma relação diminuída da taxa CD4:CD8, causada pela depleção das células T $CD4^+$ e a expansão dos linfócitos T $CD8^+$, foi associada com a progressão da doença (Serrano-Villar *et al*. 2014). Apesar dessa proporção CD4:CD8 ser aumentada no primeiro ano de ARV, raramente ela se normaliza com o tempo e apresenta-se ainda maior se o tratamento é iniciado tardiamente (Leung *et al*. 2013). Ademais, essa baixa proporção CD4:CD8 foi associada com a expressão de marcadores de ativação (CD38, HLA-DR), de exaustão (PD-1) e de senescência (CD28-CD57) em indivíduos em ARV com carga indetectável e níveis de $CD4^+$ >500 (Sainz *et al*. 2013). Recentemente, um estudo observou que o início de ARV na fase aguda (aproximadamente aos 40 dias da infecção) foi associado com um significante incremento na proporção CD4:CD8 quando

comparado com o início de tratamento, também na fase aguda, mas tardio (Hoenigl *et al*. 2016).

O impacto de ARV sobre a dinâmica de diferentes subpopulações celulares tais como as Th1, Th2, Th17 e Th1Th17 foi também avaliado. Observou-se que pacientes que começaram ARV com <200 células T CD4+ mantiveram o perfil Th2 predominante após um ano de tratamento. Essa observação sugeriu que, apesar da recuperação dos níveis de células T $CD4^+$ totais por ARV, não se tem uma recuperação homogênea entre as diferentes subpopulações de linfócitos T $CD4^+$ (Mahnke *et al*. 2016). Assim, essa predominância do perfil Th2 pode estar potencialmente influenciando a progressão à aids nos indivíduos que iniciam ARV com <200 células T $CD4^+$. Um estudo adicional de Jarrin *et al*., (2015) avaliou grupos de progressão à aids e observou que, entre o grupo dos progressores rápidos, a taxa de recuperação dos níveis de $CD4^+$ no início de ARV foi maior quando comparada com a taxa entre os progressores não rápidos. Porém os progressores rápidos foram menos propensos a atingir uma restauração ótima dos níveis de $CD4^+$ durante os primeiros 36 meses após iniciado ARV, quando comparados com os não-rápidos, sugerindo-se baixos níveis de células T $CD4^+$ no início do tratamento (Jarrin *et al*. 2015). Previamente a esse estudo Silva *et al.* (2000) já tinham observado que soropositivos que iniciaram ARV com <100 células T $CD4^+$ ganharam mais células T $CD4^+$ nos primeiros 48 meses de ARV quando comparados com soropositivos que iniciaram ARV com >100 células T $CD4^+$ (Lepej *et al*. 2006).

Como já descrito no capítulo 1 desta tese, as células T $CD4^+$ de GALT são altamente depletadas durante o curso da infecção, sendo a subpopulação de células T CD4+ com maior frequência na mucosa de GALT as células Th17. A perda das células Th17 na mucosa epitelial tem sido associada à translocação microbiana desde GALT para a corrente sanguínea, resultando em uma inflamação sistêmica e progressão da doença (Kim *et al*. 2013). Além disso, foi observado que uma ativação imune persiste em GALT apesar de um longo período de tratamento. Vários estudos mostraram que as Th17 são as células mais permissíveis à infecção pelo HIV e, por serem as primeiras a serem acometidas pelo HIV, são aquelas que dificilmente se recuperarão após iniciado ARV (Gosselin *et al*. 2010). Ainda, foi observado que a escassez das células precursoras comprometidas com a linhagem Th17 coincide com o déficit de polarização Th17 em

soropositivos crônicos sob HAART quando comparados com os controles soronegativos, o que explicaria porque essa subpopulação celular não se recupera em GALT em indivíduos com ARV (DaFonseca *et al*. 2015).

Em relação aos níveis de citocinas/quimiocinas após ARV, um estudo observou que várias citocinas/quimiocinas inflamatórias e marcadores de ativação no plasma foram normalizados em homens que fazem sexo com homens que se encontravam sob ARV e com carga viral suprimida quando comparados com aqueles indivíduos *naive* a ARV. Porém, quando comparados com os controles saudáveis, os níveis de 12 proteínas (CXCL10, CRP, sCD14, sTNFR2, TNF-α, sCD27, sGP130, IL-8, CCL13, BAFF, GM-CSF e IL-12p70) ainda estavam elevados. Ademais, 13 citocinas/quimiocinas apresentaram uma diminuição significativa no primeiro ano de ARV, mas ao longo do tempo essa diferença foi perdida quando comparados com os controles saudáveis (Wada *et al.* 2015). Fontaine *et al*. (2011) observaram que os níveis plasmáticos de CCL20 e CCL19 estavam acima dos níveis normais em progressores rápidos e progressores típicos na fase aguda e esses níveis permaneceram aumentados na fase crônica da infecção (Fontaine *et al.* 2011). Previamente, um estudo deste mesmo grupo, sugeriu que esses altos níveis de CCL20 e CCL19 poderiam estar contribuindo com o recrutamento das células dendríticas (DCs) e outras células para os locais de infecção, o que poderia ocasionar os baixos níveis de DCs observados no sangue periférico (Fontaine *et al.* 2009). Fontaine *et al.* (2011) também observaram níveis relativamente normais dessas quimiocinas em progressores lentos avirêmicos quando comparados com controles saudáveis, porém, os níveis nos progressores lentos virêmicos foram mais altos que os de indivíduos HIV soronegativos (Fontaine *et al.* 2011).

2.3 Caracterização da resposta imunológica após início da terapia: Uma coorte de pacientes progressores

Em 2011 teve início uma colaboração entre um grupo de pesquisadores da Universidade Federal do Rio Grande do Sul (UFRGS), a Fundação Estadual de Produção em Pesquisa e Saúde (FEPPS) e médicos do Serviço de Infectologia do Hospital Nossa Senhora da Conceição (HNSC), as três instituições localizadas em Porto Alegre, Brasil. O

HNSC é considerado um centro de referência no Brasil para o atendimento e cuidado humanizado de pacientes HIV soropositivos, além de integrar profissionais de alta qualidade atuando tanto na atenção médica quanto na pesquisa de doenças infecciosas, principalmente no campo do HIV.

Essa colaboração teve como objetivo identificar retrospectivamente pacientes com diferentes tipos de progressão extrema à aids (usando como marcadores principais a evolução das contagens de células T $CD4^+$ e prescrição do início de ARV) para a identificação de fatores genéticos do hospedeiro que pudessem estar influenciando a progressão da doença. Essa primeira etapa do projeto gerou dois trabalhos de mestrado onde foram avaliados fatores genéticos do sistema imune inato e adaptativo que tinham sido previamente associados com a progressão da doença (Matte 2012)(Medeiros 2012). Posteriormente, em 2013 retomou-se as análises e seleção dos prontuários, pois esses grupos de progressores representam uma baixa frequência na população HIV soropositiva e era necessário um incremento da amostragem visando abordagens estatísticas mais robustas. Assim, ao mesmo tempo em que fatores genéticos eram investigados, também se colocou em andamento a avaliação de fatores imunológicos, tais como a comparação do perfil de citocinas entre progressores em diferentes estágios clínicos da doença (de Medeiros *et al.* 2016). O projeto continuou em 2014, e neste ano as mudanças das políticas públicas sobre o uso de ARV já estavam em andamento (a mudança teve início no segundo semestre de 2013). Diante deste cenário, a identificação de progressores à aids (utilizando como um dos critérios o ponto de início de ARV pela queda do $CD4^+$) tornou-se mais difícil. Porém, ao mesmo tempo, essa foi uma grande oportunidade para analisar o impacto da terapia nos progressores que iniciaram ARV antes ou depois das mudanças das políticas públicas.

Cabe salientar que pesquisadores do HNSC, representados pelo Dr. Breno Riegel Santos chefe do Serviço de Infectologia, em colaboração com multicentros internacionais, realizaram um estudo para investigar o impacto da terapia na transmissão sexual em casais sorodiscordantes que começaram ARV com 350-550 células T $CD4^+/mm^3$ comparados com os que começaram ARV por apresentarem duas medias consecutivas de $CD4^+$ abaixo de 250 células/mm^3 (projeto HPTN 052). Esse estudo incluiu uma coorte de casais sorodiscordantes atendidos pelo HNSC. Os resultados demonstraram que houve uma

redução da taxa de transmissão nos casais que iniciaram ARV com 350-550 células/mm^3 quando comparados com os que iniciaram ARV com níveis de $CD4^+$ em declínio (Cohen *et al.* 2011). Assim, se reforçou o nosso interesse em avaliar o impacto da terapia comparando os grupos de progressores previamente identificados em nossos estudos. Os parâmetros utilizados para a caracterização desses grupos de progressores e regularização dos dados clínicos pode ser encontrado em detalhes na tese de doutorado de RM de Medeiros, 2016 (Medeiros 2016).

Essa abordagem incluiu 41 progressores rápidos e 26 progressores lentos que iniciaram ARV quando o declínio do $CD4^+$ foi observado ($CD4^+ < 350$) e, 14 progressores lentos que iniciaram ARV com $CD4^+ > 350$. Adicionalmente foi incluído um grupo de HIV soropositivos com progressão indeterminada, mas com acompanhamento entre 4-7 anos e em tratamento. Desses indivíduos 41 iniciaram ARV com $CD4^+ < 350$ e 11 com $CD4^+ > 350$. Para investigar as taxas de variação do $CD4^+$ após iniciado o tratamento, análises estatísticas utilizando modelos mistos para dados longitudinais (onde um mesmo individuo é medido várias vezes ao longo do tempo) foram realizadas com o programa *R,* utilizando o pacote *lme4.* Além disso, modelos mistos ajustados foram utilizados para a comparação entre os grupos de progressores rápidos e lentos. Atualizaram-se todos os dados clínicos de $CD4^+$ e carga viral antes e depois do tratamento. De acordo com a disponibilização de dados pelo sistema eletrônico do serviço de infectologia, foi possível obter informações clínicas no intervalo de tempo compreendido entre o ano de 2000 e dezembro de 2016.

Os resultados mostram que no grupo dos progressores rápidos para a aids, antes do início do tratamento a taxa de $CD4^+$ decaia 4,5 % ao mês, e que após o início de ARV o $CD4^+$ cresceu a uma taxa de 0,89% ao mês. Já nos progressores lentos à aids, que começaram o tratamento com $CD4^+<350$, o $CD4^+$ cresceu a uma taxa de 0,78% ao mês. No mesmo conjunto de observações, os progressores lentos que começaram o tratamento com $CD4^+>350$ apresentaram uma taxa de aumento do $CD4^+$ de 0,66% ao mês. Pacientes soropositivos com progressão indeterminada que começaram o tratamento com $CD4^+ <350$, têm taxa de variação mensal do $CD4^+$ aumentando 0,85% ao mês, enquanto que os soropositivos indeterminados que começaram o tratamento com >350 de $CD4^+$, tiveram uma taxa de aumento de $CD4^+$ de 0,34% ao mês.

Todavia, observou-se que, considerando o mesmo tempo de acompanhamento pós-ARV a média do $CD4^+$ nos progressores lentos foi 41,75% maior do que os progressores rápidos, enquanto que progressores indefinidos apresentaram uma taxa média de 35,82% maior do que os progressores rápidos.

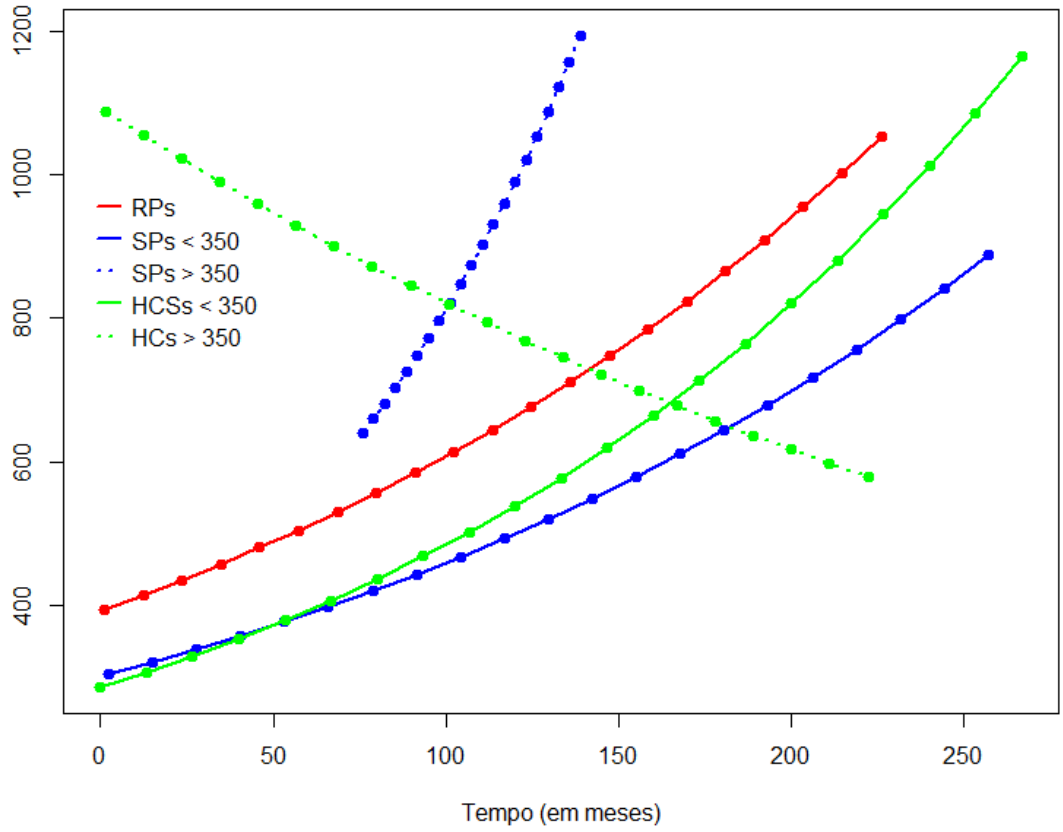


Figura 1. Predição da taxa de recuperação de células T $CD4^+$ após início de ARV, comparando os progressores rápidos (RPs) que iniciaram ARV com <350 $CD4^+$, progressores lentos (SPs) que iniciaram com <ou>350 $CD4^+$ e os HIV soropositivos com progressão indeterminada (HCSs) que iniciaram com <ou>350 $CD4^+$.

Ao mesmo tempo em que se deu continuidade ao projeto avaliando material pré-existente, novas coletas foram realizadas para darmos andamento à avaliação de fatores imunológicos e genéticos. Entre os anos de 2015 e 2016, 26 indivíduos foram coletados, a maioria deles previamente identificados por apresentarem progressão conhecida. No fim, na tentativa de homogeneizar os grupos levando em consideração a progressão, tempo

sobre o tratamento, e níveis de CD4 prévios ao início de ARV, nesta última parte da tese apresentaremos as análises de subpopulações celulares Th1, Th2, Th17, Th1Th17 e frequências de células ativadas comparando 7 progressores lentos, os quais iniciaram ARV com >350 $CD4^+$, 5 progressores rápidos que iniciaram ARV com <350 células T $CD4^+$ e 10 indivíduos controles saudáveis. Os resultados desta parte da tese são apresentados na forma de um artigo em preparação.

3 Justificativa

A literatura atualizada sobre a infecção pelo HIV e a progressão para a aids nos mostram que a fase inicial da infecção é chave para o decorrer da progressão da doença. E que as diferentes respostas dos indivíduos nessa fase inicial frente ao HIV vão depender de fatores imunológicos implicados principalmente nesta fase. Porem, os fatores genéticos do hospedeiro podem influenciar sobre esses fatores imunológicos modificando diferenciadamente essa resposta inicial. Por tanto, os estudos envolvendo indivíduos HIV+ que progridem de uma forma extrema (rápida ou lenta) são os que podem nos trazer mais informações sobre o papel de fatores imunológicos influenciados pela diversidade genética. Sem deixar do lado, os fatores imunológicos e genéticos que influenciam na susceptibilidade à infecção continuam a ser de grande interesse desde que uma melhor compreensão dos fatores que participam na relação vírus-hospedeiro antes do estabelecimento da infecção, é necessária. Assim, a investigação do papel imunológico e genético de fatores que conectam o sistema imune inato e adaptativo, como quimiocinas e receptores de quimiocinas, merece uma maior atenção desde que várias dessas moléculas estão implicadas em um primeiro momento da infecção, modulando positivamente a infecção (inibindo a entrada do vírus) ou negativamente, disseminando o vírus através do recrutamento de células alvos e sua migração celular a novos focos de infecção. Ademais, a investigação da modulação de tais fatores em diferentes estágios clínicos da doença, antes e depois de iniciado o tratamento antirretroviral, pode nos trazer mais informações sobre seu papel regulador/desregulador do sistema imune e consequentemente as propostas de uso como biomarcadores para o monitoramento clinico de indivíduos HIV+ que geralmente chegam ao hospital na fase crônica da infecção.

4 Objetivos

4.1 Geral

Investigar o papel de polimorfismos em genes de quimiocinas e receptores de quimiocinas candidatos em influenciar na susceptibilidade à infecção pelo HIV e na progressão à aids. Avaliar o papel imunológico de quimiocinas em diferentes estágios clínicos da doença e o papel de ARV na modulação de fatores imunológicos em progressores extremos.

4.2 Específicos

- Diante da complexidade da família de receptores de quimiocinas e seus ligantes, realizar uma busca na literatura sobre a diversidade genética destas proteínas para a seleção de 14 polimorfismos com potencial influencia não só no contexto do HIV, mas também em outros contextos que acometem o sistema imunológico;

- Padronizar a metodologia para a genotipagem de 14 polimorfismos, a ser analisados em conjunto, mediante a técnica molecular de minisequenciamento;

- Analisar a influência desses polimorfismos na susceptibilidade à infecção pelo HIV e na progressão à aids (fazendo uma abordagem comparativa entre progressores rápidos e não rápidos à aids);

- Realizar análises estatísticas com uma abordagem que nos traga mais informação sobre o papel da interação desses polimorfismos associados ao desfecho;

- Quantificar os níveis plasmáticos de quimiocinas selecionadas pela sua modulação dos perfis Th1, Th2, Th17 e Tregs, nos diferentes estágios clínicos da doença em progressores extremos;

- Investigar o impacto da terapia sobre a recuperação de linfócitos T $CD4^+$ comparando indivíduos que iniciaram ARV com mais ou menos de 350 células T $CD4^+/mm^3$;

- Investigar o impacto da terapia sobre subpopulações celulares e ativação imune em progressores extremos sob ARV.

Capítulo 2

Immunogenetic profiling of 23 SNPs of cytokine and chemokine receptor genes trough minisequencing technique: Design, development and validation

J. M. Valverde-Villegas, R. M. de Medeiros, S. E. M. Almeida and J. A. B. Chies

Artigo aceito para publicação em março de 2017 para a revista International Journal of Immunogenetics.

"O objetivo deste artigo foi descrever o desenho e a padronização da genotipagem de 14 polimorfismos em genes que codificam receptores de quimiocinas e seus ligantes (avaliados nesta tese) e 9 polimorfismos em genes de citocinas (avaliados em outra tese) pela técnica de minisequenciamento, e disponibilizar a metodologia para sua aplicação em outros contextos de doenças imunológicas."

Immunogenetic profiling of 23 SNPs of cytokine and chemokine receptor genes through a minisequencing technique: Design, development and validation

J. M. Valverde-Villegas[2*], R. M. de Medeiros[1,2*], , S. E. M. Almeida[1,2] and J. A. B. Chies[2]

[1] Technological and Scientific Development Center - CDCT, State Foundation in Production and Health Research - FEPPS, Rio Grande do Sul, Porto Alegre, Brazil.

[2] Post Graduation Program in Genetic and Molecular Biology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

*These authors contributed equally to this study.

Corresponding author

José Artur Bogo Chies. Email address: jabchies@terra.com.br

Laboratory of Immunogenetics. Institute of Biosciences, Departament of Genetics, UFRGS.

Av. Bento Gonçalves – 9500, Campus do Vale. 91501970

Porto Alegre, RS-Brazil. PO BOX 15053

Phone: +55 51 3308 6740; Fax: +55 51 3308 7311

Abstract

The minisequencing technique offers accuracy and robustness to genotyping of polymorphic DNA variants, being an excellent option for the identification and analyses of prognostic/susceptibility markers in human diseases. Two multiplex minisequencing assays were designed and standardized to screen 23 candidate SNPs in cytokine, chemokine receptor and ligand genes previously associated with susceptibility to cancer and autoimmune disorders as well as to infectious diseases outcome. The SNPs were displayed in two separate panels (panel 1 - *IL2* rs2069762, *TNFα* rs1800629, rs361525; *IL4* rs2243250; *IL6* rs1800795; *IL10* rs1800896, rs1800872; *IL17A* rs8193036, rs2275913; and panel 2 - *CCR3 rs309125, CCR4* rs6770096, rs2228428; *CCR6* rs968334; *CCR8* rs2853699; *CXCR3* rs34334103, rs2280964*;CXCR6* rs223435, rs2234358; *CCL20* rs13034664, rs6749704; *CCL22* rs4359426; *CXCL10/IP-10* rs3921, rs56061981). A total of 305 DNA samples from healthy individuals were genotyped by minisequencing. To validate the minisequencing technique, and in order to encompass the majority of the potential genotypes for all 23 SNPs, 20 of these samples were genotyped by Sanger sequencing. The results of both techniques were 100% in agreement. The technique of minisequencing showed high accuracy and robustness, avoiding the need for high quantities of DNA template samples. It was easily to be conducted in bulk samples derived from a highly admixed human population, being therefore an excellent option for immunogenetic studies.

Key words: SNPs, cytokine, chemokine, minisequencing, susceptibility markers.

Running head: Immunogenetic profiling through minisequencing

Introduction

The immune system acts in a complex network, involving molecular pathways and cellular components dispersed throughout the body. Health and disease outcomes depend on a delicate balance between inhibition and activation, where deficient or uncontrolled responses may result in tissue damage. This complexity requires a fine tuned communication network and cytokines are major signalling molecules in the immune system (Murphy et al., 2010). Among the main cytokine subfamilies, interleukins are importantly involved in lymphocyte signalling. Specific interleukins play defined roles in pro- and anti-inflammatory responses (Zhang and An, 2007). Also important to the homeostasis of the immune system, chemokines, small proteins belonging to the chemotactic cytokines subfamily, have pivotal roles for homing and trafficking of immune cell subsets into inflammatory sites (Sallusto et al., 2004).

The importance of genetic polymorphisms in immune-related genes, such as those coding for cytokines and chemokines, as well as chemokine receptor genes, to cancer, autoimmune and infectious diseases susceptibility is an important research topic and has already been approached in different studies (see Table 1 for a comprehensive, although not exhaustive, review). Thereby, methodologies to rapidly and accurately genotype candidate single nucleotide polymorphisms (SNPs) are avidly sought to investigate and identify prognostic/diagnostic markers in immunological diseases (Syvänen, 2001). Of special interest are methodologies able to analyse sets of genes involved in specific biochemical pathways or genetic networks. Such approaches would facilitate gene-gene interaction studies but, although different methodologies are presently available for genotyping, no technology for scoring SNPs has become a widely accepted standard (Vallone et al., 2004).

The minisequencing method is based in the use of a probe that targets a sequence immediately upstream of the SNP and which is extended by a single base by incorporation of a fluorescently labelled dideoxy nucleotide. The reactions are resolved by capillary electrophoresis and genotypes are determined by the identification of peak position and specific fluorescence emission. Minisequencing analyses give accurate and robust results and require relatively little time in comparison to other genotyping methods (Pati et al., 2004). Here we designed and standardized two multiplex assays panels for minisequencing genotyping of 23 SNPs in immune-related genes. The SNPs were displayed in two separate panels: panel 1 focuses on cytokine genes (*IL2* rs2069762; *TNFα* rs1800629, rs361525; *IL4* rs2243250; *IL6* rs1800795; *IL10* rs1800896, rs1800872; *IL17A* rs8193036, rs2275913) and panel 2 focuses on chemokine receptor/ligands genes (*CCR3 rs3091250; CCR4* rs6770096, rs2228428; *CCR6* rs968334; *CCR8* rs2853699; *CXCR3* rs34334103, rs2280964*; CXCR6* rs2234355, rs2234358; *CCL20* rs13034664, rs6749704; *CCL22* rs4359426, *CXCL10/IP-10* rs3921, rs56061981). Both panels were validated through the genotyping of a cohort of 305 healthy individuals. Of note, all evaluated SNPs were selected among candidate genes/polymorphisms previously described as involved in cancer, autoimmunity and/or infectious disease susceptibility and outcome, therefore composing a valuable tool for the evaluation of important potential immunogenetic targets.

Material and Methods

SNPs selection

The SNPs in the immune related genes were selected, as previously stated, among genes/polymorphisms previously described as involved in susceptibility or outcome of autoimmune diseases, cancer, allergy and/or infectious diseases (Table 1). In addition,

criteria for inclusion into the panels considered: (a) potential relevance of the SNP for differences on the rate of gene expression and (b) frequency of the rare allele > 5% in European- and African-descendent populations available in the Entrez SNP Database (www.ncbi.nlm.nih.gov/sites/entrezdb=snp).

Studied population

Samples were obtained from a DNA biorepository maintained at the Immunogenetics laboratory of the Federal University of Rio Grande do Sul, Brazil. The group was composed of 305 healthy individuals older than 18 years from Porto Alegre city, 222 (70%) men and 78 (30%) women. Individuals were classified as European or African ancestry according to the individual phenotypic characteristics self-assertion: 176 (58%) were European descent and 128 (42%) were African descent. This study was conducted in compliance with the principles included in the Declaration of Helsinki, all subjects contributing to the biorepository signed an informed consent to participate in this study.

Multiplex PCR-primers and minisequencing probes design

The 23 SNPs selected were combined in two panels. Panel 1 included the SNPs in interleukin and tumor necrosis factor alpha (TNF-α) genes whilst panel 2 included those for chemokines and their receptors (Table 2). Multiplex PCR-primers were designed for each panel using the Primer 3.0 program v.0.2 (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) considering two main criteria: a) similar melting temperature among primers; b) all amplicons should range from 150 to 450 bp in order to

facilitate visualization of the multiplex reactions simultaneously to minimize the risk of preferential amplification due to reagent competition.

Minisequencing probes for SNP detection were designed with the 3' end base corresponding to the last base before the SNP-position (Table 3). For panel 2, the probes were designed using BatchPrimer3 v1.0 software online adding poly (dCT) 5' tails (http://probes.pw.usda.gov/batchprimer3/). Self- and hetero-dimers annealing between pairs of primers was evaluated using the Multiple Primer Analyzer software tool from Thermo Fisher Scientific (https://www.thermofisher.com/br/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html). This tool was applied for both primer sets (multiplex PCR and minisequencing probes). For minisequencing oligoultramer probes, the recommended scale of synthesis is of 4 nmol. All primers were synthetized with purification standards and checked by mass spectrometry.

Multiplex PCR

The panel 1 and panel 2 multiplex PCR were performed separately in 25µL reaction volume with 20ng genomic DNA. In addition, 0.2mM of each primer was used according to the of Qiagen Multiplex PCR Master Mix Kit manufacturer's instructions. All reactions were performed in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems) with the following settings: pre-denaturation 95°C for 5 min followed by 35 cycles of 30s at 94°C, 90s at 57°C, 90s at 72°C, and a final extension cycle of 10 min at 72°C. Multiplex PCR products were checked in a 3% agarose gel.

In order to remove unincorporated primers and dNTPs, 2 µl of Affymetrix™ ExoSAP-IT™ was added in 5µl multiplex PCRs product (for both panels) and reactions were incubated at 37°C for 30 min. The ExoSAP-IT enzyme was inactivated by incubation at 80°C for 30 min.

Minisequencing

Minisequencing reactions were performed in a 10µL final volume using 5µL of SNaPshot™ Kit Reaction Mix, 3µL of the purified multiplex PCR product and 1.5µL of minisequencing probe mix (0.05µM of each primer, Table 3). Reactions were performed in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems) with the following settings: initialization at 95°C for 5min, followed by 25 cycles of 10s at 96°C, 5s at 50°C and 30s at 60°C.Then, 1.5 µl of Affimetrix SAP® enzyme was added, and the reaction was incubated at 37°C for 30 min to remove the 5' phosphoryl groups of the unincorporated fluorescent ddNTPs. SAP® enzyme was inactivated by incubation at 80°C for 30 min.

Capillary electrophoresis

For capillary electrophoresis 1 µL of minisequencing products purified mixed with 8.5µl of HiDi™ formamide and 0.5 µl of GeneScan-120LIZ size standard (ABI - Foster City, USA) was denatured at 95°C for 5 min. Capillary electrophoresis was performed on an ABI 3130xl genetic analyzer (ABI - Foster City, USA) with a 36 cm length capillary and POP-4™ polymer. Data analyses were performed with SNaPshot™ tool in GeneMapper 4.0 software (ABI - Foster City, USA) (see Figure 1A and B for an example).

Sanger Sequencing

To validate the minisequencing results, 20 samples were sequenced for the 23 SNPs by Sanger sequencing. These were selected amongst the previously minisequenced samples, in order to encompass the majority of the potential genotypes for all 23 SNPs evaluated. The amplified fragments were obtained by single PCR using Qiagen Multiplex PCR Master Mix Kit under the same minisequencing conditions described above. The Sanger sequencing reactions were performed using 20-30ng purified single PCR product, primers (both forward and reverse for each SNP in separated reactions) and ABI Big Dye Terminator v.3.1 cycle sequencing ready reaction kit (Applied Biosystems, Foster City, California, USA) following the manufacturer's instructions. The amplification cycling profile was performed in a Veriti 96-well Thermal Cycler (Applied Biosystems) using 35 cycles of 10 sec at 96°C, 10 sec at 54°C; 4 min at 60°C. All reactions were run in an ABI 3130xl genetic analyzer (Applied Biosystems).

Results

Validation of the minisequencing approach

Figure 1 shows representative electropherograms obtained from both panels for a control individual. Figure 1A concerns panel 1 (9 different cytokine gene SNPs) and Figure 1B concerns Panel 2 (14 different chemokine receptor or ligand gene SNPs). All SNPs are indicated by their rs nomenclature and genotyping is achieved by reading the peaks according to the size and fluorescent emission defined by each specific probe. As previously stated, Sanger sequencing was performed in 20 samples to encompass the majority of the potential genotypes for all 23 SNPs in order to validate the minisequencing technique. All genotypes obtained by the Sanger sequencing method (considering all the

23 tested SNPs for the 20 individual samples) mirrored the results from the minisequencing (data not show).

Immunogenetic profiling: allelic and genotype frequencies

Tables 4 and 5 summarize the allelic and genotypic frequencies obtained for the 305 individuals considering all 23 evaluated SNPs. In order to compare the results with a so-called standard human population, allelic and genotype frequencies for the same SNPs were extracted from the HapMap database, considering the 1000 Genomes Project Phase 3 for all populations, and are also presented in Tables 4 and 5. As expected, taking into account that our control group represents an admixed human population, the results as a whole are quite similar.

Discussion

In the development of a methodology aiming to evaluate an immunogenetical profile defined by 23 SNPs located in immune related genes, several points had to be addressed. The quality and quantity of amplified product obtained after the multiplex PCR is very important for good performance in minisequencing genotyping. Therefore, the multiplex PCR was standardized using a commercial master mix kit according to the manufacturer's instructions and all amplicons were obtained with sufficient quality and quantity for the following steps. Importantly, the multiplex product purification step can significantly affect genotyping accuracy. Failure to remove the unincorporated ddNTPs can yield extraneous fluorescence, and therefore special attention should be given to this step. It is important to mention that it is possible to use the same primary amplicon for the

annealing of two different probes in the subsequent minisequencing reaction, consequently genotyping different SNPs on the same gene. This approach was used for the identification of both rs1800629 and rs361525 of the *TNF-α* gene in Panel 1 and for the identification of rs34334103 and rs2280964 of the *CXCR3* gene in Panel 2. However, special attention should be given if these SNPs are very close to each other, due to proximity interference of the fluorescent probes, the signal intensity in the electropherogram could be reduced.

Two different principles were used to design the probes for the different panels used in this minisequencing approach. The panel 1 probes were designed with different lengths and their whole sequence encompassing and pairing to the region adjacent to the target SNP. For panel 2, only ~20 bp of the region adjacent to the SNP was selected to be incorporated in the probe and CT tails with different sizes were added. An increase in the size of the probe results in better efficiency, although simultaneously can favor nonspecifically annealing. The addition of tails can bypass this paradoxical situation. In our panels both principles to design probes worked equally well and we strongly suggest a close investigation of the regions to be genotyped to choose the appropriate criteria to design new probes.

In electropherograms, the electrophoretic migration of the shorter probes assigned by the automated sequencer were slightly different from those expected considering only the size of the synthesized fragments. Quintáns B et al., 2004 also observed this discrepancy between the expected and the observed distance of migration concluding that the length, sequence, and the dye used to label the extended primer could interfere with its electrophoretic mobility (Quintáns et al., 2004). Thus, this should be taken into consideration in the design of probes, avoiding the use of very small fragments or fragments able to fold in complex structures. When the electropherograms were analyzed,

a residual signal accumulation that does not match any SNP at the region becomes apparent, peaking around region corresponding to fragments of 40 to 45 bp in size (see Figure 1). Based on our experience, we recommend to avoid the use of probes that will fall in this region.

In the present study two separated panels were designed allowing the independent genotyping of two different groups of SNPs. In this sense, researchers can choose the most convenient panel for their specific needs. Also, other SNP targets can be added to enrich the panels (Quintáns et al., 2004; Coutinho et al., 2014; Fanis et al., 2014). Alternatively, the products of the two multiplex panels could be combined and minisequenced in a single reaction. However, in this case, some data would be lost: the allele dyes and bin positions of *CCR8* rs2853699 and *IL10* rs1800872 as well as *CCL20* rs13034664 and *IL17A* rs8193036 are the same. For this reason, it would be necessary or eliminate one marker, or make a new choice of fluorescent dyes.

In order to validate the minisequencing results 20 DNA samples from healthy individuals which included the majority genotype combinations for all 23 SNPs were sequenced by Sanger sequencing. The results were always in agreement with the minisequencing. Regarding the immunogenetic profiling of the 305 healthy individuals, the observed allelic frequencies were quite similar to those extracted from the HapMap database. Some differences can be identified in the comparison of those allelic frequencies, although this should be expected, since admixed populations are being evaluated.

In conclusion, the technique of minisequencing showed high accuracy and robustness, avoiding the need for large quantities of DNA template samples. It was easily conducted in bulk samples derived from a highly admixed human population, being therefore an excellent option for immunogenetic studies. Minisequencing allows the analyses of various

SNPs with high specificity at the same reaction conditions. Another advantage of this method is the simultaneous genotyping of several SNPs using small quantities of template DNA (approximately 20ng). Thus, minisequencing is a promising approach for multiplex high-throughput genotyping assays.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We thank to Karine Pereira de Andrade and Paula da Rosa Bettin graduate students for the supporting during the minisequencing technique standardization. This work was supported by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (Grant 2253-2551/14-2) and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

References

An P, Li R, Wang JM, Yoshimura T, Takahashi M, Samudralal R, et al. (2011). Role of Exonic Variation in Chemokine Receptor Genes on AIDS: CCRL2 F167Y Association with Pneumocystis Pneumonia. *PLoS Genet*, 7, e1002328.

Aoki T, Hirota T, Tamari M, Ichikawa K, Takeda K, Arinami T, et al. (2006). An association between asthma and TNF-308G/A polymorphism: meta-analysis. *J. Hum. Genet*, 51: 677–85.

Breunis WB, Biezeveld HM, Geissler J, Kuipers IM, Lam J, Ottenkamp J, et al. (2007). Polymorphisms in chemokine receptor genes and susceptibility to Kawasaki disease *Clin. Exp. Immunol*, 150: 83–90.

Cheong HS, Park CS, Kim LH, Park BL, Uh ST, Kim HY, et al. (2005). CXCR3 polymorphisms associated with risk of asthma, Biochem. *Biophys. Res. Commun*, 344: 1219–1225.

Choi S, Lee EK, Lee S, Chae S, Lee M, Seo G, et al. (2005). Ulcerative Colitis is Associated with Novel Polymorphisms in the Promoter Region of MIP-3α/CCL20 Gene. *Immune Netw*, 5: 205.

Coutinho A, Valverde G, Fehren-schmitz L, Cooper A, Romero B, Espinoza IF, et al. (2014).

AmericaPlex26: A SNaPshot Multiplex System for Genotyping the Main Human Mitochondrial Founder Lineages of the Americas. *PloS One*, 9: 16–21.

Duggal P, An P, Beaty TH, Strathdee S, Farzadegan H, Markham RB, et al. (2003). Genetic influence of CXCR6 chemokine receptor alleles on PCP-mediated AIDS progression among African Americans. *Genes Immun*, 4: 245–50.

Fanis P, Kousiappa I, Phylactides M, Kleanthous M. (2014). Genotyping of BCL11A and HBS1L-MYB SNPs associated with fetal haemoglobin levels: a SNaPshot minisequencing approach. *BMC Genomics*, 15: 1–12.

Fedetz M, Ndagire D, Fernandez O, Leyva L, Guerrero M, Arnal C, et al. (2009). Multiple sclerosis association study with the TENR-IL2-IL21 region in a Spanish population. *Tissue Antigens*, 74, 244–247.

Ferreira AC, Almeida S, Tavares M, Canedo P, Pereira F, Regalo G, et al. (2005). NOD2/CARD15 and TNFA, but not IL1B and IL1RN, are associated with Crohn's disease. *Inflamm. Bowel Dis*, 11: 331–9.

Foster CB, Lehrnbecher T, Samuels S, Stein S, Mol F, Metcalf JA, et al. (2000). An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. *Blood*, 96: 2562–7.

Hunninghake GM, Soto-Quirós ME, Lasky-Su J, Avila L, Ly NP, Liang C, et al. (2008). Dust mite exposure modifies the effect of functional IL10 polymorphisms on allergy and asthma exacerbations. *J. Allergy Clin. Immunol*, 122: 93–98.e5.

Hohaus S, Giachelia M, Di Febo A, Martini M, Massini G, Vannata B, et al. (2007). Polymorphism in cytokine genes as prognostic markers in Hodgkin's lymphoma. *Ann. Oncol*, 18: 1376–81.

Hirota T, Saeki H, Tomita K, Tanaka S, Ebe K, Sakashita M, et al. (2011). Variants of C-C Motif Chemokine 22 (CCL22) Are Associated with Susceptibility to Atopic Dermatitis: Case-Control Studies. *PLoSOne*, 6: e26987.

Im CH, Park JA, Kim JY, Lee EY, Lee EB, Kim Y, et al. (2014). CXCR3 polymorphism is associated with male gender and pleuritis in patients with lupus erythematosus. *Hum. Immunol*, 75: 466–469.

Kube D, Hua TD, vonBonin F, Schoof N, Zeynalova S, Kloss M, et al. (2008). Effect of Interleukin-10 Gene Polymorphisms on Clinical Outcome of Patients with Aggressive Non-Hodgkin's Lymphoma: An Exploratory Study. *Clin. Cancer Res*, 14: 3777–3784.

Kochi Y, Okada Y, Suzuki A, Ikari K, Terao C, Takahashi A, et al. (2010). A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. *Nat. Genet*, 42: 515–519.

Koumakis E, Bouaziz M, Dieudé P, Ruiz B, Riemekasten G, Airo P, et al. (2013). Brief Report: A Regulatory Variant in CCR6 Is Associated With Susceptibility to Antitopoisomerase-

Positive Systemic Sclerosis. *Arthritis Rheum*, 65: 3202–3208.

Li N, Zhu Q, Li Z, Han Q, Zhang G, Chen J, et al. (2014). IL17A Gene Polymorphisms, Serum IL-17A and IgE Levels, and Hepatocellular Carcinoma Risk in Patients With Chronic Hepatitis B Virus Infection. *Mol Carcinog*, 457: 447–457.

Limou S, Coulonges C, Herbeck JT, vanManen D, An P, LeClerc S, et al. (2010). Multiple-cohort genetic association study reveals CXCR6 as a new chemokine receptor involved in long-term nonprogression to AIDS. *J. Infect. Dis*, 202: 908–15.

Murphy, K., Travers, P., Mark, W., 2010. Imunobiologia de Janeway, 7 ed. ed. Artmed, São Paulo.

Nasi M, Riva A, Borghi V, D'Amico R, Del Giovane C, Casoli C, et al. (2013). Novel genetic association of TNF-α-238 and PDCD1-7209 polymorphisms with long-term non-progressive HIV-1 infection. *Int. J. Infect. Dis*, 17: e845–e850.

Nuñez C, Santiago JL, Varadé J, Calle H, Figueredo MA, Fernandez-Gutierrez B, et al. (2008). IL4 in the 5q31 context: association studies of type 1 diabetes and rheumatoid arthritis in the Spanish population. *Immunogenetics*, 60: 19–23.

Pati N, Schowinsky V, Kokanovic O, Magnuson V, Ghosh S. (2004). A comparison between SNaPshot, pyrosequencing and biplex invader SNP genotyping methods: accuracy, cost and throughput. *J Biochem Bioph Meth*, 60: 1–12.

Quintáns B, Álvarez-Iglesias V, Salas A, Phillips C, Lareu M, Carracedo A. (2004). Typing of mitochondrial DNA coding region SNPs of forensic and anthropological interest using SNaPshot minisequencing. *Forensic Sci. Int*, 140: 251–257.

Rajeevan MS, Dimulescu I, Murray J, Falkenberg VR, Unger ER. (2015). Pathway-focused genetic evaluation of immune and inflammation related genes with chronic fatigue syndrome. *Hum. Immunol*, 76: 553–60.

Sallusto F, Geginat J, Lanzavecchia A. (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu. Rev. Immunol*, 22: 745–763.

Sharma NK, Gupta A, Prabhakar S, Singh R, Bhatt AK, Anand A. (2013). CC chemokine receptor-3 as new target for age-related macular degeneration. *Gene*, 523: 106–111.

Shibata T, Tahara T, Hirata I, Arisawa T. (2009). Genetic polymorphism of interleukin-17A and -17F genes in gastric carcinogenesis. *Hum. Immunol*, 70: 547–551.

Shin HD, Winkler C, Stephens JC, Bream J, Young H, Goedert JJ, et al. (2000). Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10. *Proc. Natl. Acad. Sci*, 97: 14467–14472.

Sheikh G, Neela VSK, Pydi SS, Suryadevara NC, Gaddam R, Gaddam SL, et al. (2015). Genetic Association of Interferon Gamma Induced Protein-10 (IP-10), CXCL-10 Gene Polymorphisms with TB Pleurisy Susceptibility in South Indian Population. *Open J.*

348 *Immunol*, 05: 72–78.
349 Shen L, Zhang H, Yan T, Zhou G, Liu R. (2015). Association between interleukin 17A
350 polymorphisms and susceptibility to rheumatoid arthritis in a Chinese population. *Gene*,
351 566: 18–22.
352 Syvänen A. (2001). Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nat.*
353 *Rev. Genet*, 2: 930–942.
354 Tang NLS, Fan HPY, Chang KC, Ching JKL, Kong KPS, Yew WW, et al. (2009). Genetic
355 association between a chemokine gene CXCL-10 (IP-10, interferon gamma inducible
356 protein 10) and susceptibility to tuberculosis. *Clin. Chim. Acta*, 406: 98–102.
357 Vallone PM, Just RS, Coble MD, Butler JM, Parsons TJ. (2004). A multiplex allele-specific primer
358 extension assay for forensically informative SNPs distributed throughout the mitochondrial
359 genome. *Int J Legal Med*, 147–157. Coutinho, A., Valverde, G., Fehren-Schmitz, L., Cooper,
360 A., Barreto Romero, M.I., Flores Espinoza, I., Llamas, B., Haak, W., 2014. AmericaPlex26:
361 A snapshot multiplex system for genotyping the main human mitochondrial founder lineages
362 of the Americas. PLoS One 9, 16–21. doi:10.1371/journal.pone.0093292
363 Fanis, P., Kousiappa, I., Phylactides, M., Kleanthous, M., 2014. Genotyping of BCL11A and
364 HBS1L-MYB SNPs associated with fetal haemoglobin levels: a SNaPshot minisequencing
365 approach. BMC Genomics 15, 108. doi:10.1186/1471-2164-15-108
366 Murphy, K., Travers, P., Mark, W., 2010. Imunobiologia de Janeway, 7 ed. ed. Artmed, São Paulo.
367 Pati, N., Schowinsky, V., Kokanovic, O., Magnuson, V., Ghosh, S., 2004. A comparison between
368 SNaPshot, pyrosequencing, and biplex invader SNP genotyping methods: accuracy, cost, and
369 throughput. J. Biochem. Biophys. Methods 60, 1–12. doi:10.1016/j.jbbm.2003.11.005
370 Quintáns, B., Álvarez-Iglesias, V., Salas, A., Phillips, C., Lareu, M.., Carracedo, A., 2004. Typing
371 of mitochondrial DNA coding region SNPs of forensic and anthropological interest using
372 SNaPshot minisequencing. Forensic Sci. Int. 140, 251–257.
373 doi:10.1016/j.forsciint.2003.12.005
374 Sallusto, F., Geginat, J., Lanzavecchia, A., 2004. Central memory and effector memory T cell
375 subsets: function, generation, and maintenance. Annu. Rev. Immunol. 22, 745–763.
376 doi:10.1146/annurev.immunol.22.012703.104702
377 Syvänen, A., 2001. Accessing genetic variation: genotyping single nucleotide polymorphisms. Nat.
378 Rev. Genet. 2, 930–942.
379 Vallone, P.M., Just, R.S., Coble, M.D., Butler, J.M., Parsons, T.J., 2004. A multiplex allele-
380 specific primer extension assay for forensically informative SNPs distributed throughout the
381 mitochondrial genome. Int. J. Legal Med. 118, 147–157. doi:10.1007/s00414-004-0428-5
382 Zhang, J.-M., An, J., 2007. Cytokines, Inflammation and Pain. Int Anesth. Clin. 45, 27–37.

383 doi:10.1097/AIA.0b013e318034194e.
384 Zhang JM and An J. (2007). Cytokines, inflammation and Pain. *Int Anesth. Clin*, 45: 27–37.
385
386

Table 1. SNPs selected according associations in different disease contexts.

Gene	Chromosomo	SNPs	rs	Ancestral allele	MAF	Associations
Panel 1						
IL2	4	-330 G/T	rs2069762	G	0.27 (T)	Cancer, Multiple sclerosis (Wu et al., 2009; Fedetz et al., 2009)
TNFα	6	-308 G/A	rs1800629	G	0.09 (A)	Asthma, Crohn's disease, Liver cancer, Systemic lupus erythematosus
	6	-238 G/A	rs361525	G	0.06 (A)	Psoriasis, Lymphoma (Ferreira et al., 2005; Nasi et al., 2013; Aoki et al., 2006)
IL4	5	-590 C/T	rs2243250	C	0.46 (T)	Type 1 diabetes, Rheumatoid arthritis (Nuñez et al. 2008)
IL6	7	-174 C/G	rs1800795	G	0.13 (C)	Kaposi's sarcoma, Hodgkin's lymphoma, Crohn's disease (Foster et al., 2000; Hohaus et al., 2007)
IL10	1	-1082 A/G	rs1800896	A	0.27 (G)	Allergy and asthma exacerbations
	1	-598 C/A	rs1800872	C	0.43 (A)	Lymphoma, Atherosclerosis (Shin et al., 2000; Hunninghake et al., 2008; Kube et al., 2008)
IL17A	6	-737 T/C	rs8193036	T	0.38 (C)	Chronic hepatitis B, Rheumatoid arthritis,
	6	-197 G/A	rs2275913	A	0.30 (A)	Tuberculosis susceptibility, Rheumatoid arthritis, Gastric cancer (Li et al., 2014; Shibata et al., 2009; Shen et al., 2015)
Panel 2						
CCR3	3	-520 G/T	rs3091250	G	0.35 (T)	Kawasaki and macular degeneration

						diseases (Breunis et al., 2007; Sharma et al., 2013)
CCR4	3	1014 C/T	rs2228428	C	0.15 (T)	Chronic fatigue syndrome (Rajeevan et al. 2015)
	3	512 C/T	rs6770096	C	0.19 (T)	
CCR6	6	-98+594 A>G	rs968334	G	0.37 (A)	Rheumatic diseases (Kochi et al., 2010; Koumakis et al., 2013)
CCR8	3	80 G/C	rs2853699	G	0.13 (C)	AIDS progression (An et al. 2011)
CXCR6	3	7 G/A	rs2234355	G	0.14 (A)	Long-Term Nonprogression to AIDS
	3	*42 T>G	rs2234358	G	0.45 (T)	HIV infection susceptibility (Duggal et al., 2003; Limou et al., 2010)
CXCR3	X	12+213 G/A	rs34334103	G	0.06 (A)	Systemic lupus erythematosus, Asthma (Cheong et al., 2005; Im et al., 2014)
	X	12+234 C/T	rs2280964	C	0.18 (T)	
CCL20	2	-786 C>T	rs6749704	T	0.23 (C)	Ulcerative colitis, Atopic dermatitis (Choi et al. 2005)
	2	-1706 C>T	rs13034664	T	0.59 (C)	
IP-10	4	-135 C/T	rs56061981	C	0.11 (T)	Hepatitis B, malaria and tuberculosis infection diseases susceptibility (Tang et al., 2009; Wilson et al., 2013; Sheikh et al., 2015)
	4	+1642 C/G	rs3921	C	0.31 (G)	
CCL22	16	5 A>C	rs4359426	C	0.08 (A)	Atopic dermatitis, Gastric cancer susceptibility (Wang et al., 2009; Hirota et al., 2011)

MAF, minor allele frequency.

Table 2. Panel 1 and 2 multiplex primer sequences.

Gene	rs	Sequence (5'->3')	Tm	Product size (bp)
Panel 1				
IL2 Fw	rs2069762	CCATTCTGAAACAGGAAACCA	59.96	301pb
IL2 Rev		AAACCCCCAAAGACTGACTG	59.04	
IL4 Fw	rs2243250	ACCCAAACTAGGCCTCACCT	59.99	174pb
IL4 Rev		ACAGGTGGCATCTTGGAAAC	59.97	
IL6 Fw	rs1800795	TCGTGCATGACTTCAGCTTT	59.60	328pb
IL6 Rev		GCCTCAGACATCTCCAGTCC	59.80	
IL10-SNP1 Fw	rs1800896	TTCCCCAGGTAGAGCAACAC	60.11	190pb
IL10-SNP1 Rev		ATGGAGGCTGGATAGGAGGT	59.92	
IL10-SNP2 Fw	rs1800872	GGGGTCATGGTGAGCACTAC	60.29	230pb
IL10-SNP2 Rev		CAAGCAGCCCTTCCATTTTA	60.20	
IL17A-SNP1 Fw	rs2275913	GCCAAGGAATCTGTGAGGAA	60.20	328pb
IL17A-SNP1 Rev		TTCAGGGGTGACACCATTTT	60.21	
IL17A-SNP2 Fw	rs8193036	CCTTCTCTCTTTCCCCCATC	60.01	158pb
IL17A-SNP2 Rev		TGCATGCTACCAAGCAACTT	59.49	
TNFα-SNP1/2 Fw[a]	rs1800629/ rs361525	GCCCCTCCCAGTTCTAGTTC	60.07	244pb
TNFα-SNP1/2 Rev[a]		AAAGTTGGGGACACACAAGC	60.01	
Panel 2				
CCR3 Fw	rs3091250	TGACAGGAGAAATGGACATGG	60.91	282
CCR3 Ver		CTGTCTCTTACGGCATTTTGC	59.90	
CCR4-SNP1 Fw	rs2228428	TGCTGCCTTAATCCCATCAT	60.44	176
CCR4-SNP1 Ver		TCATGATCCATGGTGGACTG	60.34	
CCR4-SNP2 Fw	rs6770096	TCTTGCTTTTGCGGAACAAT	60.75	221
CCR4-SNP2 Ver		GTTTGGAAAGCAGACCTTGG	59.71	
CCR6 Fw	rs968334	TGCATTGCAGCATCAAGAAT	60.37	360
CCR6 Ver		AAATGCAGATCCCACAGACC	59.93	
CCR8 Fw	rs2853699	CAGACCACAAGGACCAGGAT	59.96	198
CCR8 Ver		CGCTGCCTTGATGGATTATAC	59.58	
CXCR3-SNP1/2 Fw[a]	rs34334103/ rs2280964	CTGTGACTGCAGGTTTCCAA	59.87	398
CXCR3-SNP1/2 Rev[a]		AGCACGCCAAGAGTCAAAGT	60.06	
CXCR6-SNP1 Fw	rs2234355	CCCCTAAATGTGGTCAATGG	60.04	240
CXCR6-SNP1 Ver		CCACAGACAAACACCACCAG	60.04	
CXCR6-SNP2 Fw	rs2234358	CCTTACCTTGGGGTCTCACA	59.96	409
CXCR6-SNP2 Ver		TCCCAATCAAGGAGAACCTG	60.04	
CCL20-SNP1 Fw	rs6749704	CTGTTATTTGACATTTGCTGTGCTG	59.0	260
CCL20-SNP1 Rev		CTGTCCGCAGTTAGAGTGGA	59.3	
CCL20-SNP2 Fw	rs13034664	GACATGAGAGAGAGGGAGGAGA	59.8	340
CCL20-SNP2 Ver		AAGGGGATTGGGGAGTGACT	60.9	
CCL22 Fw	rs4359426	AGTGAGGCTTGTGGGTGGA	60.8	130
CCL22 Ver		CCACAGCAAGGAGGACGAG	60.0	

CXCL10-SNP1 Fw	rs56061981	CCCCAACAACTTGTACAGCC	59.05	380
CXCL10-SNP1 Rev		TGCAAAAGGAAATGAGAAGGAAATCA	59.65	
CXCL10-SNP2 Fw	rs3921	GATGGACCACACAGAGGCTG	60.30	150
CXCL10-SNP2 Rev		AACATTAACCTTCCTACAGGAGTAGT	60.30	

[a] The PCR product includes two polymorphisms.
Notes: Primer final concentrations = 0.20μM for 1 Reaction final volume = 25μL.

Table 3. Panel 1 and 2 minisequencing probe sequences.

Gene	Rs	Minisequencing probes	Len (w)	w + t-tail
Panel 1				
IL2	rs2069762	F:TTATTCTTTTCATCTGTTTACTCTTGCTCTTGTCCACCACAATATGCTATTCACATGTTCAGTGTAGTTTTA	72	NA
IL4	rs2243250	F:GATACGACCTGTCCTTCTCAAAACACCTAAACTTGGGAGAACATTGT	47	NA
IL6	rs1800795	F:AAAGAAAGTAAAGGAAGAGTGGTTCTGCTTCTTAGCGCTAGCCTCAATGACGACCTAAGCTGCACTTTTCCCCCTAGTTGTGTCTTGC	88	NA
IL10	rs1800896	F:AAATCCAAGACAACACTACTAAGGCTTCTTTGGGA	35	NA
	rs1800872	F:ATCCTAATGAAATCGGGGTAAAGGAGCCTGGAACACATCCTGTGACCCCGCCTGT	55	NA
IL17A	rs2275913	F:GCATAGCAGCTCTGCTCAGCTTCTAACAAGTAAGAATGAAAAGAGGACATGGTCTTTAGGAACATGAATTTCTGCCCTTCCCATTTTCCTTCAGAAG	97	NA
TNFα	rs8193036	F:CATCACTCTCTACTCCCCCCTGCCCCCCTTTTCTCCATCT	40	NA
	rs361525	F:AAAAGAAATGGAGGCAATAGGTTTTGAGGGGCATGGGGACGGGGTTCAGCCTCCAGGGTCCTACACACAAATCAGTCAGTGGCCCAGAAGACCCCCCTCGGAATC	105	NA
	rs1800629	F:AGGAAACAGACCACAGACCTGGTCCCCAAAAGAAATGGAGGCAATAGGTTTTGAGGGGCATG	62	NA
Panel 2				
CCR3	rs3091250	F:(CT)^{14}CTTCAAGGTTCAATTTCCCCATTAACTATAATGAATG	37	65
CCR4	rs2228428	F:(CT)^{21}GCCAATACTGTGGGCTCCTCCAAATTTA	28	70
	rs6770096	F:(CT)^{4}cAGCCAGATGTATGAAGAAACAATTAG	26	35
CCR6	rs968334	F:ATGTTAGATCCACCAGCACCCCCC	24	24
CCR8	rs2853699	F:(CT)^{16}TGCCATTTGTCTGAATAAGTTCC	23	55
CXCR3	rs2280964	F:AGCCTTCGAGTCTACTTGCCCCCCGCCCCC	20	30
	rs34334103	R:*(CT)^{14}cTCTCCTCTCCTCTCTGGCTTC*	21	50
CXCR6	rs2234355	F:(CT)^{17}cGTTCATCAGAACAGACACCATGGCA	25	60
	rs2234358	F:(CT)^{27}CGAGAAGCTGCTCTGGAATTTGCAAG	26	80
CCL20	rs6749704	F:(CT)^{22}cTTCCTCAACAATTCTGAGGCTCTATATTGAGTTATATTAG	40	85
	rs13034664	R:(CT)^{9}*cTGTTCATTTCTCCTTCCTCCA*	21	40
CCL22	rs4359426	F:(CT)^{39}AGACATACAGGACAGAGCATGG	22	100
IP-10	rs56061981	F:(CT)^{26}GGGGAAGTCCCATGTTGCAGACT	23	75
	rs3921	F:(CT)^{30}AGTTTGCAGTTACACTAAAAGGTGACCAAT	30	90

Notes: Primers F:left side of the SNP and R: right side of the SNP; CT +C tails was used according the size desired (24-100pb); Primer final concentration = 0.05 μM for 1 Reaction final volume = 10 μL.
NA, not applicable.

Table 4. Allelic and genotypic frequencies of cytokine genes SNPs from healthy controls compared with the HapMap database.

Gene	SNPs/alleles/ genotypes	Controls (n=305) Frequencies	HapMap database*	Gene	SNPs/alleles/ genotypes	Controls (n=305) Frequencies	HapMap database*
	rs2069762				**rs2275913**		
	TT	0.524	0.553		AA	0.126	0.120
IL2	TG	0.390	0.353		AG	0.395	0.346
	GG	0.087	0.095		GG	0.479	0.534
	T	0.719	0.729		A	0.324	0.293
	G	0.281	0.271	***IL17A***	G	0.676	0.707
	rs2243250				**rs8193036**		
	CC	0.492	0.361		TT	0.556	0.413
IL4	CT	0.389	0.339		TC	0.403	0.413
	TT	0.118	0.300		CC	0.041	0.174
	C	0.687	0.530		T	0.757	0.620
	T	0.313	0.470		C	0.243	0.380
	rs1800795				**rs1800629**		
	GG	0.563	0.766		GG	0.725	0.827
IL6	GC	0.359	0.186		GA	0.256	0.165
	CC	0.078	0.048		AA	0.019	0.008
	G	0.742	0.859		G	0.853	0.910
	C	0.258	0.141	***TNFα***	A	0.147	0.090
	rs1800896				**rs361525**		
	GG	0.114	0.098		GG	0.858	0.883
	GA	0.485	0.348		GA	0.138	0.112
	AA	0.401	0.554		AA	0.004	0.005
	G	0.357	0.272		G	0.927	0.939
IL10	A	0.643	0.728		A	0.073	0.061
	rs1800872						
	CC	0.354	0.346				
	CA	0.513	0.438				
	AA	0.132	0.216				
	C	0.611	0.565				
	A	0.389	0.435				

* 1000 Genomes Project Phase 3 for all populations.

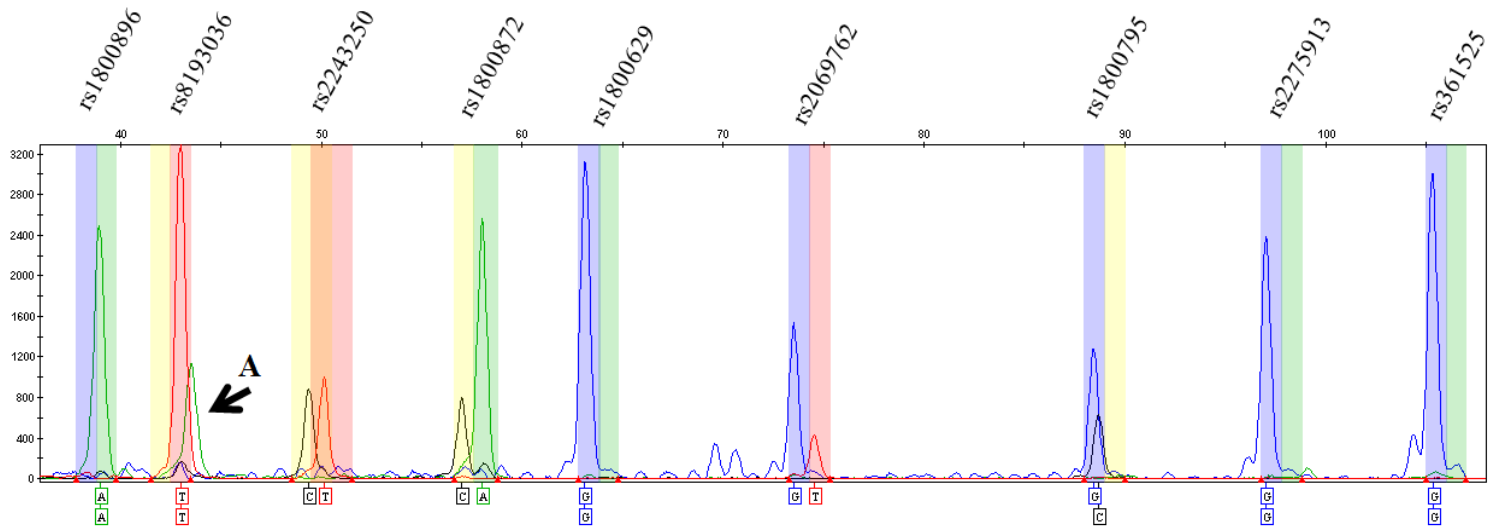
Table 5. Allelic and genotypic frequencies of chemokine receptor/ligand genes SNPs from healthtly controls compared with the HapMap database.

Gene	SNPs/alleles/ genotypes	Controls (n=305) Frequencies	HapMap database*	Gene	SNPs/alleles/ genotypes	Controls (n=305) Frequencies	HapMap database*
	rs3091250				**rs2234355**		
	GG	0.558	0.447		GG	0.847	0.786
CCR3	GT	0.361	0.414		GA	0.129	0.148
	TT	0.082	0.139		AA	0.024	0.066
	G	0.738	0.654		G	0.912	0.860
	T	0.262	0.346	***CXCR6***	A	0.088	0.140
	rs6770096				**rs2234358**		
	CC	0.821	0.675		GG	0.288	0.331
	CT	0.164	0.278		GT	0.476	0.439
	TT	0.015	0.047		TT	0.236	0.229
	C	0.903	0.814		G	0.526	0.551
	T	0.097	0.186		T	0.474	0.449
CCR4	**rs2228428**				**rs3921**		
	CC	0.582	0.742		CC	0.408	0.509
	CT	0.344	0.223		CG	0.510	0.367
	TT	0.075	0.035		GG	0.082	0.124
	C	0.753	0.853		C	0.663	0.692
	T	0.247	0.147		G	0.337	0.308
	rs968334			***CXCL10***	**rs56061981**		
	GG	0.395	0.404		CC	0.860	0.797
CCR6	GA	0.466	0.446		CT	0.140	0.192
	AA	0.139	0.151		TT	0.000	0.011
	G	0.628	0.627		C	0.930	0.893
	A	0.372	0.373		T	0.070	0.107
	rs2853699				**rs13034664**		
	GG	0.565	0.772		TT	0.204	0.210
CCR8	GC	0.388	0.198		TC	0.541	0.395
	CC	0.048	0.031		CC	0.255	0.395
	G	0.759	0.870		T	0.474	0.407
	C	0.241	0.130		C	0.526	0.593
	rs34334103			***CCL20***	**rs6749704**		
	GG ♀	0.489	0.449		TT	0.561	0.609
	GA ♀	0.011	0.056		TC	0.361	0.330
	AA ♀	0.000	0.003		CC	0.078	0.062
	G [a]	0.973	0.936		T	0.741	0.774
CXCR3	A [b]	0.027	0.064		C	0.259	0.226
	rs2280964				**rs4359426**		
	CC ♀	0.354	0.364		CC	0.888	0.845
	CT ♀	0.146	0.096		CA	0.112	0.144
	TT ♀	0.000	0.040	***CCL22***	AA	0.000	0.012
	C [a]	0.800	0.824		C	0.944	0.917
	T [b]	0.200	0.176		A	0.056	0.083

* 1000 Genomes Project Phase 3 for all populations.

[a,b] frequencies in general population

A. Panel 1



B. Panel 2

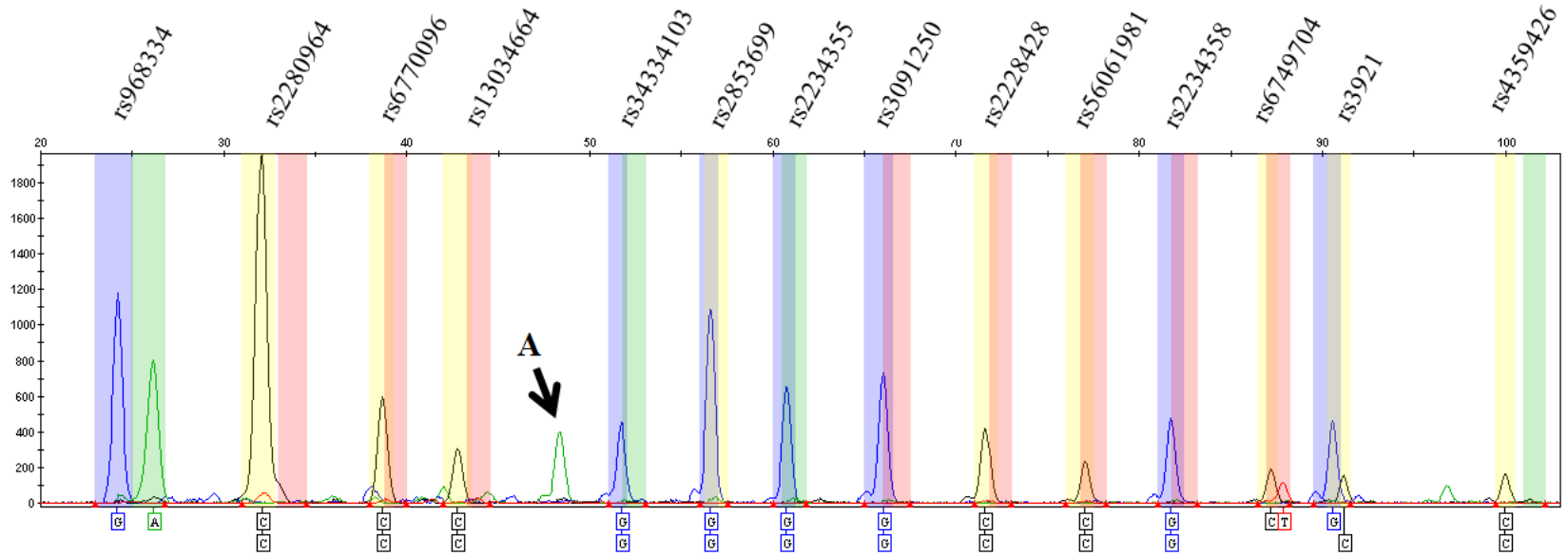


Figure 1. Detection of cytokines and chemokine receptor/ligand gene SNPs by two multiplex minisequencing assays. Panel 1 (A) shows nine cytokine gene SNPs from a healthy individual donor. Peaks correspond to the fluorescence signal detected for each SNP. Panel 2 (B) shows fourteen chemokine receptor/ligand gene SNPs. The A arrow indicates a residual signal that does not match any SNP located among 40 to 45 bp position in both panels (discussed in the text).

Capítulo 3

Novel genetic associations and gene-gene interactions of chemokine receptor and chemokine genetic polymorphisms on HIV/AIDS

Running head: Novel immunogenetic associations on HIV/AIDS

Jacqueline M. VALVERDE-VILLEGAS, Rúbia Marília DE MEDEIROS, Karine Pereira de ANDRADE, Vanessa Cristina JACOVAS, Breno Riegel DOS SANTOS, Daniel SIMON, Sabrina Esteves de Matos ALMEIDA, José Artur Bogo CHIES.

Artigo aceito para publicação em março de 2016 na revista AIDS.

"O objetivo deste artigo foi investigar a influência de 15 polimorfismos candidatos em genes de receptores de quimiocinas e seus ligantes na susceptibilidade à infecção pelo HIV e na progressão à aids".

Novel genetic associations and gene–gene interactions of chemokine receptor and chemokine genetic polymorphisms in HIV/AIDS

Running head: Novel immunogenetic associations in HIV/AIDS

Jacqueline M. VALVERDE-VILLEGAS[1,2], Rúbia Marília DE MEDEIROS[1,2], Karine Pereira de ANDRADE[2], Vanessa Cristina JACOVAS[1], Breno Riegel DOS SANTOS[3], Daniel SIMON[4], Sabrina Esteves de Matos ALMEIDA[1,2,5], José Artur Bogo CHIES[1].

[1] Departamento de Genética e Programa de Pós-graduação em Genética e Biologia Molecular (PPGBM) da Universidade Federal do Rio Grande do Sul (UFRGS)

[2] Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS)

[3] Serviço de Infectologia, Grupo Hospitalar Nossa Senhora da Conceição (GHC)

[4] Universidade Luterana do Brasil (ULBRA)

[5] Instituto de Ciências da Saúde, Universidade Feevale (FEEVALE)

Total number of words: 3,499

Corresponding author:

Dr José Artur Bogo Chies. Email address: jabchies@terra.com.br

Laboratory of Immunogenetics, Institute of Biosciences, Department of Genetics, UFRGS.

Av. Bento Gonçalves – 9500, Campus do Vale. 91501970

Porto Alegre, RS-Brazil. PO BOX 15053

Phone: +55 51 3308 6740; Fax: +55 51 3308 7311

This work was supported by Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) [Grant number 12/2151-2]; Jacqueline M. Valverde-Villegas received a scholarship from the Programa Estudantes-Convênio de Pós-Graduação (PEC-PG).

Abstract

Objective: To investigate the influence of candidate polymorphisms on chemokine receptor/ligand genes on HIV infection and AIDS progression (HIV/AIDS).

Design: Fifteen polymorphisms of the *CCR3*, *CCR4*, *CCR5*, *CCR6*, *CCR8*, *CXCR3*, *CXCR6*, *CCL20*, *CCL22* and *CXCL10* genes were analysed in 206 HIV-positive subjects classified as rapid progressors (RPs; n=40), or non-RPs (NRPs; n=166) and in 294 HIV-seronegative subjects.

Methods: The polymorphisms were genotyped using minisequencing. Genetic models were tested using binomial logistic regression; non-parametric multifactor dimensionality reduction (MDR) was used to detect gene–gene interactions.

Results: The *CCR3* rs3091250 [TT, adjusted odds ratio (AOR): 2.147, 95% confidence interval [CI] 1.076–4.287, *P*=0.030], *CCR8* rs2853699 (GC/CC, AOR: 1.577, 95% CI 1.049–2.371, *P*=0.029), *CXCL10* rs56061981 (CT/TT, AOR: 1.819, 95% CI 1.074–3.081, *P*=0.026) and *CCL22* rs4359426 (CA/AA, AOR: 1.887, 95% CI 1.021–3.487, *P*=0.043) polymorphisms were associated with susceptibility to HIV infection. The *CCL20* rs13034664 (CC, OR: 0.214, 95% CI 0.063–0.730, *P*=0.014) and *CCL22* rs4359426 (CA/AA, OR: 2.685, 95% CI 1.128–6.392, *P*=0.026) variants were associated with rapid progression to AIDS. In MDR analyses revealed that the *CXCL10* rs56061981 and *CCL22* rs4359426 combination was the best model, with 57% accuracy (*P*=0.008) for predicting susceptibility to HIV infection.

Conclusions: Our results provide new insights into the influence of candidate chemokine receptor/ligand polymorphisms and significant evidence for gene–gene interactions on HIV/AIDS susceptibility.

Key words: chemokine receptors/ligands, polymorphisms, gene–gene interaction, HIV/AIDS

Introduction

Since the discovery of the pivotal role of CCR5 and CXCR4 and their ligands in the regulation of HIV entry into target cells, investigators have focused much attention on these chemokine receptors. In recent years, some studies have addressed the immunological role of other important chemokine receptors and their ligands in HIV-1 pathogenesis. For example, CCR3, CCR8 and CXCR6 are used as minor entry coreceptors by some viral primary HIV-1 isolates and they also characterise some CD4+ T cell subpopulations targeted by HIV-1 [1] [2] [3] [4]. Moreover, memory effector CD4+ T cell subpopulations are characterised as permissive or resistant to HIV replication according to their chemokine receptor expression profiles, including CCR4, CCR6 or CXCR3 [5]. A recent review focused on the important role of these receptors in immune regulation during HIV-1 infection and disease progression [6].

Chemokines such as CCL22 (a CCR4 ligand), CCL20 (a CCR6 ligand) and CXCL10 (a CXCR3 ligand) play important roles in HIV infection, acting as inhibitors of HIV infection and acting in the recruitment of target cells to sites of infection [7][8]. Conversely, these chemokines can also upregulate HIV replication. During early infection, chemokine production by HIV-infected cells generate inflammatory site recruitment and activate different cell subsets. This generates and facilitates an environment for HIV dissemination [9]. Notably, CXCL10 stimulates HIV-1 replication in monocyte-derived macrophages and peripheral blood lymphocytes and has been suggested as a marker in AIDS [10] [11].

Several host genetic factors associated with HIV/AIDS have been described [12] [13]. In particular, genetic polymorphisms of the classic chemokine receptors such as CCR5 and CXCR4 and their ligands are associated with susceptibility to HIV infection, disease progression and antiretroviral therapy response [14] [15] [16]. Thus, there is strong evidence that the genetic diversity of such molecules is important in the context of HIV-1 infection, triggering different

phenotypes in distinct infected individuals. Nevertheless, the exact role of other chemokine receptors and their ligands in HIV-1 infection have yet to be determined. Some studies on Caucasians observed that *CXCR6*, *CCR3* and *CCR8* polymorphisms were associated with AIDS progression [17] [18] [19]. A recent genome-wide association study (GWAS) on HIV infection analysed the associations among ~8 million variants and viral load and observed a significant association signal in the chemokine receptor gene cluster on chromosome 3 [20]. In addition, the study highlighted that GWAS directed to evaluate different phenotypes in HIV-1 infection was underpowered to identify genetic variants with modest effect, which influence other complex human diseases [20].

Thus, we analysed 15 polymorphisms in 10 candidate genes chosen from the genetic variants reported in the literature as playing a role in inflammation and immunity in complex diseases and susceptibility to HIV infection and AIDS progression. Notably, the *CCR4*, *CCR8*, *CCR3* and *CXCR6* genes are located near the *CCR5* gene region on chromosome 3, which a recent GWAS described as being associated with viral load [20]. We used parametric tests for logistic regression and non-parametric tests for the multifactor dimensionality reduction algorithm (MDR) to identify single SNPs and gene–gene interactions between SNPs, respectively.

Materials and methods

Study population

The Infectious Diseases Service from Nossa Senhora da Conceição Hospital in Porto Alegre, Brazil, treats about 5,000 HIV-seropositive patients. In 2011 to 2013, the clinical histories of more than 3,500 HIV-1–infected subjects were retrospectively reviewed; subjects with specific clinical profiles were selected. The initial selection criteria included the possibility of estimating seroconversion by a previous known HIV-negative test. Additionally, eligible patients should have

97 regular clinical follow-up after positive diagnosis. Patients with highly active antiretroviral therapy
98 (HAART) recommendations up to 3 years after seroconversion were classified as rapid progressors
99 (RPs); those that remained without recommendation to initiate HAART (due to CD4+ T cell count >
100 350 cells/mm^3) for >5 years after positive diagnosis (seropositivity) were further followed and
101 classified as slow progressors (SPs) if they remained without HAART recommendation for a total
102 of at least 8 years.

103 The 206 HIV-1–positive adults selected self-identified as being of European descent or
104 African descent. Discussions regarding the skin colour–based classification criteria used in Brazil
105 are well-documented [21,22]. The AIDS progression classification was performed according to
106 longitudinal clinical and laboratory data, including CD4+ T cell count, plasmatic viral loads, HIV-
107 seronegative test, first HIV-seropositive test, initial CD4+ T cell count, clinical stage data at the
108 time of sample collection and HAART drug prescriptions. HIV-positive subjects were classified as
109 RPs or non-RPs (NRPs) according progression time. As previously stated, RPs were subjects who
110 had recommendation to initiate HAART within 3 years of seroconversion due to CD4+ T cell count
111 < 350 cells/mm^3 [23]. All other patients were included in the NRP group: SPs, elite controllers
112 (ECs) and HIV-positive subjects with ≥3 years of follow-up independently of HAART
113 recommendation after this time. SPs were defined as HIV-positive subjects asymptomatic for ≥8
114 years of follow-up after diagnosis, with average CD4+ T cell count ≥ 500 cells/mm^3 and plasma
115 viral load < 10,000 copies/mL through the years in the absence of HAART. ECs were individuals
116 with undetectable levels of viral RNA (<50 copies/mm^3) that maintained CD4+ T cell counts > 500
117 cells/mm^3 for ≥5 years [13].

118 The 294 HIV-1–seronegative control samples were from a biorepository maintained at the
119 Universidade Federal do Rio Grande do Sul (UFRGS) and were collected from healthy adult blood
120 donors. The donors were classified as being of European descent or African descent by self-

identification. Individuals with infectious diseases, cancer, autoimmune or metabolic diseases or other medical conditions were excluded from this control group.

Ethics statement

This study was conducted in compliance with the principles included in the Declaration of Helsinki and received approval from the Nossa Senhora da Conceição Hospital and Conselho Nacional de Ética em Pesquisa (002964-20.69/10-5 and 30491714.0.0000.5347 ethical process, respectively). All subjects signed an informed consent form to participate in this study.

Sample preparation

We collected 10 mL peripheral blood from the patients and controls. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient density after centrifugation at 1500 *g* for 10 min. Genomic DNA was extracted from the PBMCs using a high-salt precipitation method [24] and stored at -20°C.

Genotyping of chemokine and chemokine receptor genes

Fifteen candidate polymorphisms in 10 chemokine and chemokine receptor genes were selected according their potential influence on autoimmune diseases, cancer, allergies and infectious diseases: *CCR3* rs3091250, *CCR4* rs6770096 and rs2228428, *CCR5* rs333, *CCR6* rs968334, *CCR8* rs2853699, *CXCR3* rs34334103 and rs2280964, *CXCR6* rs2234355 and rs2234358, *CCL20* rs13034664 and rs6749704, *CCL22* rs4359426, *CXCL10* rs3921 and rs56061981. These SNPs were genotyped using a minisequencing technique developed by our group [25], with exception of the CCR5del32 (rs333) variant, which was genotyped by conventional PCR and was visualised in 3% agarose gel [26].

Statistical analysis

Compliance with expectations for Hardy–Weinberg equilibrium (HWE) was evaluated in all subjects using the χ^2 test. A 2×2 contingency table for Fisher's exact test was used to investigate differences in allelic frequencies between individuals of European and African descent; these frequencies were compared with data extracted from the HapMap project. Clinical and demographic covariate data were compared among groups using Student's *T* (or t)-test'. Codominant, dominant and recessive genetic models were tested, and genotypic associations and odds ratios (ORs) with 95% confidence intervals (CI) were estimated by binomial logistic regression. For susceptibility analyses, the logistic regression was conducted comparing HIV-positive subjects vs. controls adjusted for ethnicity and sex. For progression to AIDS analyses, we compared RPs vs. NRPs, RPs vs. SPs and RPs vs. ECs.

The gene–gene interaction analyses were performed using a non-parametric test such as MDR v.3.0.4 [27]. MDR analysis is a data-mining method used to detect and classify combinations of effect from independent variables that may interact to influence different phenotypes. The top 5 SNPs filtered with the ReliefF algorithm and the SNPs with statistical significance in univariate logistic regression were included in the MDR analyses [28] [29]. All interactions were tested using 10-fold cross-validation (CV) in an exhaustive search considering SNP combinations, and we adjusted for ethnicity [30].

The Mann–Whitney U and Kruskal–Wallis non-parametric tests were used to evaluate the relationships among the slope of CD4+ T cell counts and all SNP genotypes and genetic models. Also, ANOVA or Student's *T* (or t)-test' parametric were applied to evaluate the association of the median RNA viral load (logVL) with all genotypes/genetic models from 83 progressor patients ($P<0.001$).

Results

Demographic and clinical data

Table 1 summarises the demographic and clinical parameters of the participants. Regarding AIDS progression, 40 and 166 subjects were classified as RPs and NRPs, respectively. RPs had higher plasma viral loads and lower median CD4+ T cell counts in the first measure as compared to NRPs. CD4+ T cell counts decreased significantly faster for RPs than for NRPs. Similar differences in clinical characteristics were observed when only RPs and SPs were compared (Table S1).

Allelic and genotypic Single Nucleotide Polymorphism frequencies

Except for *CXCR6* rs2234355, which was only in accordance with HWE when analysed by ethnicity, and *CXCL10* rs3921 in control individuals (and therefore excluded from the susceptibility analyses), all other SNP frequencies were consistent with HWE expectations. Table S2 shows the allelic and genotypic frequencies of all 15 SNPs of all subjects. When allelic frequencies were compared between the controls and HIV-positive subjects stratified by European or African ancestry, we observed significant differences for the *CCR8* rs2853699, *CXCR6* rs2234355, *CXCR6* rs2234358 and *CXCL10* rs56061981 variants (Table S3).

Association of single SNPs and susceptibility to HIV infection

In univariate logistic regression adjusted by sex and ethnicity, 4 polymorphisms were associated with susceptibility to HIV infection when the HIV-positive subjects were compared to the controls: *CCR3* rs3091250, *CCR8* rs2853699, *CXCL10* rs56061981 and *CCL22* rs4359426. In multivariate logistic regression adjusted by sex and ethnicity, only rs3091250 and rs56061981 remained significant (Table 2).

Association of single SNPs and AIDS progression

In the univariate logistic regression, the frequencies of the *CCL20* rs13034664 and *CCL22* rs4359426 variants were statistically different when RPs and NRPs were compared. The differences of both polymorphisms remained significant in the multivariate logistic regression (Table 3). *CCL20* rs13034664 CC patients had a low probability of being RPs, while the CC genotype frequency was increased among NRPs. Interestingly, the CC genotype frequency was increased in ECs when compared with RPs (0.500 vs. 0.075, respectively). On the other hand, *CCL22* rs4359426 CA/AA carriers had a high probability of being RP.

Gene–gene interaction analyses

The 5 SNPs filtered by the ReliefF algorithm plus the 3 other SNPs with statistical significance in univariate logistic regression (*CCR5* rs333, *CCR3* rs3091250, *CCR6* rs968334, *CCR8* rs2853699, *CCL20* rs13034664 and rs6749704, *CCL22* rs4359426, *CXCL10* rs56061981) were further evaluated by MDR. Table 4 summarises the results of an exhaustive MDR analysis adjusted by ethnicity and sex covariates. The best model for predicting HIV infection risk indicated by MDR was the 2-factor model combination of *CCL22* rs4359426 and *CXCL10* rs56061981 [testing balance accuracy (TA)=0.5696; *P*=0.008; CV consistency (CVC)=9/10]. The criteria for selecting the best model were: *i*) it was more parsimonious, *ii*) it was significant, *iii*) the CVC was good, i.e. 9/10, and *iv*) there was closer training and testing accuracy. Regarding entropy-based interaction, there was a high redundancy effect (additive or correlation) between *CCL22* rs4359426*, CCR3* rs3091250 and *CXCL10* rs56061981 (Figure 1A). In addition, the information gain (IG) values of *CCL22* rs4359426 (3.27%), *CCR3* rs3091250 (1.86%) and *CXCL10* rs56061981 (2.01%) indicated that these variants have a large main effect on susceptibility to HIV infection. There was an epistatic effect between *CCR6* rs968334 and *CCL20* rs13034664 was observed, with a negative marginal effect between them (0.07%). Figure 1B shows the level of interaction between attributes, and the interaction between *CCL22* rs4359426, *CCL20* rs13034664 and *CCR3* rs3091250 had the highest degree of

214 correlation. None of the MDR models were statistically significant in RPs vs. NRPs, RPs vs. SPs and
215 RPs vs. ECs.

216 **Analyses stratified by ethnic origin**

217 In univariate logistic regression, the *CXCR6* rs2234355 (dominant model, GA or AA
218 genotype; OR: 2.70, 95% CI 1.29–5.64, *P*=0.008), *CCR8* rs2853699 (dominant model, GC or CC
219 genotype; OR: 1.92, 95% CI 1.23–3.01, *P*=0.004) and *CXCL10* rs56061981 (dominant model, CT or
220 TT genotype; OR: 2.33, 95% CI 1.31–4.14, *P*=0.004) polymorphisms were associated with
221 susceptibility to HIV infection in individuals of European descent (Table S4). Moreover, these
222 significant associations were maintained in multivariate logistic regression: *CXCR6* rs2234355,
223 *P*=0.006; *CCR8* rs2853699, *P*=0.004; *CXCL10* rs56061981, *P*=0.011. In individuals of African
224 descent, univariate logistic regression indicated an association between the *CXCR6* rs2234355
225 (dominant model, GA or AA genotype; OR: 0.38, 95% CI 1.18–0.77, *P*=0.008) and *CXCR6* rs2234358
226 (dominant model, GT or TT genotype; OR: 0.37, 95% CI 1.16–0.85, *P*=0.018) variants with
227 protection against HIV infection. The significance was maintained only for rs2234355 (dominant
228 model, GA or AA genotype; OR: 0.45, 95% CI 0.21–0.99, *P*=0.049) in multivariate logistic
229 regression (Table S5). No significant models were observed when gene–gene interaction analyses
230 for susceptibility to HIV or AIDS progression were applied in our sample as stratified by ethnic
231 group (data not shown).

232 **SNPs and RNA viral load**

233 There were significant associations between the *CCR5* rs333, *CXCR6* rs2234355, *CCL20*
234 rs6749704 and *CCL22* rs4359426 SNPs and the median logVL (*P*<0.001). The median plasma logVL
235 was lower among rs333 heterozygous (wt/delta32) individuals as compared to wild-type
236 homozygous individuals, and was higher among rs2234355 AA individuals as compared to the
237 other genotypes. It was also higher among rs6749704 C variant carriers as compared to subjects

238 without this SNP. Likewise, the median plasma logVL was higher among rs4359426 A allelic
239 variant carriers than among subjects with the CC genotype (data not shown).

240 Discussion

241 In this study, we explored the potential influence of candidate polymorphisms in the
242 genes of chemokine receptors and their ligands on HIV/AIDS. Multivariate logistic regression
243 revealed that the *CCR3* rs3091250 and *CXCL10* rs56061981 polymorphisms influence HIV infection
244 susceptibility. In addition, the best model of gene–gene interactions detected by MDR included
245 the effect of the *CCL22* rs4359426 and *CXCL10* rs56061981 polymorphisms on susceptibility to HIV
246 infection. A large main effect on susceptibility to HIV infection was allocated to *CCR3* rs3091250
247 (1.86%), *CXCL10* rs56061981 (2.01%) and *CCL22* rs4359426 (3.27%) (Figure 1A). Regarding AIDS
248 progression, the *CCL20* rs13034664 polymorphism was associated with low probability for rapid
249 progression whereas *CCL22* rs4359426 was associated with high probability for rapid progression
250 (Table 3). Interestingly, in multivariate logistic regression stratified by ethnicity, *CXCR6* rs2234355,
251 *CCR8* rs2853699 and *CXCL10* rs56061981 were associated with susceptibility to HIV infection
252 among subjects of European descent, while only *CXCR6* rs2234355 was protective against HIV
253 infection among subjects of African descent.

254 *CCR3* rs3091250 has been associated with increased transcription levels [31]. *CCR3* plays a
255 major role in allergies and its expression is predominantly on basophils, eosinophils and in Th2
256 effector cells [32]. In the present study, the rs3091250 TT genotype was associated with
257 susceptibility to HIV-1 infection. As stated earlier, the *CCR3* gene is located on chromosome 3
258 near the *CCR5* loci, a region associated with HIV viral load in a recent GWAS and therefore a quite
259 interesting candidate region for association studies targeting HIV infection. This variant has been
260 associated with other diseases presenting an allergic context [33] [34] [35]. The *CCR8* gene is also
261 located on chromosome 3, and increased *CCR8* expression correlates with activation in Th2 cells

[36]. Here, the GC or CC rs2853699 genotypes were associated with susceptibility to HIV infection in univariate logistic regression. A previous study reported that, together with polymorphisms within *CCR2*, *CCR5* and *CCRL2*, this polymorphism composed the best predictive model for the rate of AIDS progression in a cohort composed of individuals with 3 extreme phenotypes (resistance to HIV infection, and very rapid or slow progression to AIDS) [19]. Again, these genes are located in the same chromosome region. Notably, both *CCR3* and *CCR8* expression were increased on CD4+ T cells in HIV-1–exposed infants, and associated with enhanced immune activation and altered CD4+ T cell homing with increased HIV susceptibility [37].

Nevertheless, other molecules also interfere with the HIV infection outcome and have been suggested as predictors of HIV/AIDS. In our study, the *CCL22* rs4359426 CA or AA genotypes were associated with susceptibility to HIV infection. Furthermore, the allele A carriers had a high probability of being RPs when compared with NRPs. It has been suggested that this SNP is involved in higher mRNA expression [38] and has been associated with allergic diseases [38] [39]. Ancuta et al. (2006) showed that CCR3 and CCR4 ligands (including CCL22) produced by *in vitro* monocyte-derived macrophages deliver costimulatory signals to T cells that increase their susceptibility to productive HIV infection [40].

The inflammatory chemokine CCL20 is the only ligand for CCR6, a receptor mainly expressed by Th17 and regulatory T cells (Tregs) [41]. The CCR6–CCL20 axis plays a critical role in the homing of these T cells to the gut [42]. Polymorphisms in the *CCL20* promoter region such as the rs13034664 and rs6749704 SNPs have been associated with inflammatory bowel diseases [43]. Here, *CCL20* rs13034664 CC genotypic frequencies were statistically higher in NRPs and ECs as compared to RPs. Elevated plasma CCL20 levels were a characteristic feature throughout the course of infection in rapid and normal HIV progressors when compared with aviremic HIV SPs [7]. The Th1Th17 (CXCR3+CCR6+) cells, which in the HIV infection context produce mainly CCL20 and

286 TNF-α, are highly permissive to HIV infection and have the potential to be recruited to sites of HIV
287 persistence, such as the gut and the brain [5]. The MDR analyses revealed an epistatic effect
288 between *CCL20* rs13034664 and *CCR6* rs968334 on susceptibility to HIV infection, suggesting a
289 role for the CCR6–CCL20 axis in this phenomenon.

290 CXCL10 is one of the first chemokines whose levels in plasma increase a few days after
291 HIV infection and remain elevated throughout the infection [44]. Several studies have proven that
292 elevated CXCL10 plasma levels during the infection lead to poor outcome [10][11][45][46].
293 Polymorphisms of the *CXCL10* gene were evaluated in infectious diseases such as hepatitis B and
294 C and co-infections with HIV, malaria and tuberculosis [47][48][49][50]. In the present study, the
295 *CXCL10* rs56061981 CT or TT genotypes were associated with susceptibility to HIV infection. The
296 rs56061981 variant affects *CXCL10* promoter activity, which contributes to CXCL10 expression via
297 NF-kB transactivation [20]; therefore, evaluations of HIV-positive individuals should be done
298 taking into consideration both CXCL10 genotypes and phenotypes. Additionally, the combination
299 of *CCL22* rs4359426 and *CXCL10* rs56061981 on susceptibility to HIV infection evidenced the best
300 model through the MDR analyses, which included similar values of the training balanced accuracy
301 and TA and the highest CVC, suggesting that rs4359426 and rs56061981 could be used for
302 evaluating HIV infection risk.

303 When patients were stratified by ethnicity, our analyses revealed that *CCR8* rs2853699,
304 *CXCR6* rs2234355 and *CXCL10* rs56061981 were associated with susceptibility to HIV infection in
305 patients of European descent. In contrast, *CXCR6* rs2234355 was associated with protection
306 against HIV infection in patients of African descent. Genetic variants could vary according to
307 ethnic background. The rs2234355 allelic variant frequency is high in African Americans and rare
308 in European Americans, and this should be considered in population studies. For example, the
309 *CXCR6* rs2234355 allelic variant is protective against progression to death after *Pneumocystis*

jiroveci pneumonia in HIV-infected African American subjects [18], and also has a protective effect against HIV infection in Biaka Western Pygmies in West Central Africa [51].

As markers of progression, the median logVL and the genotypes were analysed. The median logVL was significantly decreased in heterozygous wt/delta32 HIV-positive subjects when compared with wild-type homozygous subjects. The median logVL was also higher among carriers of the rs2234355, rs6749704 and rs4359426 variants when compared with non-carriers (data not shown). Our results corroborate the association of rs333 with HIV-1 viral load [20] and demonstrate novel associations regarding rs2234355, rs6749704 and rs4359426 and HIV viral load. However, these findings require confirmation with a larger sample size.

Lastly, this study proved the importance of genes located on chromosome 3, and mainly around the *CCR5* gene region, on the outcome of HIV infections, as previously suggested by a GWAS. The selected target chemokine and chemokine receptor genes cover key factors in the response to HIV infection, and gene–gene interactions were analysed with MDR. This study demonstrates novel associations of polymorphisms of chemokines and their receptor genes and susceptibility/progression to HIV/AIDS, as well as viral load during HIV infection. Based on these results, functional analyses and replication studies are needed to fully understand the relationships between these genes and the immunological host responses in HIV pathogenesis.

Conclusions

This study provides new insights into the associations between *CCR3* rs3091250, *CXCL10* rs56061981 and *CCL22* rs4359426 and susceptibility to HIV infection. Gene–gene interaction analyses showed a significant correlation between *CXCL10* rs56061981 and *CCL22* rs4359426 in susceptibility to HIV infection. Thus, the interaction of such *CCL22–CXCL10* SNPs should be involved in HIV infection risk. Concerning AIDS progression, the *CCL20* rs13034664 polymorphism was significantly associated with a low probability to rapid progression whereas *CCL22* rs4359426

was associated with a high probability to rapid progression. Moreover, ethnic background influences HIV/AIDS infection and should be considered in case–control analyses.

Acknowledgements

We thank the Infectious Diseases Service of Nossa Senhora da Conceição Hospital for the valuable assistance and collaboration with our project and thank the study participants; most were very happy to contribute to this study.

JMV-V reviewed the clinical history of the patients, performed the statistical analyses, organised the manuscript; RMM reviewed the clinical history of the patients, analysed data; VCJ analysed data; KPA performed some of the minisequencing experiments; BRS was responsible for the clinical selection of the patients; DS analysed data; SEA and JABC conceived the project and organised the manuscript. All authors were involved in the writing and approval of the manuscript.

References

1 Jinno A, Shimizu N, Soda Y, Haraguchi Y, Kitamura T, Hoshino H. Identification of the Chemokine Receptor TER1/CCR8 Expressed in Brain-Derived Cells and T Cells as a New Coreceptor for HIV-1 Infection. *Biochem Biophys Res Commun* 1998; **243**:497–502.

2 Alkhatib G, Berger E a., Murphy PM, Pease JE. Determinants of HIV-1 coreceptor function on CC chemokine receptor 3. Importance of both extracellular and transmembrane/cytoplasmic regions. *J Biol Chem* 1997; **272**:20420–6.

3 Liao F, Alkhatib G, Peden KW, Sharma G, Berger E a, Farber JM. STRL33, A novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J Exp Med* 1997; **185**:2015–23.

4 Calado M, Matoso P, Santos-Costa Q, Espirito-Santo M, Machado J, Rosado L, *et al.* Coreceptor usage by HIV-1 and HIV-2 primary isolates: The relevance of CCR8 chemokine receptor as an alternative coreceptor. *Virology* 2010; **408**:174–182.

5 Gosselin A, Monteiro P, Chomont N, Diaz-Griffero F, Said E a, Fonseca S, *et al.* Peripheral Blood CCR4+CCR6+ and CXCR3+CCR6+ CD4+ T Cells Are Highly Permissive to HIV-1 Infection. *J Immunol* 2010; **184**:1604–1616.

6 Valverde-Villegas JM, Matte MCC, Medeiros RM De, Chies JAB. New Insights about Treg

364 and Th17 Cells in HIV Infection and Disease Progression. *J Immunol Res* 2015; **2015**:1–14.

365 7 Fontaine J, Poudrier J, Roger M. Short communication: persistence of high blood levels of
366 the chemokines CCL2, CCL19, and CCL20 during the course of HIV infection. *AIDS Res Hum*
367 *Retroviruses* 2011; **27**:655–7.

368 8 Pal R, Garzino-Demo A, Markham PD, Burns J, Brown M, Gallo RC, *et al.* Inhibition of HIV-1
369 infection by the beta-chemokine MDC. *Science* 1997; **278**:695–8.

370 9 Fantuzzi L, Belardelli F, Gessani S. Monocyte/macrophage-derived CC chemokines and
371 their modulation by HIV-1 and cytokines: a complex network of interactions influencing
372 viral replication and AIDS pathogenesis. *J Leukoc Biol* 2003; **74**:719–25.

373 10 Liovat A-S, Rey-Cuillé M-A, Lécuroux C, Jacquelin B, Girault I, Petitjean G, *et al.* Acute
374 Plasma Biomarkers of T Cell Activation Set-Point Levels and of Disease Progression in HIV-1
375 Infection. *PLoS One* 2012; **7**:e46143.

376 11 Lane BR, King SR, Bock PJ, Strieter RM, Coffey MJ, Markovitz DM. The C-X-C chemokine IP-
377 10 stimulates HIV-1 replication. *Virology* 2003; **307**:122–134.

378 12 van Manen D, van 't Wout AB, Schuitemaker H. Genome-wide association studies on HIV
379 susceptibility, pathogenesis and pharmacogenomics. *Retrovirology* 2012; **9**:70.

380 13 Poropatich K, Sullivan DJ. Human immunodeficiency virus type 1 long-term non-
381 progressors: the viral, genetic and immunological basis for disease non-progression. *J Gen*
382 *Virol* 2011; **92**:247–268.

383 14 Lama J, Planelles V. Host factors influencing susceptibility to HIV infection and AIDS
384 progression. *Retrovirology* 2007; **4**:52.

385 15 O'Brien TR, McDermott DH, Ioannidis JP, Carrington M, Murphy PM, Havlir D V, *et al.* Effect
386 of chemokine receptor gene polymorphisms on the response to potent antiretroviral
387 therapy. *AIDS* 2000; **14**:821–6.

388 16 Puissant B, Roubinet F, Massip P, Sandres-Saune K, Apoil P-A, Abbal M, *et al.* Analysis of
389 CCR5, CCR2, CX3CR1, and SDF1 polymorphisms in HIV-positive treated patients: impact on
390 response to HAART and on peripheral T lymphocyte counts. *AIDS Res Hum Retroviruses*
391 2006; **22**:153–62.

392 17 Limou S, Coulonges C, Herbeck JT, van Manen D, An P, Le Clerc S, *et al.* Multiple-cohort
393 genetic association study reveals CXCR6 as a new chemokine receptor involved in long-
394 term nonprogression to AIDS. *J Infect Dis* 2010; **202**:908–15.

395 18 Duggal P, An P, Beaty TH, Strathdee S a, Farzadegan H, Markham RB, *et al.* Genetic
396 influence of CXCR6 chemokine receptor alleles on PCP-mediated AIDS progression among
397 African Americans. *Genes Immun* 2003; **4**:245–50.

398 19 An P, Li R, Wang JM, Yoshimura T, Takahashi M, Samudralal R, *et al.* Role of exonic
399 variation in chemokine receptor genes on AIDS: CCRL2 F167Y association with
400 pneumocystis pneumonia. *PLoS Genet* 2011; **7**:e1002328.

401 20 McLaren PJ, Coulonges C, Bartha I, Lenz TL, Deutsch AJ, Bashirova A, *et al.* Polymorphisms
402 of large effect explain the majority of the host genetic contribution to variation of HIV-1
403 virus load. *Proc Natl Acad Sci* 2015; **112**:14658–14663.

404 21 Pena SDJ, di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy FDSG, *et al.* The
405 genomic ancestry of individuals from different geographical regions of Brazil is more
406 uniform than expected. *PLoS One* 2011; **6**. doi:10.1371/journal.pone.0017063

407 22 Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SDJ. Color and genomic

ancestry in Brazilians. *Proc Natl Acad Sci U S A* 2003; **100**:177–182.

23 Olson AD, Guiguet M, Zangerle R, Gill J, Perez-Hoyos S, Lodi S, *et al.* Evaluation of rapid progressors in HIV infection as an extreme phenotype. *J Acquir Immune Defic Syndr* 2014; **67**:15–21.

24 Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**:5444.

25 Valverde-Villegas JM, de Medeiros RM, Almeida SEM and Chies JAB. Immunogenetic profiling of 23 SNPs of cytokine and chemokine receptor genes through a minisequencing technique: Design, development and validation. *Int J Immunogenet* 2017; **In Press**.

26 Chies JAB, Hutz MH. High frequency of the CCR5delta32 variant among individuals from an admixed Brazilian population with sickle cell anemia. *Brazilian J Med Biol Res = Rev Bras Pesqui médicas e biológicas / Soc Bras Biofísica . [et al]* 2003; **36**:71–5.

27 Moore JH, Gilbert JC, Tsai C-T, Chiang F-T, Holden T, Barney N, *et al.* A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. *J Theor Biol* 2006; **241**:252–261.

28 Kira Kenji and Rendell Larry. A practical approach to feature selection. In: *Machine Learning Proceedings 1992: Proceedings of the Ninth International Workshop (ML92)*. Sleeman Dereck and Edwards Peter (editor). . Morgan Kaufmann Publishers Inc. San Francisco, CA, USA ©1992; 1992. pp. 249–256.

29 Greene CS, Penrod NM, Kiralis J, Moore JH. Spatially Uniform ReliefF (SURF) for computationally-efficient filtering of gene-gene interactions. *BioData Min* 2009; **2**:5.

30 Motsinger AA, Ritchie MD. The effect of reduction in cross-validation intervals on the performance of multifactor dimensionality reduction. *Genet Epidemiol* 2006; **30**:546–55.

31 Kim S-H, Yang E-M, Lee H-N, Choi G-S, Ye Y-M, Park H-S. Association of the CCR3 gene polymorphism with aspirin exacerbated respiratory disease. *Respir Med* 2010; **104**:626–632.

32 Amerio P, Frezzolini A, Feliciani C, Verdolini R, Teofoli P, De Pità O, *et al.* Eotaxins and CCR3 receptor in inflammatory and allergic skin diseases: therapeutical implications. *Curr Drug Targets Inflamm Allergy* 2003; **2**:81–94.

33 Lee J-H, Chang HS, Kim JH, Park S-M, Lee YM, Uh ST, *et al.* Genetic effect of CCR3 and IL5RA gene polymorphisms on eosinophilia in asthmatic patients. *J Allergy Clin Immunol* 2007; **120**:1110–7.

34 Kim S-H, Jeong H-H, Cho B-Y, Kim M, Lee H-Y, Lee J, *et al.* Association of Four-locus Gene Interaction with Aspirin-intolerant Asthma in Korean Asthmatics. *J Clin Immunol* 2008; **28**:336–342.

35 Breunis WB, Biezeveld MH, Geissler J, Kuipers IM, Lam J, Ottenkamp J, *et al.* Polymorphisms in chemokine receptor genes and susceptibility to Kawasaki disease. *Clin Exp Immunol* 2007; **150**:83–90.

36 Zingoni a, Soto H, Hedrick J a, Stoppacciaro A, Storlazzi CT, Sinigaglia F, *et al.* The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. *J Immunol* 1998; **161**:547–51.

37 Bunders MJ, van Hamme JL, Jansen MH, Boer K, Kootstra N a., Kuijpers TW. Fetal exposure to HIV-1 alters chemokine receptor expression by CD4+T cells and increases susceptibility

452 to HIV-1. *Sci Rep* 2014; **4**:6690.

453 38 Hirota T, Saeki H, Tomita K, Tanaka S, Ebe K, Sakashita M, *et al.* Variants of C-C motif
454 chemokine 22 (CCL22) are associated with susceptibility to atopic dermatitis: Case-control
455 studies. *PLoS One* 2011; **6**:e26987.

456 39 Shimada Y, Takehara K, Sato S. Both Th2 and Th1 chemokines (TARC/CCL17, MDC/CCL22,
457 and Mig/CXCL9) are elevated in sera from patients with atopic dermatitis. *J Dermatol Sci*
458 2004; **34**:201–208.

459 40 Ancuta P, Autissier P, Wurcel A, Zaman T, Stone D, Gabuzda D. CD16+ monocyte-derived
460 macrophages activate resting T cells for HIV infection by producing CCR3 and CCR4 ligands.
461 *J Immunol* 2006; **176**:5760–71.

462 41 Comerford I, Bunting M, Fenix K, Haylock-Jacobs S, Litchfield W, Harata-Lee Y, *et al.* An
463 immune paradox: how can the same chemokine axis regulate both immune tolerance and
464 activation?: CCR6/CCL20: a chemokine axis balancing immunological tolerance and
465 inflammation in autoimmune disease. *Bioessays* 2010; **32**:1067–76.

466 42 Lee AYS, Phan TK, Hulett MD, Körner H. The relationship between CCR6 and its binding
467 partners: does the CCR6-CCL20 axis have to be extended? *Cytokine* 2015; **72**:97–101.

468 43 Choi S, Lee E-K, Lee S, Chae S, Lee M, Seo G, *et al.* Ulcerative Colitis is Associated with
469 Novel Polymorphisms in the Promoter Region of MIP-3α/CCL20 Gene. *Immune Netw* 2005;
470 **5**:205.

471 44 Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, *et al.* Induction of a Striking
472 Systemic Cytokine Cascade prior to Peak Viremia in Acute Human Immunodeficiency Virus
473 Type 1 Infection, in Contrast to More Modest and Delayed Responses in Acute Hepatitis B
474 and C Virus Infections. *J Virol* 2009; **83**:3719–3733.

475 45 Lajoie J, Juno J, Burgener A, Rahman S, Mogk K, Wachihi C, *et al.* A distinct cytokine and
476 chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-
477 exposed seronegative commercial sex workers. *Mucosal Immunol* 2012; **5**:277–87.

478 46 Ramirez L a, Arango T a, Thompson E, Naji M, Tebas P, Boyer JD. High IP-10 levels decrease
479 T cell function in HIV-1-infected individuals on ART. *J Leukoc Biol* 2014; **96**:1055–1063.

480 47 Xu Z, Liu Y, Liu L, Li X, Bai S, Rong Y, *et al.* Association of Interferon-gamma Induced Protein
481 10 Promoter Polymorphisms with the Disease Progression of Hepatitis B Virus Infection in
482 Chinese Han Population. *PLoS One* 2013; **8**:1–5.

483 48 Tang NLS, Fan HPY, Chang KC, Ching JKL, Kong KPS, Yew WW, *et al.* Genetic association
484 between a chemokine gene CXCL-10 (IP-10, interferon gamma inducible protein 10) and
485 susceptibility to tuberculosis. *Clin Chim Acta* 2009; **406**:98–102.

486 49 Taheri M, Kouhpayeh HR, Hosseinalizadeh T, Naderi M. A Functional Polymorphism in
487 Promoter of the CXCL10 Gene (-135 G / A) Associated With Pulmonary Tuberculosis. *Arch*
488 *Clin Infect Dis* 2013; **8**:8–11.

489 50 Wilson N, Driss A, Solomon W, Dickinson-Copeland C, Salifu H, Jain V, *et al.* CXCL10 gene
490 promoter polymorphism -1447A>G correlates with plasma CXCL10 levels and is associated
491 with male susceptibility to cerebral malaria. *PLoS One* 2013; **8**:e81329.

492 51 Zhao K, Ishida Y, Oleksyk TK, Winkler C a, Roca AL. Evidence for selection at HIV host
493 susceptibility genes in a West Central African human population. *BMC Evol Biol* 2012;
494 **12**:237.

495

Table 1. Demographic and clinical characteristics of the HIV+ subjects and HIV- controls.

Characteristics	No. of subjects (%)[α]					
	HIV- (n=294)	HIV+ (n=206)	HIV- vs. HIV+	HIV+		RPs vs. NRPs
			P-value	RPs (n=40)	NRPs (n=166)	*P*-value
Demographic						
Median age ± SD[a]	44.43 ± 8.4	40.96 ± 10.33	NS	40.0 ±11.6	41.07 ±10.02	NS
Sex*						
Female	89 (30.6)	143 (69.4)	**< 0.001**	27 (67.5)	116 (69.9)	NS
Male	202 (68.7)	63 (30.6)		13 (32.5)	50 (30.1)	
Ethnicity*						
European-descent	228 (77.6)	120 (62.5)	**< 0.001**	24 (64.9)	96 (61.9)	NS
African-descent	66 (22.4)	72 (37.5)		13 (35.1)	59 (38.1)	
Coinfections (yes)[b]	NA	22/98 (22.4)	---	6/37 (16.2)	16/61 (26.2)	---
Exposure category[c]						
Het	NA	79 (76.0)	NA	31 (81.6)	48 (72.7)	---
MSM		12 (11.5)		5 (13.2)	7 (10.6)	
IDU		7 (6.70)		1 (2.6)	6 (9.1)	
others		6 (5.80)		1 (2.6)	5 (7.6)	
Clinical						
First CD4+ T cells count[d]	NA	560 ± 311.40	NA	321.00 ± 175.83	685.05 ± 315.53	**< 0.001**

First RNA viral load[e]	3.82 ± 0.98	4.29 ± 0.66	3.49 ± 1.04	< **0.001**
Slope CD4+ T-cell count[f]	0.E-7 ± 0.99	-0.66 ± 1.53	0.36 ± 0.21	< **0.001**
Median RNA viral load[g]	3.89 ± 0.76	4.28 ± 0.69	3.64 ± 0.71	< **0.001**
Time progression ± SD[h]	86.27 ± 56.8	13.50 ± 6.15	114.12 ± 39.8	< **0.001**
HAART (yes)	75 (73.5)	38 (100.0)	37 (57.8)	---

[α] Percentages are based on known data; [a] median (IQ) in years; [b] HCV and/or HBV and/or HTLV; [c] exposure category: Het - heterosexual, MSM - men who have sex with men; IDU - injecting drug user; [d] median (IQ) in cells/mm3 available for 83 patients; [e] median (IQ) in log10 copies/mL available for 78 patients; [f] estimated for data pre-HAART available for 83 patients; [g] estimated for data pre-HAART in log10 copies/mL; [h] median (IQ) in months.
RPs, rapid progressors; NRPs, non-rapid progressors.

* Missing data (14 HIV+ individuals ethnic origin unknown and 3 HIV- individuals with sex unknown); SD, standard deviation; NA, not applicable; NS, not significant.

P< 0.001

Table 2. Binomial logistic regression in susceptibility to HIV infection comparing HIV+ and HIV- subjects.

Gene	SNPs	Models	Genotypes	HIV+ (n=192)	HIV- (n=291)	Univariate logistic regression		Multivariate logistic regression	
				n (frequency)	n (frequency)	AOR* (95% CI)	*P-value*	AOR* (95% CI)	*P-value*
CCR3	rs3091250	Codominant	GG	90 (0.47)	162 (0.56)	1		1	
			GT	76 (0.40)	106 (0.36)	1.18 (0.76-1.81)	0.462	1.15 (0.74-1.81)	0.533
			TT	25 (0.13)	23 (0.08)	2.15 (1.08-4.29)	**0.030**	2.26 (1.09-4.70)	**0.029**
CCR8	rs2853699	Dominant	GG	94 (0.49)	163 (0.56)	1		1	
			GC+CC	98 (0.51)	128 (0.44)	1.58 (1.05-2.37)	**0.029**	1.51 (0.99-2.31)	0.056
CXCL10	rs56061981	Dominant	CC	139 (0.76)	243 (0.86)	1		1	
			CT+TT	43 (0.24)	39 (0.14)	1.82 (1.07-3.08)	**0.026**	1.75 (1.02-3.01)	**0.042**
CCL22	rs4359426	Dominant	CC	163 (0.86)	258 (0.89)	1		1	
			CA+AA	27 (0.14)	33 (0.11)	1.89 (1.02-3.49)	**0.043**	1.84 (0.96-3.52)	0.067

$P < 0.05$ univariate and multivariate logistic regression analyses, * AOR, adjusted odds ratio for sex and ethnic origin (only patients that have all data were included in these analyses); CI, confidence interval.

Significant association is shown in boldface.

Table 3. Binomial logistic regression in AIDS progression.

Gene	SNPs	Models	Genotypes	RPs (n=40) n (frequency)	NRPs (n=166) n (frequency)	Univariate logistic regression		Multivariate logistic regression	
						OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
CCL20	rs13034664	Recessive	TT+CT	37 (0.92)	117 (0.71)	1		1	
			CC	3 (0.07)	47 (0.29)	0.21 (0.06-0.73)	**0.014**	0.21 (0.06-0.72)	**0.013**
CCL22	rs4359426	Dominant	CC	30 (0.75)	137 (0.89)	1		1	
			CA+AA	10 (0.25)	18 (0.11)	2.68 (1.13-6.39)	**0.026**	2.73 (1.12-6.66)	**0.028**

$P < 0.05$ univariate and multivariate logistic regression analyses, OR, Odds ratio; CI, confidence interval. RPs, rapid progressors; NRPs, non-rapid progressors.
Significant association is shown in boldface.

Table 4. The best model of locus interaction by the multifactor dimensionality reduction (MDR) to predict susceptibility to HIV infection.

Models	**Training balanced accuracy**	**Testing balanced accuracy**	**Cross-validation consistency**	***P*-value***
CCR3 rs56061981	0.5591	0.5270	7/10	0.3
***CCL22* rs4359426, *CXCL10* rs56061981**	**0.5832**	**0.5696**	**9/10**	**0.008**
CCL20 rs13034664, *CCR6 rs968334*, *CXCL10* rs56061981	0.6122	0.5718	9/10	0.007
CCL20 rs13034664, CCL22 rs4359426, *CCR6 rs968334*, *CXCL10* rs56061981	0.6375	0.5817	7/10	< 0.001

*Evaluated using a 1000-fold permutation test to compare observed testing accuracies with those expected under the null hypothesis of null association. In bold, the best model by MDR.

Figure 1

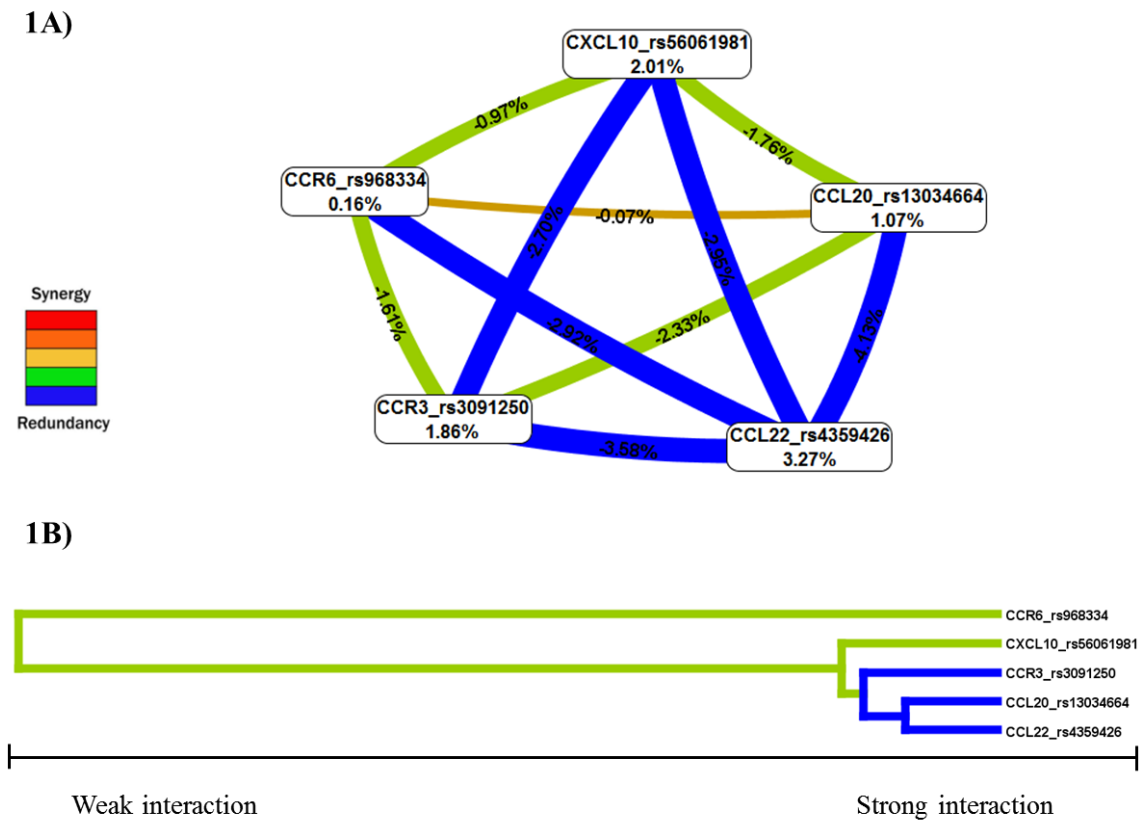


Figure 1. Multifactor dimensionality reduction (MDR) interaction model generated by MDR software. (A) The interaction circle graph comprised of nodes with pairwise connections between them. Values in nodes represent information gain (IG) of individual genes (main effect); values between nodes are the IG of each pairwise combination (interaction effects). A positive IG indicates a synergistic or non-additive effect; a negative IG indicates redundancy or correlation. The line colours indicate the type of interaction: Red and orange, synergistic (i.e. epistasis); green and blue, redundancy or correlation. Our results show the great percentages of the redundancy entropy interaction between the loci analysed. *CCR3* rs3091250 (1.86%), *CXCL10* rs56061981 (2.01%) and *CCL22* rs4359426 (3.27%) had a large main effect on susceptibility to HIV infection. In addition, the predominant additive effects between pairs were for *CCL22* rs4359426, *CCL20* rs13034664, *CCR3* rs3091250. Notably, there was an epistatic effect between *CCR6* rs968334 and *CCL20* rs13034664. (B) The dendrogram graphic showing the level of interaction between attributes. The colours used are attributed the same meanings described in (A).

Table S1. Demographic and clinical characteristics of HIV progressor subjects.

Characteristics	RPs (n=40)	SPs (n=87)	ECs (n=8)	*P-value* RPs vs. SPs	*P-value* RPs vs. ECs
Demographic					
Median age ± SD[a]	40.0±11.6	42.0±9.6	36.5±8.6	NS	NS
Sex					
Female	27 (67.5)	64 (73.6)	1 (12.5)	NS	NS
Male	13 (32.5)	23 (26.4)	7 (87.5)		
Ethnicity*					
European-descent	24/37 (64.9)	44/84 (52.4)	8 (100)	NS	0.048
African-descent	13/37 (35.1)	40/84 (47.6)	0 (0.0)		
Coinfections (yes)[b]	6/37 (16.2)	16/61 (26.2)	0 (0.0)	NS	
Exposure category[c]					
Het	31 (77.5)	47 (72.3)	1 (12.5)	NS	NS
MSM	5 (12.5)	7 (10.8)	0 (0.0)		
IDU	1 (2.5)	6 (9.2)	0 (0.0)		
others	1 (2.5)	5 (7.7)	7 (87.5)		
Clinical					
First CD4+ T cells count[d]	321.00 ± 175.83	589.00 ± 315.53	NA	**< 0.001**	NA
First RNA viral load[e]	4.29 ± 0.66	3.665 ± 1.04	NA	**< 0.001**	
Slope CD4+ T-cell count[f]	-0.66 ± 1.53	0.400 ± 0.22	NA	**< 0.001**	
Median RNA viral load[g]	4.28 ± 0.69	3.74 ± 0.71	NA	**< 0.001**	
Time progression ± SD[h]	13.50 ± 6.15	114.12 ± 39.8	NA	**< 0.001**	
HAART (yes)	38 (100.0)	37 (57.8)	0 (0.0)	---	

RPs, rapid progressors; SPs, slow progressors; ECs, elite controllers. * Missing data; SD, standard deviation; NA, not applicable; NS, not significant.

[α] Percentages are based on known data; [a] median (IQ) in years; [b] HCV and/or HBV and/or HTLV; [c] exposure category: Het - heterosexual, MSM - men who have sex with men; IDU - injecting drug user; [d] median (IQ) in cells/mm3; [e] median (IQ) in log10 copies/mL; [f] estimated for data pre-HAART; [g] estimated for data pre-HAART in log10 copies/mL; [h] median (IQ) in months. $P < 0.001$

Table S2. Allelic and genotypic frequencies for all 15 SNPs in HIV+ subjects and controls.

Gene	SNPs	HIV+ (n=206)	HIV- (n=294)	RPs (n=40)	NRPs (n=166)	SPs (n=87)	ECs (n=8)
CCR3	rs3091250						
	GG	97 (0.475)	164 (0.558)	17 (0.436)	80 (0.485)	38 (0.442)	5 (0.625)
	GT	79 (0.387)	106 (0.361)	15 (0.385)	64 (0.388)	34 (0.395)	3 (0.375)
	TT	28 (0.137)	24 (0.082)	7 (0.179)	21 (0.127)	14 (0.163)	0 (0.000)
	G	273 (0.669)	434 (0.738)	49 (0.628)	224 (0.679)	110 (0.640)	13 (0.813)
	T	135 (0.331)	154 (0.262)	29 (0.372)	106 (0.321)	62 (0.360)	3 (0.188)
CCR4	rs6770096						
	CC	135 (0.754)	220 (0.821)	27 (0.771)	108 (0.750)	50 (0.725)	5 (0.714)
	CT	41 (0.229)	44 (0.164)	8 (0.229)	33 (0.229)	17 (0.246)	2 (0.286)
	TT	3 (0.017)	4 (0.015)	0 (0.000)	3 (0.021)	2 (0.029)	0 (0.000)
	C	311 (0.869)	484 (0.903)	62 (0.886)	249 (0.865)	117 (0.848)	12 (0.857)
	T	47 (0.131)	52 (0.097)	8 (0.114)	39 (0.135)	21 (0.152)	2 (0.143)
	rs2228428						
	CC	136 (0.663)	171 (0.582)	28 (0.700)	108 (0.655)	58 (0.674)	5 (0.625)
	CT	58 (0.283)	101 (0.344)	11 (0.275)	47 (0.285)	24 (0.279)	3 (0.375)
	TT	11 (0.054)	22 (0.075)	1 (0.025)	10 (0.061)	4 (0.047)	0 (0.000)
	C	330 (0.805)	443 (0.753)	67 (0.838)	263 (0.797)	140 (0.814)	13 (0.813)
	T	80 (0.195)	145 (0.247)	13 (0.163)	67 (0.203)	32 (0.186)	3 (0.188)
CCR5	rs333						
	wt/wt	170 (0.876)	246 (0.875)	33 (0.917)	137 (0.867)	74 (0.860)	7 (0.875)
	wt/del32	24 (0.124)	33 (0.117)	3 (0.083)	21 (0.133)	12 (0.140)	1 (0.125)
	del32/del32	0 (0.000)	2 (0.007)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
	wt	382 (0.938)	525 (0.934)	69 (0.958)	295 (0.934)	160 (0.930)	15 (0.938)
	del32	24 (0.062)	37 (0.066)	3 (0.042)	21 (0.066)	12 (0.070)	1 (0.062)
CCR6	rs968334						
	GG	83 (0.403)	116 (0.395)	19 (0.475)	64 (0.386)	29 (0.333)	3 (0.375)
	GA	101 (0.490)	137 (0.466)	17 (0.425)	84 (0.506)	47 (0.540)	3 (0.375)
	AA	22 (0.107)	41 (0.139)	4 (0.100)	18 (0.108)	11 (0.126)	2 (0.250)
	G	267 (0.648)	369 (0.628)	55 (0.688)	212 (0.639)	105 (0.603)	9 (0.563)
	A	145 (0.352)	219 (0.372)	25 (0.313)	120 (0.361)	69 (0.397)	7 (0.438)
CCR8	rs2853699						
	GG	101 (0.493)	166 (0.565)	20 (0.500)	81 (0.491)	45 (0.523)	4 (0.500)
	GC	89 (0.434)	114 (0.388)	16 (0.400)	73 (0.442)	36 (0.419)	4 (0.500)
	CC	15 (0.073)	14 (0.048)	4 (0.100)	11 (0.067)	5 (0.058)	0 (0.00)
	G	291 (0.710)	446 (0.759)	56 (0.700)	235 (0.712)	126 (0.733)	12 (0.750)
	C	119 (0.290)	142 (0.241)	24 (0.300)	95 (0.288)	46 (0.267)	4 (0.250)
CXCR3[a]	rs34334103						
	GG ♀	128 (0.901)	87 (0.978)	22 (0.815)	106 (0.922)	60 (0.952)	6 (0.857)
	GA ♀	14 (0.099)	2 (0.022)	5 (0.185)	9 (0.078)	3 (0.048)	1 (0.143)

	AA ♀	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
	G ♀	270 (0.951)	176 (0.989)	49 (0.907)	221 (0.961)	123 (0.976)	13 (0.929)
	A ♀	14 (0.049)	2 (0.011)	5 (0.093)	9 (0.039)	3 (0.024)	1 (0.071)
	G/- ♂	60 (1.00)	195 (0.965)	13 (1.000)	47 (1.000)	21 (1.000)	1 (1.000)
	A/- ♂	0 (0.000)	7 (0.035)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
	rs2280964						
	CC ♀	89 (0.622)	63 (0.708)	18 (0.667)	71 (0.612)	42 (0.656)	3 (0.429)
	CT ♀	49 (0.343)	26 (0.292)	9 (0.333)	40 (0.345)	19 (0.297)	4 (0.571)
	TT ♀	5 (0.035)	0 (0.000)	0 (0.000)	5 (0.043)	3 (0.047)	0 (0.000)
	C ♀	227 (0.794)	152 (0.854)	45 (0.833)	182 (0.784)	103 (0.805)	10 (0.714)
	T ♀	59 (0.206)	26 (0.146)	9 (0.167)	50 (0.216)	25 (0.195)	4 (0.286)
	C/- ♂	48 (0.828)	151 (0.778)	10 (0.833)	38 (0.826)	18 (0.857)	1 (1.000)
	T/- ♂	10 (0.172)	43 (0.222)	2 (0.167)	8 (0.174)	3 (0.143)	0 (0.000)
	rs2234355						
	GG	167 (0.815)	249 (0.847)	36 (0.900)	131 (0.794)	62 (0.721)	7 (0.875)
	GA	33 (0.161)	38 (0.129)	3 (0.075)	30 (0.182)	21 (0.244)	1 (0.125)
	AA	5 (0.024)	7 (0.024)	1 (0.025)	4 (0.024)	3 (0.035)	0 (0.000)
	G	367 (0.895)	536 (0.912)	75 (0.938)	292 (0.885)	145 (0.843)	15 (0.938)
CXCR6	A	43 (0.105)	52 (0.088)	5 (0.063)	38 (0.115)	27 (0.157)	1 (0.063)
	rs2234358						
	GG	61 (0.338)	84 (0.288)	13 (0.325)	53 (0.342)	28 (0.354)	3 (0.375)
	GT	77 (0.431)	139 (0.476)	17 (0.425)	67 (0.432)	32 (0.405)	4 (0.500)
	TT	42 (0.231)	69 (0.236)	10 (0.250)	35 (0.226)	19 (0.241)	1 (0.125)
	G	199 (0.553)	307 (0.526)	43 (0.538)	173 (0.558)	88 (0.557)	10 (0.625)
	T	161 (0.447)	277 (0.474)	37 (0.463)	137 (0.442)	70 (0.443)	6 (0.375)
	rs3921						
	CC	84 (0.410)	120 (0.408)	20 (0.500)	64 (0.388)	41 (0.477)	4 (0.500)
	GC	98 (0.478)	150 (0.510)	16 (0.400)	82 (0.497)	35 (0.407)	2 (0.250)
	GG	23 (0.112)	24 (0.082)	4 (0.100)	19 (0.115)	10 (0.116)	2 (0.250)
	C	266 (0.649)	390 (0.663)	56 (0.700)	210 (0.636)	117 (0.680)	10 (0.625)
CXCL10	G	144 (0.351)	198 (0.337)	24 (0.300)	120 (0.364)	55 (0.320)	6 (0.375)
	rs56061981						
	CC	149 (0.768)	245 (0.860)	31 (0.861)	118 (0.747)	57 (0.704)	7 (0.875)
	CT	39 (0.201)	40 (0.140)	4 (0.111)	35 (0.222)	22 (0.271)	1 (0.125)
	TT	6 (0.031)	0 (0.000)	1 (0.028)	5 (0.032)	2 (0.025)	0 (0.000)
	C	337 (0.869)	530 (0.930)	66 (0.917)	271 (0.858)	136 (0.840)	15 (0.937)
	T	51 (0.131)	40 (0.070)	6 (0.083)	45 (0.142)	26 (0.160)	1 (0.063)
	rs13034664						
	TT	47 (0.230)	60 (0.204)	13 (0.325)	32 (0.195)	25 (0.294)	1 (0.125)
	CT	109 (0.534)	159 (0.541)	24 (0.600)	85 (0.518)	46 (0.541)	3 (0.375)
CCL20	CC	48 (0.235)	75 (0.255)	3 (0.075)	47 (0.287)	14 (0.165)	4 (0.500)
	T	203 (0.498)	279 (0.474)	50 (0.625)	149 (0.454)	96 (0.565)	5 (0.313)
	C	205 (0.502)	309 (0.526)	30 (0.375)	179 (0.546)	74 (0.435)	11 (0.688)

	rs6749704						
	TT	110 (0.539)	165 (0.561)	20 (0.513)	90 (0.545)	49 (0.570)	3 (0.375)
	CT	78 (0.382)	106 (0.361)	13 (0.333)	65 (0.394)	32 (0.372)	4 (0.500)
	CC	16 (0.078)	23 (0.078)	6 (0.154)	10 (0.061)	5 (0.058)	1 (0.125)
	T	298 (0.730)	436 (0.741)	53 (0.679)	245 (0.742)	130 (0.756)	10 (0.625)
	C	110 (0.270)	152 (0.259)	25 (0.321)	85 (0.258)	42 (0.244)	6 (0.375)
	rs4359426						
	CC	175 (0.862)	261 (0.888)	30 (0.750)	145 (0.890)	69 (0.821)	7 (0.875)
CCL22	CA	25 (0.123)	33 (0.112)	9 (0.225)	16 (0.098)	14 (0.167)	1 (0.125)
	AA	3 (0.015)	0 (0.000)	1 (0.025)	2 (0.012)	1 (0.012)	0 (0.000)
	C	375 (0.924)	555 (0.944)	69 (0.863)	306 (0.939)	152 (0.905)	15 (0.938)
	A	31 (0.076)	33 (0.056)	11 (0.138)	20 (0.061)	16 (0.095)	1 (0.063)

RPs, rapid progressors; NRPs, non-rapid progressors; SPs, slow progressors; Ecs, ellite controllers.
[a]*CXCR3* is a gene located on X chromosome; therefore data is shown by sex.

Table S3. Allelic frequencies of SNPs analyzed in this study compared with the HapMap database frequencies.

Gene	SNP/ Alleles / Genotypes	HapMap database		This study (HIV+) n=206		This study (HIV-) n=294	
		European-descent population	African-descent population	European-descent n (f)	African-descent n (f)	European-descent n (f)	African-descent n (f)
CCR3	rs3091250						
	G	0.735	0.787	161 (0.676)	95 (0.660)	332 (0.728)	102 (0.773)
	T	0.265	0.213	77 (0.324)	49 (0.340)	124 (0.272)	30 (0.227)
	GG	0.539	0.610	55 (0.462)	35 (0.486)	123 (0.539)	41 (0.621)
	GT	0.392	0.354	51 (0.429)	25 (0.347)	86 (0.377)	20 (0.303)
	TT	0.070	0.036	13 (0.109)	12 (0.167)	19 (0.083)	5 (0.076)
CCR4	rs2228428						
	C	0.686	0.964	189 (0.788)	121 (0.840)	326 (0.715)	117 (0.886)
	T	0.314	0.036	51 (0.213)	23 (0.160)	130 (0.285)	15 (0.114)
	CC	0.467	0.929	75 (0.625)	53 (0.736)	120 (0.526)	51 (0.773)
	CT	0.437	0.071	39 (0.325)	15 (0.208)	86 (0.377)	15 (0.227)
	TT	0.095	0.000	6 (0.050)	4 (0.056)	22 (0.096)	0 (0.000)
CCR5	rs6770096						
	C	0.931	0.744	186 (0.869)	108 (0.871)	404 (0.898)	80 (0.930)
	T	0.069	0.256	28 (0.131)	16 (0.129)	46 (0.102)	6 (0.070)
	CC	0.875	0.558	80 (0.748)	48 (0.774)	183 (0.813)	37 (0.860)
	CT	0.113	0.372	26 (0.243)	12 (0.194)	38 (0.169)	6 (0.140)
	TT	0.012	0.070	1 (0.009)	2 (0.032)	4 (0.018)	0 (0.000)
	rs333						
	wt	0.952	1.000	219 (0.936)	134 (0.944)	417 (0.923)	108 (0.982)
	del32	0.048	0.000	15 (0.064)	8 (0.056)	35 (0.077)	2 (0.018)
	wt/wt	0.903	1.000	102 (0.872)	63 (0.887)	193 (0.854)	53 (0.964)
	wt/del32	0.097	0.000	15 (0.128)	8 (0.113)	31 (0.137)	2 (0.036)
	del32/del32	0.000	0.000	0 (0.000)	0 (0.000)	2 (0.009)	0 (0.000)
CCR6	rs968334						
	G	0.573	0.694	153 (0.638)	95 (0.660)	279 (0.612)	90 (0.682)
	A	0.427	0.306	87 (0.363)	49 (0.340)	177 (0.338)	42 (0.318)
	GG	0.342	0.481	47 (0.392)	31 (0.431)	85 (0.373)	31 (0.470)
	GA	0.461	0.425	59 (0.492)	33 (0.458)	109 (0.478)	28 (0.424)
	AA	0.197	0.094	14 (0.117)	8 (0.111)	34 (0.149)	7 (0.106)
CCR8	rs2853699						
	C	0.702	0.899	**160 (0.667)[a]**	112 (0.778)	**351 (0.770)[b]**	95 (0.720)
	G	0.298	0.101	**80 (0.333)[a]**	32 (0.222)	**105 (0.230)[b]**	37 (0.280)
	CC	0.495	0.811	50 (0.417)	44 (0.611)	132 (0.579)	34 (0.515)
	CG	0.414	0.175	60 (0.500)	24 (0.333)	87 (0.382)	27 (0.409)
	GG	0.091	0.014	10 (0.083)	4 (0.056)	9 (0.039)	5 (0.076)
CXCR6	rs2234355						

	G	0.995	0.509	**221 (0.921)[c]**	**122 (0.847)[d]**	**442 (0.969)[e]**	**94 (0.712)[f]**
	A	0.005	0.491	**19 (0.079)[c]**	**22 (0.153)[d]**	**14 (0.031)[e]**	**38 (0.288)[f]**
	GG	0.990	0.268	102 (0.850)	54 (0.750)	214 (0.939)	35 (0.530)
	GA	0.010	0.483	17 (0.142)	14 (0.194)	14 (0.061)	24 (0.364)
	AA	0.000	0.250	1 (0.008)	4 (0.056)	0 (0.000)	7 (0.106)
	rs2234358						
	G	0.531	0.315	133 (0.578)	74 (0.544)	254 (0.559)	53 (0.408)
	T	0.469	0.685	97 (0.422)	62 (0.456)	200 (0.441)	77 (0.592)
	GG	0.288	0.104	40 (0.578)	24 (0.353)	73 (0.322)	11 (0.169)
	GT	0.485	0.421	53 (0.461)	26 (0.382)	108 (0.476)	31 (0.477)
	TT	0.227	0.475	22 (0.191)	18 (0.265)	46 (0.203)	23 (0.354)
	rs34334103						
	G ♀	0.999	0.994	160 (0.941)	98 (0.980)	144 (0.986)	32 (1.000)
	A ♀	0.001	0.006	10 (0.059)	2 (0.020)	2 (0.014)	0 (0.000)
	GG ♀	0.521	0.510	75 (0.882)	48 (0.960)	71 (0.973)	16 (1.000)
	GA ♀	0.002	0.006	10 (0.118)	2 (0.004)	2 (0.027)	0 (0.000)
CXCR3	AA ♀	NA	0.002	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
	rs2280964						
	C ♀	0.748	0.653	134 (0.788)	81 (0.810)	124 (0.849)	28 (0.875)
	T ♀	0.252	0.347	36 (0.212)	19 (0.190)	22 (0.151)	4 (0.125)
	CC ♀	0.664	0.551	53 (0.624)	31 (0.620)	51 (0.699)	12 (0.750)
	CT ♀	0.168	0.204	28 (0.329)	19 (0.380)	22 (0.301)	4 (0.250)
	TT ♀	0.168	0.245	4 (0.047)	0 (0.000)	0 (0.000)	0 (0.000)
	rs3921						
	C	0.492	0.263	148 (0.617)	100 (0.694)	298 (0.654)	92 (0.697)
	G	0.508	0.737	92 (0.383)	44 (0.306)	158 (0.346)	40 (0.303)
	CC	0.235	0.076	45 (0.375)	33 (0.458)	92 (0.404)	28 (0.424)
	CG	0.515	0.367	58 (0.483)	34 (0.472)	114 (0.500)	36 (0.545)
	GG	0.250	0.509	17 (0.142)	5 (0.069)	22 (0.096)	2 (0.030)
CXCL10	rs56061981						
	C	0.966	0.864	**194 (0.858)[g]**	121 (0.877)	**421 (0.936)[h]**	109 (0.908)
	T	0.034	0.136	**32 (0.142)[g]**	17 (0.123)	**29 (0.064)[h]**	11 (0.092)
	CC	0.934	0.741	84 (0.743)	55 (0.797)	196 (0.871)	49 (0.817)
	CT	0.064	0.245	26 (0.230)	11 (0.159)	29 (0.129)	11 (0.183)
	TT	0.002	0.014	3 (0.027)	3 (0.043)	0 (0.000)	0 (0.000)
	rs13034664						
	T	0.256	0.732	108 (0.450)	83 (0.585)	202 (0.443)	77 (0.583)
	C	0.744	0.268	132 (0.550)	59 (0.415)	254 (0.557)	55 (0.417)
	TT	0.072	0.545	21 (0.175)	23 (0.324)	41 (0.180)	19 (0.288)
CCL20	TC	0.370	0.375	66 (0.550)	37 (0.521)	120 (0.526)	39 (0.591)
	CC	0.559	0.080	33 (0.275)	11 (0.155)	67 (0.294)	8 (0.121)
	rs6749704						
	T	0.766	0.859	171 (0.718)	108 (0.692)	332 (0.728)	104 (0.788)
	C	0.234	0.141	67 (0.282)	48 (0.308)	124 (0.272)	28 (0.212)

	TT	0.590	0.746	64 (0.538)	39 (0.500)	124 (0.544)	41 (0.621)
	TC	0.352	0.027	43 (0.361)	30 (0.385)	84 (0.368)	22 (0.333)
	CC	0.058	0.227	12 (0.101)	9 (0.115)	20 (0.088)	3 (0.045)
	rs4359426						
	C	0.965	0.952	215 (0.903)	135 (0.951)	428 (0.939)	127 (0.962)
CCL22	A	0.035	0.048	23 (0097)	7 (0.049)	28 (0.061)	5 (0.038)
	CC	0.930	0.909	99 (0.832)	64 (0.901)	200 (0.877)	61 (0.924)
	CA	0.070	0.085	17 (0.143)	7 (0.099)	28 (0.123)	5 (0.076)
	AA	0.000	0.006	3 (0.025)	0 (0.000)	0 (0.000)	0 (0.000)

Fisher's exact test P<0.05: $\boldsymbol{P_{axb}}$**=0.09**; cxe $\boldsymbol{P_{cxe}}$**=0.016**; $\boldsymbol{P_{dxf}}$**=0.027**; $\boldsymbol{P_{gxh}}$**=0.02**

Table S4. Binomial logistic regression in European descent individuals comparing HIV+ and HIV-.

Genes	SNPs	Models	Genotypes	HIV+ (n=120)	HIV- (n=228)	Univariate logistic regression		Multivariate logistic regression	
				n (Frequency)	n (Frequency)	OR (95% CI)	*P-value*[*]	OR (95% CI)	*P-value*[*]
CXCR6	rs2234355	Dominant	GA+AA	18 (0.15)	14 (0.06)	2.70 (1.29-5.64)	**0.008**	2.98 (1.37-6.49)	**0.006**
			GG	102 (0.85)	214 (0.94)	1		1	
CCR8	rs2853699	Dominant	GC+CC	104 (0.51)	128 (0.44)	1.92 (1.23-3.01)	**0.004**	2.02 (1.25-3.24)	**0.004**
			GG	101 (0.49)	166 (0.56)	1		1	
IP-10	rs56061981	Dominant	CT+TT	45 (0.23)	40 (0.14)	2.33 (1.31-4.14)	**0.004**	2.14 (1.19-3.87)	**0.011**
			CC	149 (0.77)	245 (0.86)	1		1	

[*]$P < 0.05$ univariate and multivariate logistic regression analyses, OR, odds ratio; CI, confidence interval.

Significant association is shown in boldface.

Table S5. Binomial logistic regression in African descent individuals comparing HIV+ and HIV-.

Gene	SNPs	Models	Genotypes	HIV+ (n=72)	HIV- (n=66)	Univariate logistic regression		Multivariate logistic regression	
				n (Frequency)	n (Frequency)	OR (95% CI)	*P-value*[*]	OR (95% CI)	*P-value*[*]
CXCR6	rs2234355	dominant	GA+AA	18 (0.25)	31 (0.47)	0.38 (0.18-0.77)	**0.008**	0.45 (0.21-0.99)	**0.049**
			GG	54 (0.75)	35 (0.53)	1		1	
	rs2234358	dominant	GT+TT	44 (0.65)	54 (0.83)	0.37 (0.16-0.85)	**0.018**	0.51 (0.21-1.22)	0.131
			GG	24 (0.35)	11 (0.17)	1		1	

[*]$P < 0.05$ univariate and multivariate logistic regression analyses, OR, odds ratio; CI, confidence interval.

Significant association is shown in boldface.

Capítulo 4

Chemokine levels in AIDS progression: CXCL10/IP-10 is an immunological biomarker in pre-HAART clinical stage

Running head: CXCL10 is a biomarker in pre-HAART

Jacqueline M. VALVERDE-VILLEGAS, Rúbia Marília DE MEDEIROS, Joel Henrique ELLWANGER, Breno Riegel DOS SANTOS, Sabrina Esteves de Matos ALMEIDA, José Artur Bogo CHIES.

Artigo submetido para a revista JAIDS.

"O objetivo deste artigo foi investigar os níveis plasmáticos das quimiocinas CXCL10, CCL17, CCL22, CCL2, CCL24, CCL20, em progressores extremos, em diferentes estágios clínicos da infecção pré- e pós-HAART."

Chemokine levels in AIDS progression: CXCL10/IP-10 is an immunological biomarker in pre-HAART clinical stage

Running head: CXCL10 is a biomarker in pre-HAART

Jacqueline M. VALVERDE-VILLEGAS[1,2], Rúbia Marília DE MEDEIROS[1,2], Joel Henrique ELLWANGER[1], Breno Riegel dos SANTOS[3], Marineide Gonçalves de MELLO[3], Sabrina Esteves de Matos ALMEIDA[1,2,5], José Artur Bogo CHIES[1].

[1] Departamento de Genética e Programa de Pós-graduação em Genética e Biologia Molecular da UFRGS (PPGBM)

[2] Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS)

[3] Serviço de Infectologia, Grupo Hospitalar Nossa Senhora da Conceição (GHC)

[5] Instituto de Ciências da Saúde, Universidade Feevale (FEEVALE)

Total number of words: 2,844

Corresponding author:

Dr José Artur Bogo Chies. Email address: jabchies@terra.com.br

Laboratory of Immunogenetics, Institute of Biosciences, Department of Genetics, UFRGS.

Av. Bento Gonçalves – 9500, Campus do Vale. 91501970

Porto Alegre, RS-Brazil. PO BOX 15053

Phone: +55 51 3308 6740; Fax: +55 51 3308 7311

This work was supported by Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) [Grant number 12/2151-2]; Jacqueline M. Valverde-Villegas received a scholarship from the Programa Estudantes-Convênio de Pós-Graduação (CAPES PEC-PG).

Abstract

Objective: To investigate plasma levels of CXCL10/IP-10, CCL20/MIP-3α and CCL2/MCP-1, CCL17/TARC, CCL24/eotaxin-2 and CCL22/MDC, evaluating their potential as biomarkers of HIV/AIDS and their correlation with specific immune profiles.

Design: HIV-positive patients with extreme phenotypes of progression to AIDS (n=58) at different stages of infection (chronic individuals, both pre–highly active antiretroviral therapy [HAART] and under HAART) and HIV-negative controls (n=20) were evaluated. Also, HIV-positive individuals that initiated HAART with >350 $CD4^+$T cells/mm^3 were compared with those who initiated treatment with <350 $CD4^+$T cells/mm^3.

Methods: Chemokine plasma levels were quantified using Human Cytokine/Chemokine magnetic bead panels by Luminex assay.

Results: Higher CXCL10 levels were observed in individuals immediately before their $CD4^+$T cell levels were indicative for HAART, independently of their progressor status, i.e. slow (SP) or rapid progressor (RP). SPs pre-HAART showed higher CXCL10 levels compared to elite controllers and RPs under HAART (p_c=0.009 and p_c=0.007, respectively). CXCL10 levels were higher in SPs HAART CD4<350 (initiated HAART with <350 $CD4^+$T cells) when compared with SPs HAART CD4>350 (initiated HAART with >350 $CD4^+$T cells) (1096 vs. 360.33 pg/mL, p=0.0101). Thus, normalisation of plasma CXCL10 levels is dependent on the $CD4^+$T-cell nadir at HAART initiation. CCL20 levels were higher in chronic SPs, SPs pre-HAART, SPs HAART and RPs HAART compared with the HIV-negative controls, indicating persistent expression of CCL20.

Conclusions: Our results encourage measuring CXCL10 and CCL20 during the clinical monitoring of HIV-positive subjects. CXCL10 levels could be used as an auxiliary tool in decision-making related to HAART initiation (even in chronic individuals with >500 $CD4^+$T cells).

Key words: CXCL10; CCL20; AIDS progression; HAART.

Introduction

Chemokines are chemotactic cytokines [1] that control immune cell homing and trafficking throughout the body, mediating various human health and disease processes [2]. In HIV pathogenesis, MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5) and SDF-1 (CXCL12) were initially shown to be able to suppress HIV-1 infection. Distinct chemokines expressed by activated T cells with different profiles (Th1, Th2, Th17 or Treg) are of great interest in the field of HIV research due to their effect as chemoattractants of key immune cells to sites of infection. Furthermore, such molecules can activate T cells, mainly at the initial stage of infection, which is crucial and determinant for AIDS progression [3][4].

Several studies have been done with a focus on chemokine levels in the context of HIV infection. For example, IP-10 (CXCL10) produced by Th1 cells stimulates HIV-1 replication [5]. In addition, high CXCL10 plasma levels during early infection are considered predictive of rapid progression to AIDS [6]. CCL20 participates in the migration and trafficking of Th17, Tregs and dendritic cells (DCs) to sites of infection through its unique receptor CCR6 [7]. Plasma CCL20 levels are significantly higher in HIV-positive men with <200 $CD4^+$ T cells/mm^3 compared to HIV-positive men with >200 $CD4^+$ T cells/mm^3 [8]. Moreover, CCL20 is persistent in the HIV infection course in HIV-positive subjects under highly active antiretroviral therapy (HAART) [9]. Th2 cells can be characterised by the expression of a specific chemokine subset, including CCL2, CCL17, CCL22 and CCL24, suggested to play a role in HIV/AIDS infection [10][11][12][10]. CCL24 and CCL2, with stimulation via CD3/CD28, render T cells highly permissive for productive HIV infection [12]. Furthermore, high levels of CCL2 have been found in the cerebrospinal fluid (CSF) of HIV-positive subjects and associated with viral load in HIV infection [13], and CCL17 was associated with allergic situations in HIV-infected individuals, such as eosinophilic folliculitis [10].

74 HAART is a procedure that plays an important role in the recovery of $CD4^+$ T counts and
75 increases survival in HIV-infected individuals [14]. Several studies strongly suggest that better
76 results are obtained if HAART is initiated early [15]. Moreover, pro-inflammatory cytokine and
77 chemokine levels are normalised 1–2 years after HAART initiation, while the levels of other
78 molecules, such as CXCL10, CCL2, CCL20 and CCL5, remain elevated [16][9]. Recent studies have
79 suggested that adequate immunological system recovery depends on the $CD4^+$ T cell count when
80 HAART is initiated [17]. For instance, after HAART initiation, Jarrin et al. [18] observed that rapid
81 progressors (RPs) experienced faster initial $CD4^+$ T cell count increases when compared to non-RPs
82 or slow progressors (SPs). However, these patients were less likely to achieve optimal immune
83 restoration during the first 36 months after HAART, probably due to the lower $CD4^+$ T cell counts
84 at HAART initiation [18]. Thus, $CD4^+$ T cell counts at HAART initiation could be critical in the
85 modulation of cytokines/chemokines in the time after HAART initiation in HIV-1–positive
86 individuals with extreme phenotypes (RPs and SPs).

87 Taking the above into consideration, the aim of the present study was to investigate
88 differences in plasma CXCL10, CCL20, CCL22, CCL2, CCL17 and CCL24 levels in HIV-positive
89 patients grouped according to extreme phenotypes of progression to AIDS (RPs, SPs, elite
90 controllers [EC]), and at different stages of HIV infection. We also investigated if $CD4^+$ T cell counts
91 at HAART initiation correlated with chemokine plasma levels during AIDS progression.

92

93 **Material and methods**

94 *Design, study population and ethical aspects*

95 Table 1 describes the clinical baseline and demographic characteristics of the HIV-positive
96 progressors and HIV-negative controls included in the study. Our group has detailed the

97 enrolment and classification of extreme progressors at different clinical stages in a previous study
98 [19]. Additionally, we included a group of ECs (n=8). ECs were characterised as seropositive
99 aviremic individuals ≥5 years after diagnosis and with average ≥1065 $CD4^+$ T cells/mm^3 without
100 HAART through the years. SPs in the chronic clinical stage (SPs chronic, n=12) had stable >500
101 $CD4^+$ T cells/mm^3 with <10,000 copies/mL plasma viral load for up to 8 years; SPs HAART (n=9)
102 were subjects sampled before HAART initiation but who had clinical features at sampling
103 indicative for therapy [221 $CD4^+$ T cells/mm^3 (range, 163–334) and in decline; Viral load (VL) (log)
104 4.9 copies/mL (range 4.0–5.6)]; SPs HAART (n=12) were subjects under HAART; of these, 5
105 initiated HAART according old Brazilian public health recommendations, before 2013 (with <350
106 $CD4^+$ T cells/mm^3, SPs HAART CD4<350) and 7 initiated HAART according new Brazilian public
107 health recommendations, established after mid 2013 (>500 $CD4^+$ T cells/mm^3, SPs HAART
108 CD4>350). RPs pre-HAART (n=6) were sampled close to HAART initiation [254 $CD4^+$ T cells/mm^3 in
109 decline (range, 40–317) and VL (log) 3.7 copies/mL (range, 3.7–5.0)]; RPs HAART (n=11) were
110 already under HAART; all RPs HAART initiated treatment according old Brazilian public health
111 recommendations, meaning with <350 $CD4^+$ T cells/mm^3.

112 HIV-seronegative blood donors (n=20) were from Hospital de Clínicas de Porto Alegre (Rio
113 Grande do Sul, Brazil) and had no metabolic or autoimmune disorders or other medical
114 conditions. The HIV-seronegative samples were matched with HIV-positive individuals based on
115 age, sex and ethnic origin. The study protocol was approved by the Ethics Committees of the
116 Hospital Nossa Senhora da Conceição (protocol number: 002964-20.69/10-5) and Universidade
117 Federal do Rio Grande do Sul (protocol number: 30491714.0.0000.5347) in Porto Alegre. All
118 participants signed informed consent forms developed according to the Declaration of Helsinki.

119

120

Chemokine quantification

The plasma levels of CCL2, CCL17, CCL20, CCL22, CCL24 and CXCL10 were quantified by Bio-Plex methodology using 3.1 Xponent software (Luminex Corp., Austin, TX, USA) in a Luminex 200 System (Bio-Rad Laboratories Inc., Hercules, CA, USA). Data were corrected using Milliplex Analyst software. Human Cytokine/Chemokine Magnetic Bead panels (MILLIPLEX MAP Kit, EMD Millipore, Darmstadt, Germany). The kits were used according to the manufacturer's instructions. All samples were analysed in the same experiment and the results are expressed in pg/mL.

Statistical analyses

Data were analysed and graphs were plotted using GraphPad Prism 5.01 software (GraphPad Software, Inc., San Diego, CA, USA). Comparisons among ≥3 groups were performed with the non-parametric Kruskal–Wallis test followed by Dunn's post-tests to investigate differences between paired groups; *p*-values were adjusted for multiple comparisons (p_c). The non-parametric Mann–Whitney test was used to compare two groups of subjects. A Spearman correlation test was used to assess the significance of correlations between $CD4^+$ T cell counts, VL (log) measurements and chemokine levels. The significance level was set at $p<0.05$ and adjusted *p*-values (p_c) are presented.

Results

Plasma chemokine levels in progressors at different stages of HIV infection

Figure 1A shows comparisons of the plasma CXCL10 levels of the HIV-negative controls, ECs, SPs chronic, SPs pre-HAART, SPs HAART [SPs HAART (CD4>350) + SPs HAART (CD4<350)], RPs pre-HAART and RPs HAART (CD4<350). Plasma CXCL10 levels were higher in SPs pre-HAART and

RPs pre-HAART when compared to the HIV-negative controls ($p_c \leq 0.0001$ and $p_c = 0.003$, respectively). In addition, SPs pre-HAART had higher CXCL10 plasma levels when compared to ECs and RPs HAART ($p_c = 0.009$ and $p_c = 0.007$, respectively). Notably, there were significant differences between SPs pre-HAART and SPs HAART and between RPs pre-HAART and RPs HAART (Dunn's post-test: $p = 0.021$ and $p = 0.007$, respectively), although significance was lost after p adjustments for multiple comparisons. There were no significant differences between SPs pre-HAART and RPs pre-HAART. Figure 1B shows the comparison of plasma CCL20 levels among all groups; no significant differences were observed after p-value adjustment. Notably, the plasma CCL20 levels of SPs chronic, SPs pre-HAART, SPs HAART and RPs HAART were significantly higher when compared to the HIV-negative controls (Dunn's post-test: $p = 0.043$, $p = 0.011$, $p = 0.004$ and $p = 0.037$, respectively) without adjusting for multiple comparisons. No significant differences were observed for the remaining evaluated chemokines (Figures 1C–F).

Correlations of plasma chemokine levels, $CD4^+$ T cell counts and VL (log)

$CD4^+$ T cells and VL (log) measurements were used for correlations with plasma chemokine levels in HAART-naïve individuals (SPs chronic, SPs pre-HAART, RPs pre-HAART). Figure 2A shows a negative correlation between the $CD4^+$ T cell counts and plasma CXCL10 levels in the HAART-naïve SPs (SPs chronic + SPs pre-HAART, n=21) ($r = -0.4532$, $p = 0.0391$). However, there was a positive correlation between VL (log) and CXCL10 levels in this group ($r = 0.7173$, $p = 0.0003$) (Figure 2B). The $CD4^+$ T cell counts and VL (log) levels pre-HAART were used for correlation in subjects under HAART (SPs HAART and RPs HAART). CXCL10 levels and VL (log) were positively correlated in SPs HAART ($r = 0.648$, $p = 0.0227$) (Figure 2C). Additionally, the CCL17 and CXCL10 levels in RPs pre-HAART were positively correlated ($r = 0.9429$, $p = 0.0167$) (Figure S1A). In SPs pre-HAART, there was positive correlation between CCL2 and CXCL10 levels ($r = 0.7500$, $p = 0.0255$),

166 CCL24 and CXCL10 levels (r=0.786, p=0.036) and CCL17 and CXCL10 levels (r=0.786, p=0.036)
167 (Figure S1B–D).

168 *Plasma CXCL10 levels based on CD4$^+$ T cell nadir at HAART initiation*

169 The Mann–Whitney non-parametric test was used to compare paired groups. Figure 3A
170 shows the comparison of plasma CXCL10 levels among SPs HAART (CD4<350) and SPs HAART
171 (CD4>350). There were higher CXCL10 levels in SPs HAART (CD4<350) as compared to SPs HAART
172 (CD4>350) (median 1096.00 vs. 360.33 pg/mL, p=0.0101). Similarly, higher levels were observed in
173 SPs HAART (CD4<350) when compared to RPs HAART (CD4<350) (median 1096.00 vs. 328 pg/mL,
174 p=0.0235) (Figure 2B).

175 **Discussion**

176 The acute phase of HIV infection is characterised by a peak in viremia accompanied by
177 increased immune activation, high production of pro-inflammatory cytokines/chemokines and
178 CD4$^+$ T cell depletion, mainly from gut-associated lymphoid tissues [20]. Although some
179 cytokines/chemokines produced during this clinical phase may contribute to the control of viral
180 replication, a strong, uncontrolled response and the persistence of such chemokines at high levels
181 could contribute to a poor outcome and disease progression.

182 Stacey et al. observed that CXCL10 is rapidly produced and presents increased levels
183 during the acute phase of HIV infection [21]. Liovat et al. observed that, during the primary
184 infection, plasma CXCL10 levels were predictive of rapid progression, and taking these
185 observations into consideration, suggested it as a diagnostic marker of increased viremia in
186 subjects with early HIV-1 infection [6]. Our results show that plasma CXCL10 levels were increased
187 in the pre-HAART progressors when compared with the HIV-negative controls and in SPs pre-
188 HAART when compared with the ECs and RPs HAART (Figure 1A). CXCL10 is a marker of viremia

189 and it correlates with low $CD4^+$ T cell counts during early infection. Thus, CXCL10 could also be a
190 marker of the pre-AIDS stage, a clinical point where HIV-positive individuals have declining $CD4^+$ T
191 cell counts and increasing viral loads. Importantly, there were no significant differences in the
192 CXCL10 levels between the SPs pre-HAART and RPs pre-HAART despite the obvious difference in
193 the period of disease after infection. This likely indicates that high levels of plasmatic CXCL10 in
194 the pre-HAART stage are not dependent on individual phenotypes, but rather are a characteristic
195 of this specific clinical stage. There were no significant differences in CXCL10 levels when ECs and
196 controls were compared (Figure 1A). Again, this corroborates a correlation between CXCL10 levels
197 and specific clinical stages. In agreement with these results, Card et al. also observed that the
198 CXCL10 levels of 18 HIV controllers (a combined group of ECs + viremic controllers) were similar to
199 that of healthy donors [22]. It appears that this special group of individuals is capable of
200 maintaining plasma CXCL10 levels close to a normal range, although the data in the literature are
201 controversial (Noel et al. (2014)) [23]. We cannot rule out the possibility that demographic
202 differences, interfere with these results. In our study, for example, 7 of the 8 ECs were women,
203 while Noel et al. included a similar number of males and females in their study.

204 Furthermore, in HAART-naïve SPs, there was a significant negative correlation between
205 $CD4^+$ T cell counts at sampling with CXCL10 levels and a positive correlation between VL (log) with
206 CXCL10 levels (Figure 2A, 2B). These results are in agreement with several studies that positively
207 correlated CXCL10 levels with high viral loads and negatively correlated this marker with $CD4^+$ T
208 cell counts in early HIV infection [16][6][24]. Furthermore, high CXCL10 levels have been
209 correlated with increased T cell activation [16]. Studies with HIV-exposed seronegative
210 commercial sex workers and HIV-serodiscordant couples have suggested that a low CXCL10 level
211 is potentially protective against HIV-1 although high levels may increase HIV susceptibility and
212 transmission [24][25]. Interestingly, we observed a significant positive correlation of plasma levels
213 between CXCL10 (Th1) and CCL2 (Th2), CXCL10 (Th1) and CCL24 (Th2), CXCL10 (Th1) and CCL17

(Th2) in SPs pre-HAART, and between CXCL10 (Th1) and CCL17 (Th2) in RPs pre-HAART (Figure 1S). Previous studies have suggested that Th2 may weaken the defenses against HIV, and in the presence of high IgE and IL-4 levels trigger eosinophilia and allergic clinical manifestations, which are found in a proportion of HIV-1–infected individuals [10][26]. Again, our observations suggest that high plasma CXCL10 (Th1) levels together with the CCL2, CCL17 and CCL24 (Th2) chemokines in SPs and RPs pre-HAART are associated with a specific clinical point, characteristic of progression to AIDS.

Plasma CCL20 levels were higher in SPs chronic, SPs pre-HAART, SPs HAART and RPs HAART when compared to the controls. Although significance was lost after adjusting for multiple comparisons, these observations are noteworthy in the context of a previous study that showed higher CCL20 levels in viremic HAART-naïve SPs and RPs under HAART when compared to HIV-negative donors [9]. These data suggest a certain level of inflammation or an active mechanism of control of disease progression in SPs chronic (>500 $CD4^+$ T cells/mm^3) or viremic SPs, and persistent expression of CCL20 independently of HAART. On the other hand, the relatively similar CCL20 levels observed in aviremic ECs in the present study and in Fontaine et al. (2011) when compared to HIV-negative controls could reflect a potential for controlling disease progression in such individuals [9]. $CCR6^+$ $CD4^+$ cells are highly permissive to infection [27] and have the potential to be recruited to the gastrointestinal and vaginal mucosa through a CCR6−CCL20 axis–dependent mechanism, significantly contributing to HIV dissemination and persistence [28]. Although CCL20 measurements in specific microenvironments such as the gut are quite challenging, peripheral blood concentrations may be indicative of CCL20 concentrations and action at those locations.

Previously, we reported that IL-6 and IL-10 cytokine levels were higher in seropositive individuals pre-HAART when compared with healthy controls and individuals under HAART [19]. Taken together, these results suggest that IL-6, IL-10 and CXCL10 (and likely CCL20) levels should

238 be monitored in HIV-positive patients even during the chronic phase of the disease. These
239 biomarkers could help both the decision-making concerning HAART initiation as well as the
240 surveillance of the immune activation/inflammation state of patients. Nevertheless, it should be
241 pointed out that, since 2013 and in Brazil specifically, all HIV-positive individuals are
242 recommended to initiate HAART independently of $CD4^+$ T cell count. Before this date, HAART was
243 only recommended to HIV-positive subjects with <350 $CD4^+$ T cells/mm^3. Thus, it was possible to
244 evaluate the chemokine levels of SPs who initiated HAART according to the indications of both
245 therapeutic schemes. We observed significant differences in CXCL10 levels between SPs who
246 initiated HAART with >350 $CD4^+$ T cells/mm^3 compared with SPs who initiated HAART with <350
247 $CD4^+$ T cells/mm^3 (360.33 vs. 1096.00 pg/mL) (Figure 3). This observation provides more
248 information on the modulation of plasmatic pro-inflammatory biomarkers, which appears
249 dependent on the immune condition of the individual at the beginning of the therapy. Some
250 studies have suggested that HAART initiation during the acute phase of infection contributes to
251 plasma cytokine/chemokine level normalisation and to the maintenance of an adequate CD4:CD8
252 ratio in the time [29]. In addition, HIV-positive individuals who initiated HAART in the chronic
253 stage with >350 $CD4^+$ T cells/mm^3 also kept CXCL10 at levels similar to that of healthy individuals.
254 Again, these are important data to be considered in decision-making concerning HAART initiation.

255 The observed difference between SPs HAART (CD4<350) and RPs HAART (CD4<350)
256 (Figure 3) can also be due to the time under HAART treatment. RPs HAART had been under
257 treatment for a median 25 months, while SPs HAART had been under treatment for a median 12
258 months. Notably, in the design of this study and in an attempt to homogenise the groups, 2 SPs
259 HAART were withdrawn from the analysis due to an extremely long period under HAART (40 and
260 66 months). Interestingly these individuals had practically restored normal CXCL10 levels (216.7
261 and 172.59 pg/mL, respectively). Although Lee et al. [16] have shown that CXCL10 levels remained
262 elevated in HIV-positive male subjects >24 months under HAART, our RPs HAART normalised their

CXCL10 levels within 25 months of HAART treatment, indicating, CXCL10 as an interesting marker of the general clinical state of HIV-positive patients.

Conclusions

Plasma CXCL10 levels are significantly higher in SPs pre-HAART and RPs pre-HAART when compared with HIV-negative controls. Moreover, the normalisation of plasma CXCL10 levels after HAART appears dependent on the $CD4^+$ T cell nadir at the time of HAART initiation and on the duration under HAART. Thus, the measurement of CXCL10 (and potentially CCL20) levels should be considered in the clinical monitoring of HIV-positive subjects as a biomarker to aid both decision-making concerning HAART initiation and as an auxiliary tool for surveying the general state of activation/inflammation of the immune system during disease progression.

Acknowledgements

We thank the Infectious Diseases Service of Nossa Senhora da Conceição Hospital for the valuable help and collaboration with our project, and thank the study participants; most were very happy to contribute to this study.

JMV-V reviewed the clinical history of the patients, performed the statistical analyses, organised the manuscript; RMM reviewed the clinical history of the patients and analysed data; JHE analysed data; BRS and MGM were responsible for the clinical selection of the patients; SEA and JABC conceived the project and organised the manuscript. All authors were involved in the writing and approval of the manuscript.

References

1 Epstein FH, Luster AD. Chemokines — Chemotactic Cytokines That Mediate Inflammation. *N Engl J Med* 1998; **338**:436–445.

2 Qidwai T. Chemokine genetic polymorphism in human health and disease. *Immunol Lett* 2016; **176**:128–38.

3 Deeks SG. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 2004; **104**:942–947.

4 Lee AYS, Körner H. The CCR6/CCL20 Chemokine Axis in HIV Immunity and Pathogenesis. *J Gen Virol* Published Online First: 21 December 2016. doi:10.1099/jgv.0.000691

5 Lane BR, King SR, Bock PJ, Strieter RM, Coffey MJ, Markovitz DM. The C-X-C chemokine IP-10 stimulates HIV-1 replication. *Virology* 2003; **307**:122–134.

6 Liovat A-S, Rey-Cuillé M-A, Lécuroux C, Jacquelin B, Girault I, Petitjean G, *et al.* Acute Plasma Biomarkers of T Cell Activation Set-Point Levels and of Disease Progression in HIV-1 Infection. *PLoS One* 2012; **7**:e46143.

7 Valverde-Villegas JM, Matte MCC, Medeiros RM De, Chies JAB. New Insights about Treg and Th17 Cells in HIV Infection and Disease Progression. *J Immunol Res* 2015; **2015**:1–14.

8 Aziz N, Detels R, Chang LC, Butch AW. Macrophage Inflammatory Protein-3 Alpha (MIP-3α)/CCL20 in HIV-1-Infected Individuals. *J AIDS Clin Res* 2016; **7**. doi:10.4172/2155-6113.1000587

9 Fontaine J, Poudrier J, Roger M. Short communication: persistence of high blood levels of the chemokines CCL2, CCL19, and CCL20 during the course of HIV infection. *AIDS Res Hum Retroviruses* 2011; **27**:655–7.

10 Yokobayashi H, Sugaya M, Miyagaki T, Kai H, Suga H, Yamada D, *et al.* Analysis of serum chemokine levels in patients with HIV-associated eosinophilic folliculitis. *J Eur Acad Dermatology Venereol* 2013; **27**:e212–e216.

11 Pal R, Garzino-Demo A, Markham PD, Burns J, Brown M, Gallo RC, *et al.* Inhibition of HIV-1 infection by the beta-chemokine MDC. *Science* 1997; **278**:695–8.

12 Ancuta P, Autissier P, Wurcel A, Zaman T, Stone D, Gabuzda D. CD16+ monocyte-derived macrophages activate resting T cells for HIV infection by producing CCR3 and CCR4 ligands. *J Immunol* 2006; **176**:5760–71.

13 Ansari AW. Host chemokine (C-C motif) ligand-2 (CCL2) is differentially regulated in HIV type 1 (HIV-1)-infected individuals. *Int Immunol* 2006; **18**:1443–1451.

14 Marins JRP, Jamal LF, Chen SY, Barros MB, Hudes ES, Barbosa AA, *et al.* Dramatic improvement in survival among adult Brazilian AIDS patients. *AIDS* 2003; **17**:1675–82.

15 Grinsztejn B, Hosseinipour MC, Ribaudo HJ, Swindells S, Eron J, Chen YQ, *et al.* Effects of early versus delayed initiation of antiretroviral treatment on clinical outcomes of HIV-1 infection: results from the phase 3 HPTN 052 randomised controlled trial. *Lancet Infect Dis* 2014; **14**:281–290.

16 Lee S, Fernandez S, French M, Price P. Chemokine receptor expression on dendritic cells is normal in HIV-infected patients with a stable response to art, but chemokine levels remain elevated. *J Med Virol* 2011; **83**:1128–33.

17 INSIGHT START Study Group, Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, *et al.*

Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med* 2015; **373**:795–807.

18 Jarrin I, Pantazis N, Dalmau J, Phillips AN, Olson A, Mussini C, *et al.* Does rapid HIV disease progression prior to combination antiretroviral therapy hinder optimal CD4+ T-cell recovery once HIV-1 suppression is achieved? *AIDS* 2015; **29**:2323–33.

19 de Medeiros RM, Valverde-Villegas JM, Junqueira DM, Gräf T, Lindenau JD, de Mello MG, *et al.* Rapid and Slow Progressors Show Increased IL-6 and IL-10 Levels in the Pre-AIDS Stage of HIV Infection. *PLoS One* 2016; **11**:e0156163.

20 Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, *et al.* Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med* 2004; **200**:761–70.

21 Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, *et al.* Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. *J Virol* 2009; **83**:3719–33.

22 Card CM, Keynan Y, Lajoie J, Bell CP, Dawood M, Becker M, *et al.* HIV Controllers Are Distinguished by Chemokine Expression Profile and HIV-Specific T-Cell Proliferative Potential. *JAIDS J Acquir Immune Defic Syndr* 2012; **59**:427–437.

23 Noel N, Boufassa F, Lécuroux C, Saez-Cirion A, Bourgeois C, Dunyach-Remy C, *et al.* Elevated IP10 levels are associated with immune activation and low $CD4^+$ T-cell counts in HIV controller patients. *AIDS* 2014; **28**:467–76.

24 Lajoie J, Juno J, Burgener A, Rahman S, Mogk K, Wachihi C, *et al.* A distinct cytokine and chemokine profile at the genital mucosa is associated with HIV-1 protection among HIV-exposed seronegative commercial sex workers. *Mucosal Immunol* 2012; **5**:277–87.

25 Kahle EM, Bolton M, Hughes JP, Donnell D, Celum C, Lingappa JR, *et al.* Plasma Cytokine Levels and Risk of HIV Type 1 (HIV-1) Transmission and Acquisition: A Nested Case-Control Study Among HIV-1-Serodiscordant Couples. *J Infect Dis* 2015; **211**:1451–1460.

26 Becker Y. The changes in the T helper 1 (Th1) and T helper 2 (Th2) cytokine balance during HIV-1 infection are indicative of an allergic response to viral proteins that may be reversed by Th2 cytokine inhibitors and immune response modifiers--a review and hypothesi. *Virus Genes* 2004; **28**:5–18.

27 Gosselin A, Monteiro P, Chomont N, Diaz-Griffero F, Said E a, Fonseca S, *et al.* Peripheral Blood CCR4+CCR6+ and CXCR3+CCR6+ CD4+ T Cells Are Highly Permissive to HIV-1 Infection. *J Immunol* 2010; **184**:1604–1616.

28 Monteiro P, Gosselin A, Wacleche VS, El-Far M, Said EA, Kared H, *et al.* Memory CCR6+CD4+ T cells are preferential targets for productive HIV type 1 infection regardless of their expression of integrin β7. *J Immunol* 2011; **186**:4618–30.

29 Hoenigl M, Chaillon A, Little SJ. CD4/CD8 Cell Ratio in Acute HIV Infection and the Impact of Early Antiretroviral Therapy. *Clin Infect Dis* 2016; **63**:425–6.

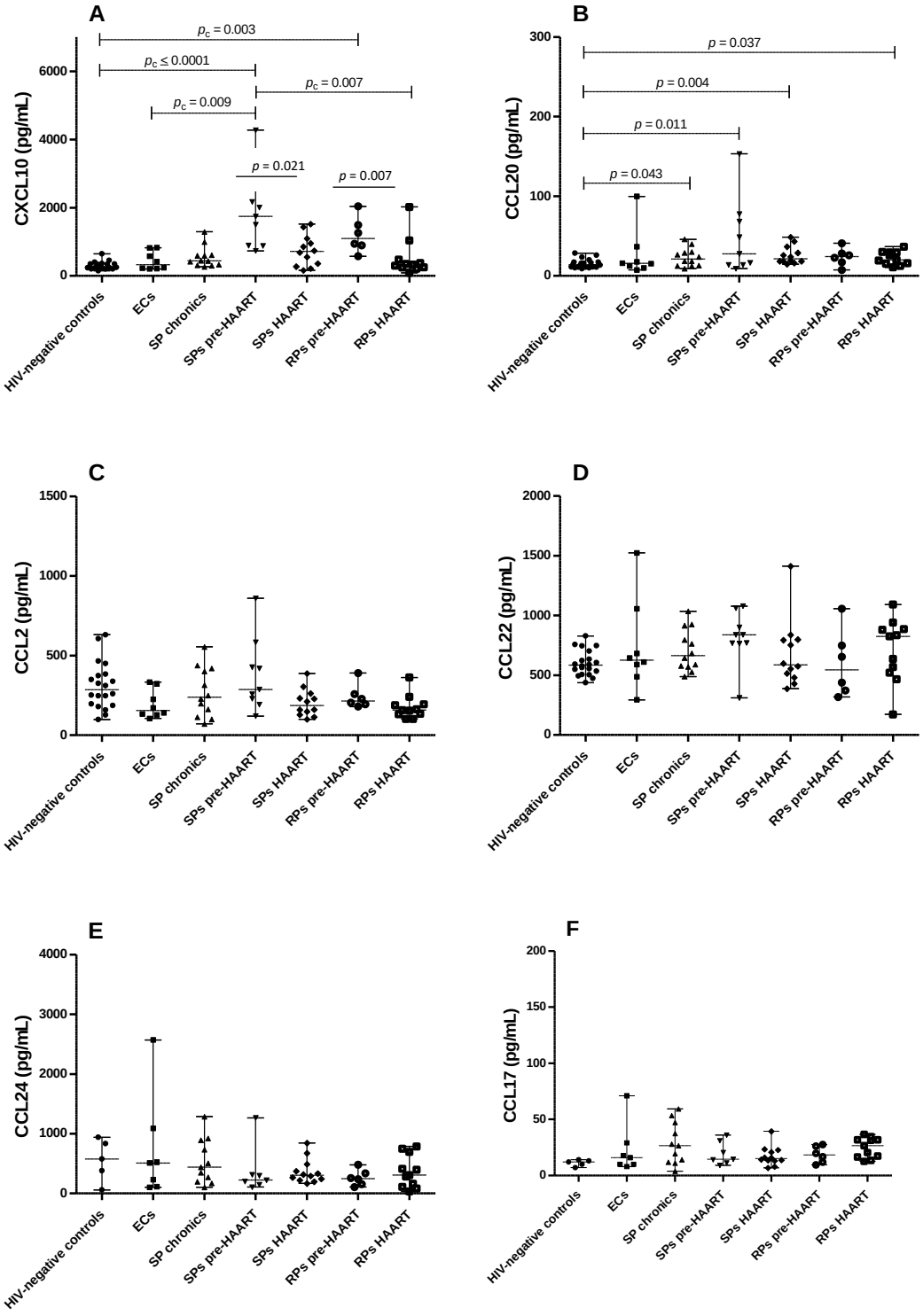


Figure 1. Plasma chemokine levels in HIV+ progressors in different clinical stages. Kruskal-Wallis non-parametric test comparing HIV-negative controls (n=20), ECs (n=8), SPs chronic (n=12), SPs pre-HAART (n=9), SPs HAART (n=12), RPs pre-HAART (n=6), RPs HAART (n=11). Significant differences were observed after controlling for multiple comparisons are showed by p_c and significant differences from Dunn's post-tests are showed for p<0.05.

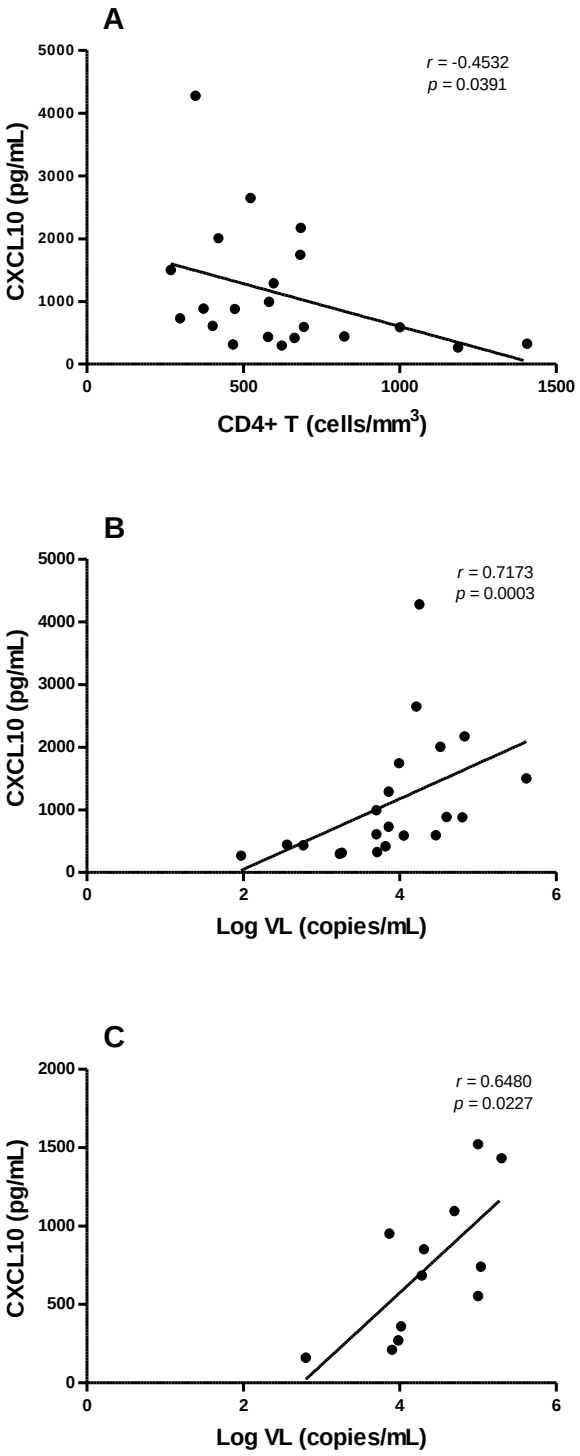


Figure 2. Correlation of CD4$^+$ T cell counts and VL (log) and plasma CXCL10 levels. A) shows significant negative correlation between the CD4$^+$ T cell counts and plasma CXCL10 levels in the group of SPs HAART-naïve (SPs chronic + SPs pre-HAART, n=21); B) shows significant positive correlation between VL (log) and CXCL10 levels in SPs HAART-naïve; C) shows a positive correlation among CXCL10 levels and VL (log) in SPs HAART. Data was analysed by Spearman correlation, with $p<0.05$ considered significant.

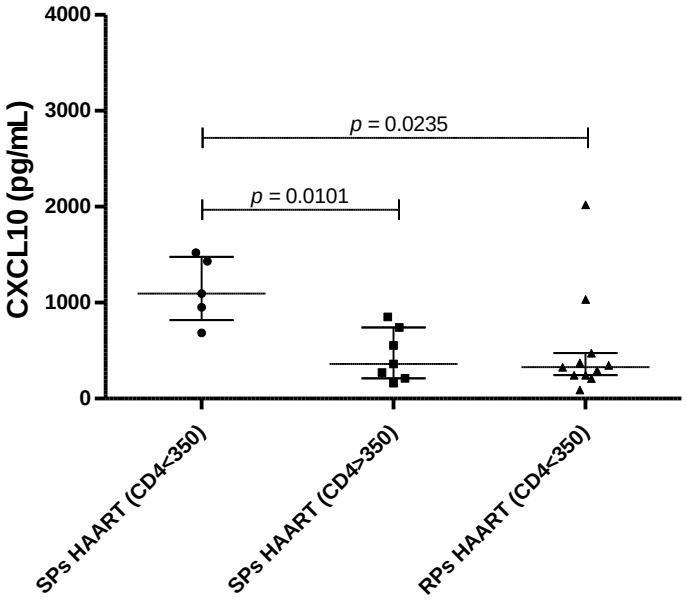


Figure 3. Comparison of plasma CXCL10 levels between HIV+ progressors under-HAART. Shows significant differences among SPs HAART who initiated HAART with $CD4^+$ T cells>350 vs. SPs HAART who initiated HAART with $CD4^+$ T cells<350. Also, higher CXCL10 levels in SP HAART who initiated HAART with $CD4^+$ T cells <350 were observed when compared to RP HAART who initiated HAART with $CD4^+$ T cells <350. Mann-Whitney non-parametric test was used with $p<0.05$ considered significant.

Table 1. Baseline measurements and demographic characteristics of HIV+ progressors and HIV seronegative controls.

	Clinical stages							
	HIV seronegative	**ECs**	**SPs chronic**	**SP pre-HAART***	**SPs HAART**		**RPs pre-HAART***	**RPs HAART (CD4<350)α**
	(n=20)	**(n=8)**	**(n=12)**	**(n=9)**	**SPs HAART (CD4<350)α (n=5)**	**SPs HAART (CD4>350)β (n=7)**	**(n=6)**	**(n=11)**
Baseline measurements								
Time progression[a]	NA	6 (5-24)	11 (8-15)	13 (9-17)	11 (9-15)	12 (8-17)	01 (1-2)	01 (1-3)
$CD4^+$ T cells nadir before HAART[b]	NA	NA	NA	221 (163-334)	203 (58-290)	391 (350-854)	254 (40-317)	287 (105-350)
VL (log) before HAART[c]	NA	NA	NA	4.9 (4.0-5.6)	4.7 (3.9-5.3)	4.0 (2.8-5.0)	3.7 (3.7-5.0)	4.3 (3.0-5.2)
$CD4^+$ T cells at sampling[b]	NA	1202 (729-1798)	644 (402-1407)	420 (268-684)	398 (284-581)	769 (411-918)	283 (153-391)	538 (185-867)
VL (log) at sampling[c]	NA	1.6 (1.6-1.6)	3.7 (2.0-4.5)	4.5 (3.9-5.6)	1.6 (1.6-4.6)	1.6 (1.6-1.6)	3.9 (3.7-5.2)	1.6 (1.6-1.6)
Time of HAART at sampling[d]	NA	NA	NA	NA	12 (12-28)	18 (8-28)	NA	25 (13-39)
Demographic characteristics								
Median age (min-max)	43 (26-60)	37.5 (27-52)	37.5 (27-64)	42 (34-53)	46 (35-52)	45 (32-54)	32.5 (22-69)	36 (23-53)
Sex								
female	16 (0.80)	7 (0.88)	11 (0.92)	9 (1.00)	4 (0.80)	7 (1.00)	4 (0.67)	5 (0.55)
male	4 (0.20)	1 (0.13)	1 (0.08)	0 (0.00)	1 (0.20)	0 (0.00)	2 (0.33)	6 (0.45)
Ethnicity								
European descents		19 (0.95)	6 (0.50)	6 (0.67)	2 (0.40)	5 (0.71)	5 (0.83)	7 (0.64)
African descents		1 (0.05)	6 (0.50)	3 (0.33)	3 (0.60)	2 (0.29)	1 (0.17)	4 (0.36)

[a]median (IQ), years; [b]median (IQ) in cells/mm^3; [c]median (IQ) in log10 copies/mL; [d]median (IQ), months; NA, not applicable; HAART, highly active antiretroviral therapy

* pre-HAART collected naïve and started HAART after 12- 24 months

αaccording old public health HAART recommendations

βaccording new public health HAART recommendations

Table 2. Plasma chemokine levels in HIV+ progressors naive-HAART and under-HAART.

Chemokine	HIV-negative controls n=20	ECs n=8	SPs chronic n=12	SPs pre-HAART n=9	SPs HAART n=12	RPs pre-HAART n=6	RPs HAART n=11
CXCL10	254.97 (149-646)	328.32 (201-828)	440.15 (267-1291)	1743.00 (729-4275)	712.08 (160-1522)	1094.75 (573-2037)	328 (93-2022)
CCL20	13.97 (9-29)	15.69 (7-100)	21.12 (9-46)	27.63 (9-153)	21.47 (15-49)	24.15 (7-41)	19.80 (11-37)
CCL2	286.84 (100-631)	155.93 (106-334)	239.80 (72-555)	288.32 (120-860)	187.06 (100-388)	215.82 (181-390)	158.43 (103-363)
CCL17	12 (7-14)	15.97 (8-71)	26.66 (4-59)	14.41 (9-36)	15.01 (7-39)	18.27 (9-28)	26.61 (13-37)
CCL22	583.77 (438-829)	627.44 (293-1524)	664.23 (490-1035)	838.49 (311-1079)	587.13 (388-1413)	545.73 (318-1057)	826.53 (172-1092)
CCL24	577 (62-942)	512.99 (104-2571)	442.38 (106-1289)	224.37 (109-1267)	306.06 (167-844)	247.44 (110-481)	307.57 (35-787)

[a] Values are represented by median IQR (minimum-maximum) in pg/mL.

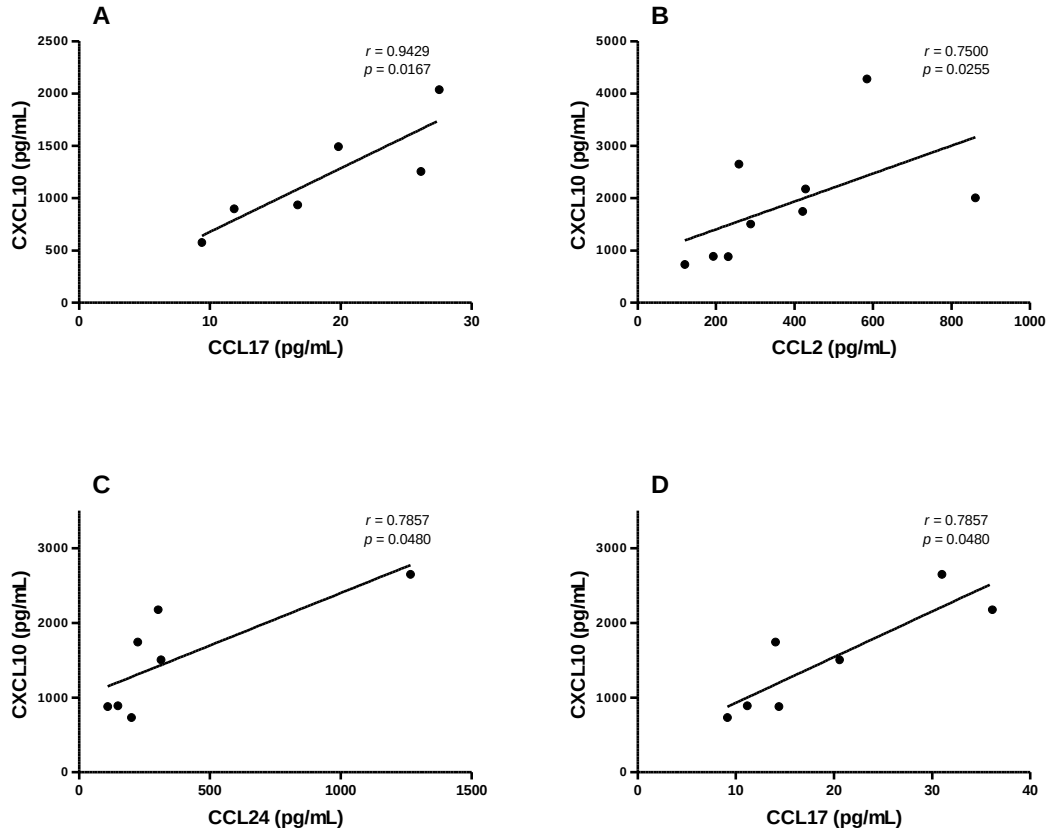


Figure 1S. Correlation between chemokine plasma levels. A) Shows a positive correlation among CCL17 and CXCL10 levels in RPs pre-HAART (*r*=0.9429, *p*=0.0167). B, C and D) Shows positive correlations between CCL2 and CXCL10 levels (*r*=0.7500, *p*=0.0255), CCL24 and CXCL10 levels (*r*=0.7857, *p*=0.0480) and CCL17 and CXCL10 levels (*r*=0.7857, *p*=0.0480), respectively in SPs pre-HAART. Data was analyzed by Spearman correlation, with p<0.05 considered significant.

Table S1. Chemokine plasma levels between groups of SPs HAART.

Chemokines	SPs HAART [a]	
	$CD4^+$ T cells <350 (n=5)	**$CD4^+$ T cells >350 (n=7)**
CXCL10	1096.00 (684-1522)	360.33 (160-853)
CCL20	22.43 (18-37)	23.84 (16-49)
CCL2	229.03 (129-388)	160.96 (100-306)
CCL17	15.95 (7-39)	13.71 (8-23)
CCL22	598.75 (515-838)	554.18 (388-1413)
CCL24	318.01 (221-492)	269.00 (167-844)

[a] Values are represented by median IQR (minimum-maximum) in pg/mL.

nadir $CD4^+$ T cells <350: Initiated HAART according old public health HAART recommendations.

nadir $CD4^+$ T cells >350: Initiated HAART according new public health HAART recommendations.

Capítulo 5

Immunodynamic characterization of HIV+ extreme progressors under-HAART: Imbalance of Th cell subsets

(Artigo em preparação)

"O objetivo deste artigo é avaliar o impacto de ARV como modulador da resposta imune nos progressores extremos"

Immunodynamic characterization of HIV+ extreme progressors under-HAART: Imbalance of Th cell subsets

Jacqueline María Valverde-Villegas[1], Rúbia Marília de Medeiros[1], Priscila Vianna[1], Valéria Kaminski[1], Breno Riegel dos Santos[3], Sabrina Esteves de Matos Almeida[2], José Artur Bogo Chies[1]

[1] Programa de Pós-graduação em Genética e Biologia Molecular da UFRGS (PPGBM)

[2] Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS)

[3] Serviço de infectologia, Grupo Hospitalar Nossa Senhora da Conceição (GHC)

Corresponding author:

Dr. José Artur Bogo Chies. Email address: jabchies@terra.com.br

Laboratory of Immunogenetics. Institute of Biosciences, Departament of Genetics, UFRGS.

Av. Bento Gonçalves – 9500, Campus do Vale. 91501970

Porto Alegre, RS-Brazil. PO BOX 15053

Phone: +55 51 3308 6740; Fax: +55 51 3308 7311

This work was supported by Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) [Grant number 12/2151-2 and 14/2253-2]; Jacqueline M. Valverde-Villegas received a scholarship by the Programa Estudantes-Convênio de Pós-Graduação (PEC-PG).

Abstract

Key words: HAART, extreme progressors, immune activation, Th subsets

Introduction

In Brazil, in the middle of 2013, recommendation rules to initiate highly active antiretroviral therapy (HAART) were changed and HAART became available for any HIV seropositive individual independently of their CD4+ T cell counts. Studies showed that an effective CD4+ T-cells restoration is depending of the CD4+T-cells nadir at HAART beginning since patients who initiate HAART with a lower CD4+ T-cell nadir took longer time to normalize this cell subset (1). Recently studies are showing that HAART initiation during HIV soroconversion or at the primary stage phase is also the most effective intervention to limit the extent of viral reservoirs and could preserve gut T-cell homeostasis and the integrity of the epithelial barrier (2) when compared with HAART initiation during the chronic clinical phase (3)(4). Interestingly, immune activation was observed even in individuals with a long-term of HAART and it was associated with delayed treatment-mediated CD4+ T cell gains (5).

Mahnke Y et al., (2016) observed that in HIV+ individuals who initiated HAART with <200 CD4+/mm^3, the CD4+ T-cell total subset recovered was not representative of the different cell subsets and resulted in a predominant Th2 profile (6). Different cell subsets are target to HIV infection and some cell subpopulations are more permissive to HIV infection than others. Permissivity to HIV infection also likely depends of the local of infection, for example Th17 and Th17Th1 cell subsets are more represented in mucosal sites and exhaustion of these cellular compartment at early infection, was already described at the gut and peripheral blood (7) , and this can dramatically impact disease progression, especially if that exhaustion affect precursor cells. Thus, it was suggested that early HAART initiation in HIV-infected subjects contribute to the preservation of Th17 (8).

Together, the aim of this study was to evaluate the impact of HAART in extreme progressors, focusing on those who initiated HAART with <350 CD4+ T cells versus >350 CD4+ T.

Also, the dynamic of different CD4+ T subsets and CD4+ and CD8 T-cell activated frequencies was investigated in subgroups of extreme progressors under ARV.

Material and methods

Study cohort

Extreme progressors were identified retrospectively from Nossa Senhora da Conceição hospital (HNSC) in Porto Alegre, Brazil. Rapid Progressors (RPs) were defined as subjects who had recommendation to initiate HAART within 3 years after seroconversion (due to two or more CD4+ T cell measurements <350 cells/mm3 during that period of time). Also, a previous HIV seronegative test was used to identify this group. Slow progressors (SPs) were defined as HIV+ subjects asymptomatic for ≥ 8 years of follow-up after diagnosis, with average CD4+ T cells measurements ≥ 500 cells/mm^3 and plasma viral load <10,000 copies/mL through the years without recommendation to initiate HAART. Details about enrolment and classification of extreme progressors are described in a previous study of our group (9). HIV-seronegative blood donors were voluntary donors from the Universidade Federal do Rio Grande do Sul (UFRGS, Porto Alegre), with no metabolic or autoimmune disorders or other medical conditions. The HIV-seronegative samples were matched with HIV+ individuals based on age, sex and ethnic origin. The study protocol was approved by the Ethic Committees of the HNSC (protocol number: 002964-20.69/10-5) and UFRGS (protocol number: 30491714.0.0000.5347). Informed consent developed according to the Declaration of Helsinki was signed by all participants.

PBMCs isolation

Peripheral blood was collected by venipuncture and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient with Ficoll-Paque (Sigma-Aldrich, St. Louis, MO). The cell pellet was resuspensed in RPMI-1640 (Sigma-Aldrich, St. Louis, MO) supplemented with 10%

of FBS (Fetal Bovine Serum, Sigma-Aldrich, St. Louis, MO), and 100 U/ml penicillin-0.1 mg/ml streptomycin (Sigma-Aldrich, St. Louis, MO). Cells counting was performed with Neubauer chamber by means of microscopy (100 x) and viability was verified using Trypan Blue (Sigma-Aldrich, St. Louis, MO). After cell isolation cells were immediately labelled with antibodies from the panel described below.

Immunophenotyping

Immunophenotyping of Th1, Th2 Th17, Th1Th17 cell subsets and activation markers were performed in cells isolated from fresh peripheral blood. Positivity to chemokine receptors was used to characterize distinct memory CD4+ T cell subsets: Th17 ($CXCR3^{-}CCR4^{+}CCR6^{+}$), Th2 ($CXCR3^{-}CCR4^{+}CCR6^{-}$), Th1Th17 ($CXCR3^{+}CCR4^{-}CCR6^{+}$) and Th1 ($CXCR3^{+}CCR4^{-}CCR6^{-}$) as described by Gosselin A et al., (2010) (10). Activated T cells were CD4 or CD8 positive cells identified as $CD38^{+}HLA\text{-}DR^{+}$. Populations positive to the activation markers CD38 and HLA-DR were gated from the $CD4^{+}$ and $CD8^{+}$ cells. T cell activation levels were reported as the percentages of $CD4^{+}$ and $CD8^{+}$ T cells expressing both HLA-DR and CD38. The PBMCs were labelled with specific fluorochrome-conjugated antibodies used for multicolor flow cytometry analysis, such as: anti-CD45RA-FITC, anti-CD3 Alexa 647, anti-CD4 APC-H7, anti-CCR4 PE-CY7, anti-CCR6 PERCP-CY5.5, anti-CXCR3-PE, anti-CD8-PERCPCy5.5, anti-CD38 PE, and anti-HLA-DR PE-Cy7. Fluorescence minor one (FMO) was used for CCR4, CCR6, CXCR3 to define positive and negative populations. All antibodies were purchased from BD Biosciences, San Diego, CA. Cells were analyzed by FACS using the BD FACSAria III and BD Diva software (BD Biosciences, San Jose, CA). FlowJo, LLC V.10 software (Tree Star Corporation, Ashland, 163 OR) was used for gating and evaluation of the frequencies of cell populations.

Quantification of plasma chemokine levels

Plasma levels of CCL2, CCL17, CCL20, CCL22, CCL24, and CXCL10 were quantified by a Bio-Plex methodology using the 3.1 Xponent software (Luminex Corp., Austin, TX, USA) in a Luminex 200 System (Bio-Rad Laboratories Inc., Hercules, CA). Human Cytokine / Chemokine Magnetic Bead panels (MILLIPLEX MAP Kit, EMD Millipore, Darmstadt, Germany) were used in the assays. The kits were used following the manufacturer's instructions. All samples were analyzed in the same experiment and the results were expressed in pg/mL. Data were corrected using the software Milliplex Analys.

Statistical analyses

Frequencies of cell subsets were analyzed and graphs were plotted using the GraphPad Prism 5.01 software (GraphPad Software, Inc.; San Diego, CA, USA). Comparisons between three or more groups were performed with the non-parametric Kruskal-Wallis test followed by Dunn's post-tests to investigate differences between paired groups and *p*-values were adjusted for multiple comparisons. A Spearman correlation test was used to assess the significance of correlations between the CD4+ T cell subsets, activation cell markers and chemokine levels. The significance level was set at $p<0.05$.

Results

Demographic and clinical characteristics

Table 1 shows clinical and demographic characteristics of RPs HAART (n=5) who initiated therapy with <350 (201-340) CD4+ T cells/mm^3 according to the previous HAART recommendation rules in Brazil (valid until 2013) and they were in average 45 [32-66] months under therapy); and SPs HAART (n=7) who initiated therapy with >350-500 (371-854) CD4+T cells/mm^3 according to

144 the most recently established recommendation rules of 2013 and they were in average 21 [11-28]
145 months under therapy. Additionally, demographic characteristics of healthy controls are shown.

146 *T-cell activation in extreme progressors*

147 Healthy controls had lower median percentages of activated CD4+ T cells ($CD38^+HLA$-
148 DR^+CD4^+) when compared with SPs and RPs (1.3% vs. 5.4%, *pc*=0.044 and 1.3% vs. 13.6%,
149 p_c=0.008, respectively). Also, RPs had higher median percentages of activated $CD8^+$ T cells
150 ($CD38^+HLA\text{-}DR^+CD8^+$) when compared with healthy controls (0.32% vs. 8.7%; p_c=0.001) (Figure 1).

151

152 *Frequency of memory T cells: Th1, Th2, Th17 and Th1Th17 in extreme progressors*

153 Memory T cells were identified as cells lacking the expression of the naive T cell CD45RA
154 marker, as previously described (11). In this study, we gated memory $CD4^+$ T cell subsets
155 (CD3+CD4+CD45RA-) and evaluated positivity to CCR4, CXCR3, and CCR6. Expression of CCR4 and
156 CXCR3 distinguishes four memory subsets, including CCR4+CXCR3- and CCR4-CXCR3+ subsets (Fig.
157 2A). The expression of CCR6 further identifies CCR4+CCR6+, CCR4+CCR6-, CXCR3+CCR6+, and
158 CXCR3+CCR6- T cell subsets (Fig. 2B). In figure 2C, Th1 cell frequency in SPs (CD4+ T cells>350) was
159 significantly lower when compared with healthy controls (14.4±2.8 vs. 30.2±2.4, p_c=0.007). The
160 frequency of Th2 cells were significantly lower in RPs (CD4+ T cells<350) when compared with
161 healthy controls and SPs (CD4+ T cells>350) (7.8±1.1 vs. 17.5±2.1, p_c =0.027 and 7.8±1.1 vs.
162 21.2±2.2, p_c=0.002, respectively). The frequency of Th17 cell subset was significantly higher in SPs
163 (CD4+ T cells>350) when compared with healthy controls and RPs (CD4+ T cells<350) (10.2±2.5 vs.
164 2.5±0.7, p_c =0.042 and 10.2±2.5 vs. 1.1±0.4, p_c=0.012, respectively).

165

166

Correlation between plasma chemokine levels and cell subsets

In SPs, negative correlations were observed between CCL22 plasma levels and frequency of Th17 cells and among CCL22 levels and frequency of Th1Th17 cells (r=-0.857, p=0.014 and r=-0.786, p=0.036, respectively). In RPs, negative correlations were observed between CXCL10 levels and frequency of Th1Th17 cells (r=0.900, p=0.037) (Figure 4).

Discussion

Several studies observed a persistent cellular activation and inflammation in HIV positive patients even under sustained viral suppression by HAART. In several cases this was related to subsequent mortality and non-AIDS morbidities, for example cardiovascular diseases (5). Also, despite $CD4^+$ T cell counts restoration by HAART, there is still an imbalance of specific cell subsets recovered in individuals who initiated HAART with <200 $CD4^+$ T–cells (6). These phenomena, meaning the $CD4^+$ T cell counts restoration as well as an imbalance of specific cell subsets on the recovered patient, seems to depend on the $CD4^+$ T cell nadir at HAARTs beginning, but may also be associated to phenotypic characteristic of the subjects.

Regarding immune activation, as expected, higher frequencies of activated CD4+ T cells were observed in SPs and RPs under HAART when compared to healthy controls. Also, RPs had higher frequencies activated CD8+ T cells when compared to healthy controls. Studies showed that immune activation is persistent even in individuals with long-time of HAART (5). Hunt et al., (2011) observed that cellular activation biomarkers predict mortality even in HIV+ individuals with CD4+ T cell counts >500 cells/mm^3, suggesting that inflammation may be a risk even in subjects with optimal CD4+ T cell recovery (12). Also, a persistent activation progressively disrupts the functional organization of the immune system leaving HIV subject susceptible to co-infections resulting in AIDS.

When memory Th cell subsets were compared between extreme progressors under HAART, we observed an imbalance of the restoration of $CD4^+$ T cells. In SPs, despite they were under HAART in median 21 months (12-28), Th1 cell frequencies were lower when compared with healthy control (Figure 2C). This fact could be explained because these individuals likely maintain a high Th1 profile at acute/primary clinical stage for controlling the viral load and subsequent a better response of disease progression. Thus, these cell subsets would be the most committed and exhausted during the early phase of infection that after 21 months under HAART still not recover to normal levels. It was observed that Th1 effector memory cells are short-lived and do not efficiently develop into long-term memory Th1 cells and the total number of progeny they produce upon stimulation and restimulation is smaller and they are rapidly exhausted (13). On the other hand, SPs had higher Th17 frequencies when compared to healthy controls and RPs (Figure 2C). Recently, El-Far et al., (2016) showed that IL-32 correlated with the loss of virological control and CD4 decline and was suggested that sustained inflammation and activation of Th17 cells by IL-32 could contribute to a significant of these cells in vivo (14). In this study, five SPs showed CD4 decline (data not shown) and the higher frequency of Th17 cells observed in this group could be explained by IL-32. Of note, our SPs were in average 21 months under HAART, showing that during this period of time under HAART they were not able to normalize their Th17 cell frequencies. In RPs, lower frequency of Th2 cells were observed when compared to healthy controls and SPs (Figure 2C). Clerici and Shearer presented a hypothesis whereby Th1 cell profile declines and Th2 activity increases (the Th1 --> Th2 switch hypothesis) in HIV-1 infected people. Subsequent studies associated Th2 profile with a bad outcome of the disease. Recently a work observed that levels of IL-10, IL-4 and TNF-α were increased in the acute HIV type 1 infection when compared to control group (15). Since that IL-10 and IL-4 are cytokines of Th2 profile and it seems be associated with rapid progression, this cell subsets would be the most affected and exhausted in the initial stage of the infection and difficult to recover even with 41 months under

HAART. However, the relationship between Th2 and rapid progression should be better investigated taking into account, in addition to immunological factors, the host genetic factors.

Furthermore, in SPs, negative correlation between frequencies of Th17 and plasma CCL22 levels and among Th1Th17 and CCL22 was observed (Figure 4). These observations could be due to CCL22, which besides being produced by Th2 is also produced by Treg cells (16). While Th17 cells are increased in this progressor group, the CCL22 levels could be decreasing together with Tregs cells due to opposite roles. On the other hand, the negative correlation between plasma CXCL10 levels and Th1Th17 cell frequencies in RPs is likely due to Th1Th17 is one of the cellular population highly permissive to HIV infection (10) and CXCL10 is positively correlated with HIV viral load. Thus, situations where low levels of CXCL10 were observed (maybe because of HAART) in the same time, a restoration of Th1Th17 cell frequencies could be observed.

Conclusion

References

1. Autran B. Effects of antiretroviral therapy on immune reconstitution. Antivir Ther. 1999;4 Suppl 3:3–6.

2. Loiseau C, Requena M, Mavigner M, Cazabat M, Carrere N, Suc B, et al. CCR6− regulatory T cells blunt the restoration of gut Th17 cells along the CCR6–CCL20 axis in treated HIV-1-infected individuals. Mucosal Immunol. Nature Publishing Group; 2016 Sep 17;9(February):1–14.

3. Ananworanich J, Dubé K, Chomont N. How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs? Curr Opin HIV AIDS. 2015 Jan;10(1):18–28.

4. Malatinkova E, Spiegelaere W De, Bonczkowski P, Kiselinova M, Vervisch K, Trypsteen W, et al. Impact of a decade of successful antiretroviral therapy initiated at HIV-1 seroconversion on blood and rectal reservoirs. Elife. 2015 Oct 6;4(OCTOBER2015):1–17.

5. Hunt PW. HIV and Inflammation: Mechanisms and Consequences. Curr HIV/AIDS Rep. 2012 Jun 18;9(2):139–47.

6. Mahnke YD, Fletez-Brant K, Sereti I, Roederer M. Reconstitution of Peripheral T Cells by Tissue-Derived CCR4+ Central Memory Cells Following HIV-1 Antiretroviral Therapy. Pathog Immun. 2016 Oct 13;1(2):260.

7. Kim CJ, McKinnon LR, Kovacs C, Kandel G, Huibner S, Chege D, et al. Mucosal Th17 cell function is altered during HIV infection and is an independent predictor of systemic immune activation. J Immunol. 2013 Sep 1;191(5):2164–73.

8. DaFonseca S, Niessl J, Pouvreau S, Wacleche VS, Gosselin A, Cleret-Buhot A, et al. Impaired Th17 polarization of phenotypically naive CD4(+) T-cells during chronic HIV-1 infection and potential restoration with early ART. Retrovirology. 2015 Apr 30;12:38.

9. de Medeiros RM, Valverde-Villegas JM, Junqueira DM, Gräf T, Lindenau JD, de Mello MG, et al. Rapid and Slow Progressors Show Increased IL-6 and IL-10 Levels in the Pre-AIDS Stage of HIV Infection. Kumar A, editor. PLoS One. 2016 May 23;11(5):e0156163.

10. Gosselin A, Monteiro P, Chomont N, Diaz-Griffero F, Said E a, Fonseca S, et al. Peripheral Blood CCR4+CCR6+ and CXCR3+CCR6+ CD4+ T Cells Are Highly Permissive to HIV-1 Infection. J Immunol. 2010 Feb 1;184(3):1604–16.

11. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol. 2004;22:745–63.

12. Hunt PW, Cao HL, Muzoora C, Ssewanyana I, Bennett J, Emenyonu N, et al. Impact of CD8+ T-cell activation on CD4+ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy. AIDS. 2011 Nov 13;25(17):2123–31.

13. Wu C-Y, Kirman JR, Rotte MJ, Davey DF, Perfetto SP, Rhee EG, et al. Distinct lineages of T(H)1 cells have differential capacities for memory cell generation in vivo. Nat Immunol. 2002 Sep;3(9):852–8.

14. El-Far M, Kouassi P, Sylla M, Zhang Y, Fouda A, Fabre T, et al. Proinflammatory isoforms of IL-32 as novel and robust biomarkers for control failure in HIV-infected slow progressors. Sci Rep. Nature Publishing Group; 2016 Mar 15;6(February):22902.

15. Gorenec L, Zidovec Lepej S, Grgic I, Planinic A, Iscic Bes J, Vince A, et al. The comparison of Th1, Th2, Th9, Th17 and Th22 cytokine profiles in acute and chronic HIV-1 infection. Microb Pathog. Elsevier Ltd; 2016 Aug;97:125–30.

16. Gobert M, Treilleux I, Bendriss-Vermare N, Bachelot T, Goddard-Leon S, Arfi V, et al. Regulatory T Cells Recruited through CCL22/CCR4 Are Selectively Activated in Lymphoid Infiltrates Surrounding Primary Breast Tumors and Lead to an Adverse Clinical Outcome. Cancer Res. 2009 Feb 10;69(5):2000–9.

Table 1. Baseline measurements and demographic characteristics of HIV+ progressors and HIV seronegative controls.

	Clinical stages		
	HIV seronegative (n=10)	**SPs HAART (CD4>350)$^{\beta}$ (n=7)**	**RPs HAART (CD4<350)$^{\alpha}$ (n=5)**
Baseline measurements			
Time progression[a]	NA	12 (8-17)	01 (1-3)
CD4+ T cells nadir before HAART[b]	NA	391 (371-854)	287 (201-340)
VL (log) before HAART[c]	NA	4.0 (2.8-5.0)	4.3 (3.0-5.2)
CD4+ T cells at sampling[b]	NA	769 (411-918)	538 (185-867)
VL (log) at sampling[c]	NA	1.6 (1.6-1.6)	1.6 (1.6-1.6)
Time of HAART at sampling[d]	NA	21 (11-28)	45 (32-66)
Demographic characteristics			
Median age (min-max)	43 (26-60)	45 (32-54)	36 (23-53)
Sex			
female	4 (0.40)	7 (1.00)	5 (0.55)
male	6 (0.60)	0 (0.00)	6 (0.45)
Ethnicity			
European descents	8 (0.80)	5 (0.71)	7 (0.64)
African descents	2 (0.20)	2 (0.29)	4 (0.36)

[a]median (IQ), years; [b]median (IQ) in cells/mm^3; [c]median (IQ) in log10 copies/mL; [d]median (IQ), months; NA, not applicable; HAART, highly active antiretroviral therapy.

$^{\alpha}$according old public health HAART recommendations

$^{\beta}$according new public health HAART recommendations

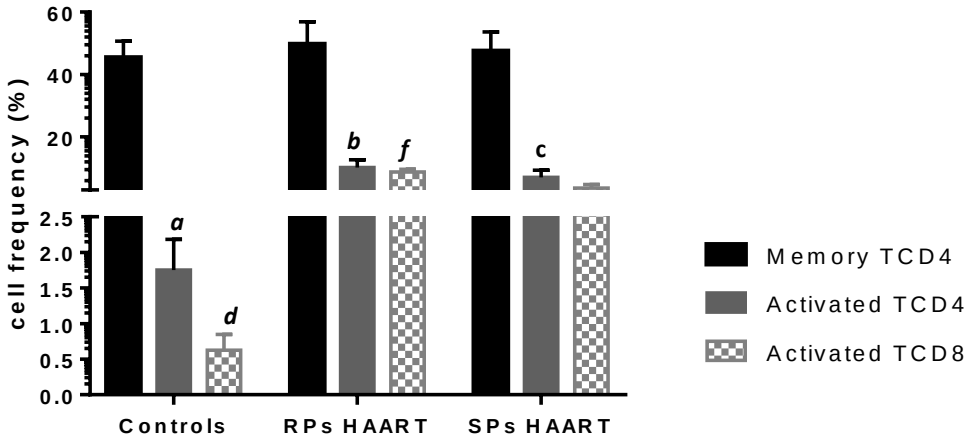


Figure 1. Cell frequencies of T cell activated trough immunophenotyping in extreme progressors and healthy controls. Kruskal-Wallis non-parametric test comparing healthy controls (n=10), SPs (n=7) and RPs (n=5). The CD4+ T cell activated frequencies were gated as $CD3^{+}CD4^{+}CD38^{+}HLA\text{-}DR^{+}$ and CD8+ T cell activated frequencies as $CD3^{+}CD8^{+}CD38^{+}HLA\text{-}DR^{+}$. SPs and RPs had higher levels of CD4+ T-cells active when compared with healthy controls [p_c=0.044 (axc) and p_c=0.008 (axb), respectively]. Also, RPs had higher levels of CD8+ T-cells activated when compared with healthy controls [(p_c=0.001 (dxf)]. Significant differences were observed after controlling for multiple comparisons are showed by *p* corrected (p_c) <0.05.

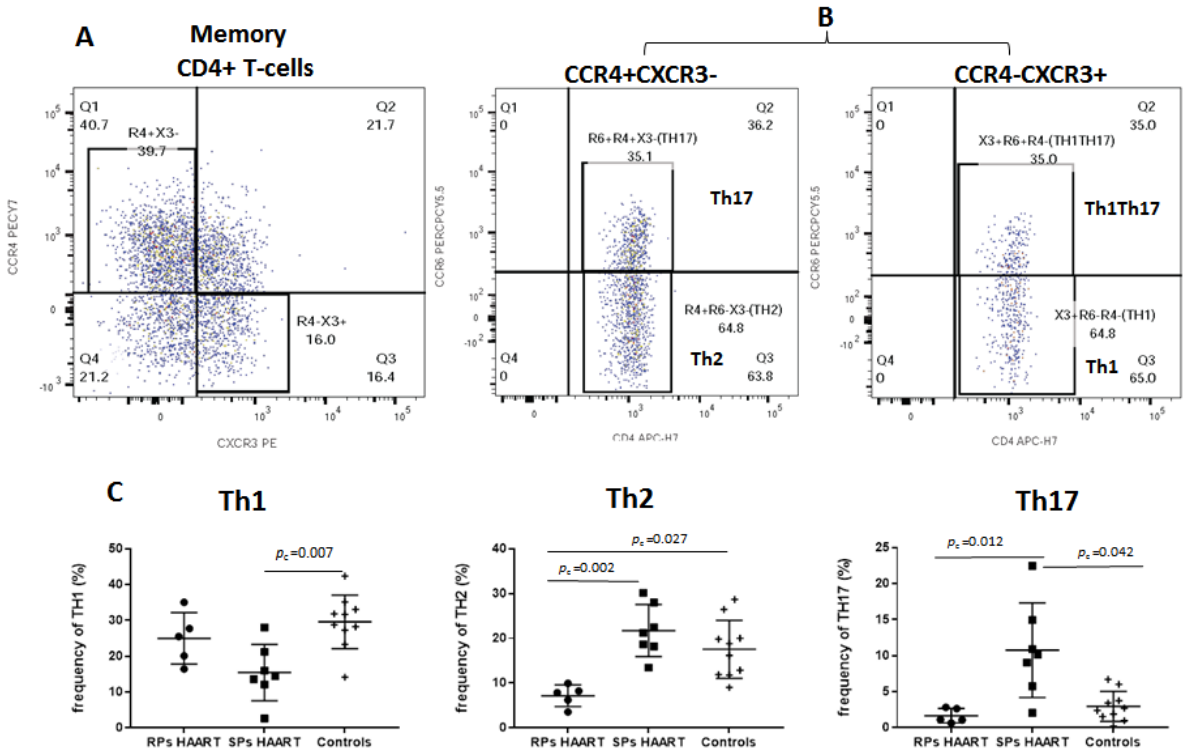


Figure 2. Immunophenotyping of Th cell subsets and comparisons between extreme progressors. The chemokine receptors CCR4, CXCR3, and CCR6 identify CD4+ T cells with distinct Th17, Th2, Th1Th17, and Th1 profiles. PBMCs isolated from uninfected individuals were stained with CD3, CD4, CD45RA, CCR4, CXCR3, and CCR6 Abs and then analysed by multicolour flow cytometer. A, CCR4 and CXCR3 expression identified four subsets of memory ($CD45RA^-$) $CD4^+$ T cells, including $CCR4^+CXCR3^-$ and $CCR4^-CXCR3^+$ subsets. B, CCR6 expression distinguished four cell subsets within the $CCR4^+CXCR3^-$ and $CCR4^-CXCR3^+$ T cell subsets. Figures 2A and 2B are representative of experiment from one patient. C, Kruskall-Wallis non-parametric test for comparisons between groups SPs (n=7), RPs (n=5) and healthy controls (n=10), *p*-values corrected (p_c) for multiple comparisons are indicated in the figure. Horizontal lines indicate median values.

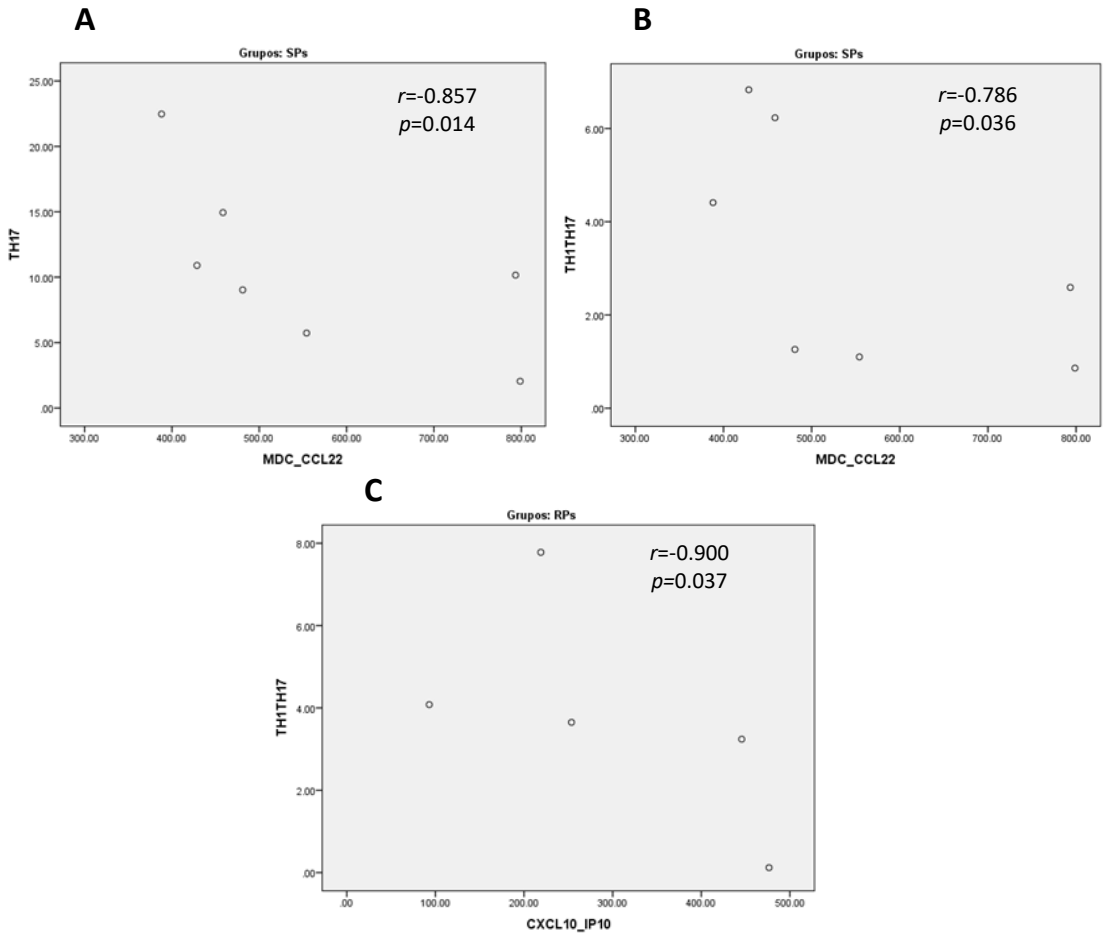


Figure 3. Correlation between Th cell subsets and chemokine plasma levels in extreme progressors. A) and B) Show a negative correlation among CCL22 and Th17 cell frequencies (*r*=0.857, *p*=0.014) and among CCL22 and Th1Th17 (*r*=0.786, *p*=0.036) in SPs. C) Shows negative correlation between CXCL10 and Th1Th17 cell frequencies (r=0.900, *p*=0.037) in RPs. Data were analyzed by Spearman correlation, with *p*<0.05 considered significant.

Capítulo 6

6.1 Discussão

A fase aguda da infecção pelo HIV é um período chave, pois sabe-se que a forma como respondem os fatores imunológicos do hospedeiro nesse primeiro momento frente ao HIV, irá direcionar a progressão da doença. Além disso, muito provavelmente a resposta de tais fatores imunológicos será influenciada pela diversidade genética. Como já descrito, citocinas e quimiocinas são as encarregadas de conectar o sistema imune inato com o adaptativo, ou seja, elas agem desde esse primeiro contato do vírus com o hospedeiro e o papel delas continua a ser constante, na fase crônica, e até a última fase da infecção pelo HIV. Inicialmente estas moléculas são produzidas por células do sistema imune inato, as quais após reconhecerem o vírus, desencadeiam uma resposta pró-inflamatória para detê-lo. No entanto, essa resposta, ao mesmo tempo, ativará mais células dando ao vírus a oportunidade de se replicar intensamente. Assim, em um primeiro momento da infecção, citocinas/quimiocinas têm o papel de controlar a infecção pelo vírus, porém logo terminam sendo as responsáveis por uma ativação imune exacerbada, propiciando mais replicação descontrolada. Interessantemente observou-se que há indivíduos que controlam muito bem essa resposta inicial da infecção, mantendo níveis baixos ou indetectáveis da carga viral. O lado oposto a essa resposta é apresentado pelos indivíduos que progridem de uma forma rápida à aids, pois muito provavelmente durante a fase aguda ou primária não conseguem controlar a carga do vírus, mantendo níveis virais altos e uma queda acentuada e rápida de linfócitos T $CD4^+$. Já há outro grupo distinto de pacientes HIV+, que tem um melhor controle da carga viral, mantendo níveis estáveis de linfócitos T $CD4^+$ por um período longo de tempo. Voltando ao papel das citocinas/quimiocinas pró-inflamatórias, alguns estudos observaram que a sua expressão na fase aguda ou primária da infecção é transitória e que seus níveis se reestabelecem na fase crônica, após o controle da carga viral, porém outros trabalhos têm demonstrado que esses níveis aumentados persistem, e junto com isso,

ocorre uma persistência da ativação celular, persistência essa ainda observada mesmo em indivíduos sob ARV.

Contudo, esta tese explorou o papel genético de quimiocinas e receptores de quimiocinas muito pouco estudados no contexto do HIV e ademais, seu papel imunológico antes e depois dos ARVs. Os alvos de estudo foram escolhidos após revisão da literatura, selecionando-se moléculas que potencialmente pudessem nos dar algumas informações sobre essas diferentes respostas observadas entre os indivíduos HIV+. Foram escolhidos então polimorfismos nos genes que codificam CCR3, CCR4, CCR8 (principais receptores das células Th2), CCR6 (principal receptor das células Th17 e Tregs), CXCR3 (principal receptor das Th1), CCL20 (produzida principalmente pelas células Th17), CXCL10 (produzida principalmente pelas células Th1) e CCL22 (produzida principalmente pelas células Th2). Assim, também na tentativa de correlacionar os achados da primeira parte dos polimorfismos, foram selecionadas seis quimiocinas para avaliação dos níveis plasmáticos: CXCL10, CCL20, CCL22 e CCL2, CCL17, CCL24 (estas últimas três quimiocinas foram incluídas no painel para uma melhor exploração do perfil Th2 e porque são ligantes do CCR3 e CCR4). Os níveis foram quantificados em progressores extremos em diferentes estágios clínicos da infecção. Adicionalmente, se avaliou o papel de ARV na expressão dessas quimiocinas, na ativação imune e na modulação de subpopulações celulares que têm um papel específico frente ao HIV, em um grupo de progressores extremos à aids.

Na primeira parte da tese, após uma análise robusta utilizando-se regressão logística multivariada foi encontrada uma associação dos polimorfismos rs56061981 no *CXCL10* e rs3091250 no *CCR3* na susceptibilidade à infecção pelo HIV. Ainda, foi aplicada uma análise de interação gene-gene pelo MDR (*Multifactor Dimensionality Reduction*), a qual é uma ferramenta interessante para entendermos melhor os efeitos pequenos ou medianos dos polimorfismos e que quando combinados se somam para determinar o desfecho (Moore *et al*. 2006). O melhor modelo dado pela regressão do MDR agrupou dois polimorfismos: rs56061981 no *CXCL10* e rs4359426 no *CCL22*, os quais juntos predizem 57% da susceptibilidade à infecção pelo HIV. Já na abordagem para investigar a influência desses polimorfismos na progressão à aids, através da regressão logística multivariada observamos associação do rs4359426 no *CCL22* e do rs13034664 no *CCL20* com

progressão rápida à aids. Porém, nas análises de interação com o MDR nenhum modelo foi estatisticamente significativo para predizer a progressão à aids.

Esses primeiros resultados evidenciam o papel do rs56061981 no *CXCL10*, rs3091250 no *CCR3* e rs4359426 no *CCL22* na susceptibilidade à infecção pelo HIV. É importante relatar que CXCL10 é produzido pelas células Th1 e, CCR3 é expresso nas células Th2, as quais produzem CCL22. Assim, nossos resultados podem contribuir, do ponto de vista genético, para a compreensão dessa relação Th1:Th2 já bastante associada com a progressão à aids. Estudos *in vitro* para entender o papel das quimiocinas produzidas pelas células Th1 (CXCL10, CXCL9, CXCL11) e sua modulação com as células Th2, as quais produzem citocinas/quimiocinas que ativam eosinófilos, mastócitos e basófilos, no contexto de uma inflamação alérgica são essenciais, pois essa relação Th1:Th2 é bastante observada nesse contexto. Jinquan *et al*., (2000) observaram que o CXCR3, receptor predominantemente expresso em células de memória ativadas Th1, também é um receptor expresso em eosinófilos em humanos. Todavia, se observou que CXCL10 ativa e induz a quimiotaxia dos eosinófilos via CXCR3 (Jinquan *et al*. 2000). Logo depois, Dajotoy *et al*., (2004) observaram que, após a estimulação de eosinófilos com IFN-γ e TNF-α (citocinas do perfil Th1), essas células produziram CXCL10 e CXCL9, porém na presença de IL-4 a síntese de CXCL10 e CXCL9 foi diminuída (Dajotoy *et al*. 2004). Por outro lado, outros estudos observaram que o CCR3, receptor altamente expresso em células do perfil Th2, como eosinófilos, é bloqueado na presença do CXCL10, este último então atuando como um antagonista natural do CCR3 (Loetscher *et al*. 2001; Fulkerson et al. 2004). Em contrapartida, eotaxin-1 (CCL11), uma quimiocina produzida pelas células Th2, compete com o CXCL10 pela união com o CXCR3, bloqueando assim a ativação das células Th1. Assim, se observamos que há uma modulação agonista e antagonista de quimiocinas com perfil Th1 e Th2 mediadas principalmente pela expressão dos receptores CXCR3 e CCR3, resta uma questão: Qual resposta predominará? No contexto do HIV, recentemente um estudo observou níveis aumentados de citocinas do perfil Th2 (IL-10, IL-4 e TNF-α) na fase aguda da infecção. Além disso, os pesquisadores observaram que esse perfil predominou até a fase crônica (Gorenec *et al*. 2016). Outros achados interessantes foram os de Miguez-Burbano *et al*., (1995) que reportaram na fase inicial da infecção pelo HIV, que níveis incrementados de IgE (marcador de resposta alérgica) no plasma precediam o declínio das células T $CD4^+$. Esses resultados sugeriram que IgE poderia ser utilizada

como um marcador para monitorar a progressão da doença em indivíduos HIV+ pertencentes a diferentes grupos de risco (Miguez-Burbano *et al*. 1995). Outros dois estudos mostraram que houve indução de IgE no sangue de crianças HIV+ mediada pelas proteínas do HIV: gp160, p24 e p17 (Khalife *et al*. 1988; Secord *et al*. 1996). Ademais, a proteína viral gp160 regulou positivamente a expressão de IgE, induzida por IL-4 (Dugas *et al*. 2000). Esses resultados sugerem que peptídeos tipo alergenos, como as proteínas virais do HIV, induzem a síntese de IgE. Além disso, outros dois estudos demonstraram que existe uma replicação preferencial do HIV nas células Th2 e nas células T virgens (Maggi *et al*. 1994; Romagnani *et al*. 1994). Contudo, a fase inicial da infecção pelo HIV, desencadeando uma resposta com níveis incrementados de Th2 e IgE, pode contribuir para o enfraquecimento do sistema imunológico contra o HIV levando a manifestações clínicas como alergias e eosinofilia - as quais são efetivamente encontradas em indivíduos HIV+ (Becker 2004; Yokobayashi *et al*. 2013). Todos esses resultados contrastam com a hipótese do *shift* Th1→Th2 na infecção pelo HIV, a qual propõe que inicialmente se desenvolve um perfil Th1 e que o perfil Th2 já é um indicador da doença avançada (Clerici and Shearer 1993). No entanto, esta hipótese tem sido bastante discutida devido a diferenças encontradas em diferentes estudos (Becker 2004), assim ainda precisa ser melhor investigada. Além dos fatores imunológicos que vem sendo estudados para entendermos essas respostas na fase inicial da infecção, outros pontos importantes, como o papel da variabilidade genética do hospedeiro, envolvendo quimiocinas e receptores de quimiocinas, merecem ser avaliados.

O polimorfismo rs56061981 no *CXCL10* corresponde a uma variante na região promotora do gene e estimula a expressão do CXCL10 via transativação pelo fator de transcrição NFkβ. Em um estudo com modelo de SIV observaram-se níveis aumentados do mRNA do *CXCL10* nas PBMCs e nos nódulos linfoides associados com progressão rápida em primatas não humanos infectados com SIV (Durudas *et al*. 2009). Estudos funcionais mostraram que o polimorfismo rs3091250 no *CCR3* e o rs4359426 no *CCL22* estavam associados com níveis aumentados da expressão do mRNA (Kim *et al*. 2008; Hirota *et al*. 2011). Para melhor compreensão desses primeiros resultados, quantificamos os níveis plasmáticos de CXCL10 e CCL22 de 29 HIV soropositivos (pertencentes aos grupos de progressores rápidos e lentos) que ainda não tinham iniciado o tratamento e que tinham sido previamente genotipados para os polimorfismos rs56061981 (*CXCL10*) e rs4359426

(*CCL22*). Adicionalmente, estes polimorfismos foram genotipados em soronegativos pertencentes a um grupo controle de 18 indivíduos saudáveis e os níveis plasmáticos dessas duas quimiocinas foram quantificados. Infelizmente, devido à baixa frequência das variantes alélicas rs56061981 e rs4359426 não foi possível atingir um grupo representativo que pudesse dar robustez estatística aos resultados (dados não apresentados).

Em relação à progressão à aids, a frequência do genótipo homozigoto mutante CC do rs13034664 no *CCL20* foi mais baixa nos progressores rápidos quando comparados com os não rápidos (0,07 vs. 0,29, *P*=0,013). E, dentro dos não rápidos, observamos que a frequência desse genótipo foi mais alta nos progressores lentos e nos controladores de elite quando comparados com os progressores rápidos (0,17, 0,50 e 0,07, respectivamente) (Tabela S2 no artigo do capítulo 3). Interessantemente, em um grupo de 8 controladores de elite, 4 apresentaram o genótipo CC e a frequência foi significativamente diferente quando comparada com os progressores rápidos (*P*=0,006). Nossos achados sobre a influência da variante rs13034664 do *CCL20* nos níveis plasmáticos foram: progressores lentos com o genótipo CC (n=3) apresentaram níveis mais baixos do CCL20 quando comparados com os portadores dos genótipos CT+TT (n=18), porém essa diferença não foi estatisticamente significativa (13,14 vs. 27,13 pg/mL, *P*=0,0617). Nos controles saudáveis, esses níveis foram mais baixos nos indivíduos com o genótipo CC (n=8) quando comparados com os portadores de CT+TT (n=11) (13,97 vs. 17,01 pg/mL, *P*=0,3511), também sem diferença significativa. Nenhum progressor rápido com o genótipo CC foi possível comparar com os portadores de CT+TT devido à baixa frequência do genótipo CC neste grupo de progressão. Apesar desses dados não terem alcançado valores estatisticamente significativos, esses resultados merecem ser levados em conta para estudos funcionais e/ou de bioinformática avaliando esse polimorfismo, assim como os reportados aqui do *CCR3*, *CXCL10* e *CCL22*. O rs13034664 está localizado na região promotora do gene *CCL20* e apenas dois trabalhos evidenciaram seu papel em outras doenças: na dermatite atópica e colitis ulcerativa (Choi *et al*. 2005; Rafaels *et al*. 2009). Porém, uma ampla variedade de estudos associando níveis alterados do CCL20 com diferentes desfechos, principalmente em doenças autoimunes e câncer, já foi realizada (Jafarzadeh *et al*. 2014; Frick 2016; Liu *et al*. 2016). A região promotora do gene *CCL20* está regulada por pelo menos cinco fatores de transcrição e receptores com perfil inflamatório, que regulam negativa ou positivamente os níveis de transcrição do gene *CCL20* (Zhao *et al*. 2014). Uma

particularidade do CCL20 é que é o único ligante que se liga ao CCR6, e vários estudos já observaram o papel chave do eixo CCL20-CCR6 na regulação da resposta imune (Schutyser *et al*. 2003; Comerford *et al*. 2010; Lee *et al*. 2015). Apesar de já ser bastante conhecido seu papel particular na resposta imune, pouco foi avaliado acerca a influência da variabilidade do *CCL20* em diferentes contextos. No contexto da infecção pelo HIV, está se elucidando cada vez mais seu impacto no recrutamento de células da mucosa (lugar onde essa quimiocina é altamente expressa) via CCR6 e na propagação do vírus em novos focos de infecção (Arnold *et al*. 2015). Foi observado níveis aumentados do CCL20 no fluido cérvico-vaginal em mulheres HIV+ na fase primária da infecção (McKinnon *et al*. 2015) e outros estudos observaram níveis plasmáticos aumentados de CCL20 em progressores rápidos e progressores típicos quando comparados com os controladores de elite aviremicos e controles saudáveis (Fontaine *et al*. 2011; Gauvin *et al*. 2016). Este trabalho é o primeiro a avaliar polimorfismos candidatos tanto do *CCL20* como do *CCR6* no contexto do HIV.

Desde que essa primeira abordagem foi realizada pela regressão logística ajustando as análises pela variável etnia, e sabendo que esse é um fator que potencialmente pode influenciar nas análises genéticas de caso-controle, também foram realizadas as análises estratificando pela etnia. Observou-se que o rs2234355 do *CXCR6*, rs2853699 do *CCR8* e rs56061981 do *CXCL10* foram associados com susceptibilidade à infecção pelo HIV na população euro-descendente. De acordo com esses achados, o rs2853699 já foi previamente associado com a progressão a aids em uma população europeia (An *et al*. 2011). Entretanto, o rs2234355 do *CXCR6* foi associado com proteção à infecção pelo HIV nos indivíduos afrodescendentes neste estudo, corroborando prévios achados sobre o efeito protetor deste polimorfismo na população afrodescendente (Duggal *et al*. 2003; Zhao *et al*. 2012).

Quando os níveis plasmáticos das quimiocinas já citadas foram quantificados nos diferentes estágios clínicos da infecção, foram observados níveis maiores de CXCL10 nos grupos de indivíduos HIV+ que estavam na fase pré-aids (os quais iniciaram ARV entre 1-2 anos após a coleta) quando comparados com os controles saudáveis. Estudos já observaram que níveis aumentados do CXCL10 são encontrados na fase aguda e primária da infecção junto com alta carga viral do HIV (Stacey *et al*. 2009; Liovat *et al*. 2012). Neste estudo, os progressores extremos que se encontravam na fase crônica da infecção e

especificamente na fase pré-aids, apresentaram níveis significativamente aumentados do CXCL10 quando comparados com os controles saudáveis. Interessantemente, não houve diferenças significativas quando comparados os progressores lentos e rápidos pré-aids, mostrando que essa modulação dos níveis de CXCL10 é dependente do estágio clínico da doença e não das diferenças fenotípicas entre os indivíduos. Todavia, nesta parte do estudo, foi possível avaliar o impacto de ARV sobre os níveis de CXCL10 quando comparados os indivíduos que iniciaram ARV com <350 células T $CD4^+$ e os que iniciaram com >350 células T $CD4^+$. Assim, quando comparados os progressores lentos que estavam com o mesmo tempo de ARV, observou se que os níveis de CXCL10 foram praticamente normalizados nos indivíduos que iniciaram ARV com >350 células T $CD4^+$. Por outro lado, níveis persistentemente altos de CCL20 foram medidos mesmo em progressores lentos crônicos com >350 células $CD4^+$ e em pacientes sob ARV. Apesar da perda da significância estatística após a correção por múltiplas comparações, esses dados estão de acordo com prévios estudos, que observaram a persistência do CCL20 em pacientes sob ARV (Fontaine *et al*. 2011; Gauvin *et al*. 2016; Aziz *et al*. 2016). Assim, CCL20 tem um potencial papel na disseminação do vírus como já descrito, e é importante considerá-lo em pesquisas futuras.

Esses dados chamam a atenção para o reforço nas recomendações sobre início de ARV em indivíduos crônicos, mesmo estes apresentando números estáveis de células T $CD4^+$. Somado a isto, nós avaliamos a ativação celular e perfil de subpopulações celulares no grupo de progressores lentos que iniciaram ARV com >350 células T $CD4^+$ (dados apresentados no capítulo 5 em relação ao impacto de ARV). Os nossos resultados confirmam o que outros estudos já vêm observando, que há uma ativação persistente do sistema imunológico, mesmo em indivíduos sob ARV e com carga viral indetectável. Ante essas observações, estudos têm proposto que iniciar ARV na fase inicial da infecção vai reestabelecer mais rapidamente o sistema imune que se iniciado tardiamente na fase crônica, onde o sistema imune já foi bastante comprometido. Ademais, o início tardio de ARV pode, por alterar as subpopulações celulares já estabelecidas no indivíduo, fornecer um sinal que leve a um desequilíbrio, visto que algumas subpopulações celulares foram mais acometidas no início da infecção e, logo serão mais dificilmente recuperadas, mesmo sob ARV. Neste estudo os progressores rápidos que iniciaram ARV com <350 tiveram uma frequência diminuída das células Th2 quando comparados com os controles saudáveis

e já os progressores lentos que iniciaram ARV >350 na fase crônica da infecção também apresentaram uma desregulação nas frequências das células com perfil Th1 (diminuídas) e Th17 (aumentadas) em relação aos controles saudáveis.

Porém, a limitação da proposta de iniciar ARV na fase inicial da infecção é muito grande já que a maioria da população HIV+ só descobre sua sorologia na fase crônica da infecção: ao chegar ao hospital para doar sangue, na internação por alguma outra doença, no acompanhamento pré-natal, quando da identificação de sintomas relacionados à aids, entre outras causas. As propostas de recomendação para o início de ARV independentemente do nível de $CD4^+$ permitiu comparar pacientes que iniciam ARV com $CD4^+$ estável (350-550 células T $CD4^+$) com aqueles que iniciaram ARV com <200-350 células T $CD4^+$. Estudos anteriores observaram que iniciar ARV com $CD4^+$ estável leva a uma taxa de recuperação melhor quando comparado com o outro grupo (Cohen *et al*. 2011; Grinsztejn *et al*. 2014). Contudo, ante essa limitação de atingir HIV soropositivos na fase inicial da infecção, sugere-se o monitoramento de pacientes na fase crônica, mesmo com níveis estáveis do $CD4^+$ usando biomarcadores, tais como o CXCL10, e muito provavelmente o CCL20, para as recomendações do início imediato de ARV.

Finalmente, os controladores de elite, neste estudo apresentaram níveis de CXCL10 similarmente baixos aos controles saudáveis e isso pode potencialmente ser explicado devido ao fato deles apresentarem carga viral indetectável, pois esta é uma quimiocina expressa na presença de infecção viral. Em relação ao CCL20, os níveis também foram similarmente baixos aos controles saudáveis. Existe na literatura dados controversos sobre este grupo especial de indivíduos HIV+. Alguns estudos observaram que eles apresentam uma ativação celular aumentada quando comparados com os controles saudáveis, e tal ativação poderá eventualmente contribuir para uma desestabilização do sistema imune (Hunt *et al*. 2008). Porém, outros trabalhos mostraram uma ativação celular similar aos controles saudáveis e uma manutenção da integridade da mucosa intestinal (Sankaran *et al*. 2005). Diferenças entre essas observações provavelmente são dadas pela caracterização que cada grupo usa para definir os controladores de elite.

6.2 Conclusão

Uma parte dos resultados desta tese, avaliando fatores genéticos e imunológicos do hospedeiro são esquematicamente representados na figura 5, e sugerem que: o papel dos polimorfismos rs56061981 do *CXCL10* e o rs3091250 do *CCR3* aumentam a susceptibilidade do indivíduo frente à infecção pelo HIV, enquanto que o polimorfismo rs13034664 do *CCL20* influencia na progressão da doença. Já o polimorfismo rs4359426 do *CCL22* influencia tanto na susceptibilidade e na progressão à aids. Levando em conta que esses polimorfismos podem modificar o padrão de expressão dos níveis de CCR3, CXCL10, CCL20 e CCL22, prejudicando a resposta inicial do hospedeiro dada pelo padrão Th1:Th2 frente ao HIV, e consequentemente afetam a progressão da doença, este é um campo a ser investigado para um melhor entendimento dessa relação. Além disso, os níveis plasmáticos do CXCL10 estão aumentados na fase pré-aids, o monitoramento do individuo na fase crônica da infecção e ainda com boa manutenção dos níveis de CD4, usando biomarcadores tais como o CXCL10 e CCL20, pode auxiliar na tomada de decisões para o início imediato de ARV. Já o papel da terapia influenciou sobre os níveis de CXCL10 chegando aos níveis normais, enquanto que ativação celular e os níveis de CCL20 persistem nos progressores extremos sob ARV. Ademais, uma desregulação de subpopulações T $CD4^+$ específicas em progressores extremos sob ARV foi observada.

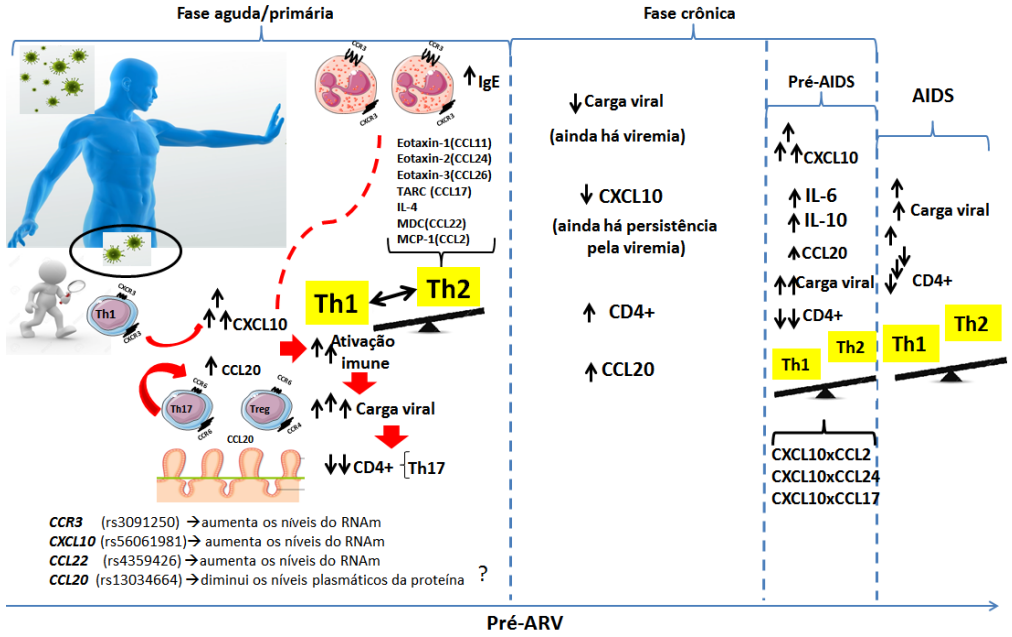


Figura 5. Fatores imunológicos e genéticos do hospedeiro que potencialmente influenciam na susceptibilidade e na progressão à aids. Contexto: na fase aguda e primária da infecção, o CXCL10 é uma das primeiras quimiocinas produzidas e altos níveis plasmáticos desta molécula são encontrados nesta fase inicial. Além disso, uma ativação imune exacerbada e consequentemente uma replicação viral descontrolada leva a um acentuado esgotamento de células T $CD4^+$ (principalmente daquelas que se encontram na área da mucosa, como as Th17), tal esgotamento é negativamente correlacionado com os níveis plasmáticos do CXCL10. O CXCL10, que é produzido principalmente pelas Th1, vai recrutar também células Th2, (as quais expressam predominantemente CCR3, porém também expressam o CXCR3) e estas a sua vez vão produzir também o CXCL10. Recentemente um perfil predominante Th2 foi observado na fase aguda da infecção (presença de altos níveis de IL-10 e IL-4), e se manteve até a fase crônica. Vários trabalhos observaram uma predominância do perfil Th2 na fase da aids. Hipóteses: será que o perfil Th2 na fase inicial da infecção vai determinar a progressão da doença e esse perfil vai se manter o longo da infecção na fase pré-ARV? será que o gatilho do perfil Th2 é dado pelos altos níveis do CXCL10 na fase inicial da infecção? será que a variabilidade genética de moléculas do hospedeiro, que são chaves desde o primeiro momento da infecção, pode influenciar no padrão de expressão delas influenciando na infecção pelo HIV/AIDS? Nesta tese: Observou-se a influência da diversidade genética de CCR3, CXCL10, CCL22 e CCL20 na susceptibilidade à infecção pelo HIV e progressão para a aids. Já na fase pré-aids, observou-se níveis plasmáticos aumentados do CXCL10 os quais foram positivamente correlacionados com carga viral e negativamente com linfócitos T $CD4^+$. Ademais, uma correlação positiva com quimiocinas de perfil Th2 foi observada nesta fase clínica da doença. Os níveis plasmáticos de CXCL10 de normalizaram após um tempo sob ARV. Já os níveis do CCL20 estavam aumentados na fase crônica e pré-aids, e uma persistência destes níveis aumentados encontrou-se mesmo após um tempo com o tratamento.

6.3 Perspectivas

- Está em andamento um estudo de replicação avaliando esse painel de polimorfismos em uma coorte de transmissão vertical de Recife-Pernambuco;
- Está em andamento um projeto para quantificação da expressão de fatores de transcrição das subpopulações celulares Th1, Th2, Th17 e Tregs;
- Incluir as análises das células T regulatórias (Tregs) e precursoras no último artigo em preparação (a imunofenotipagem já foi realizada).

REFERÊNCIAS BIBLIOGRÁFICAS

Aasa-Chapman MMI, Seymour CR, Williams I and McKnight A (2006) Novel envelope determinants for CCR3 use by human immunodeficiency virus. J Virol 80:10884–9. doi: 10.1128/JVI.01030-06

Abbas AK, Litchman AH PS (2012) Imunologia Celular e Molecular, Saunders. Elsevier Editora Ltda

Altfeld M and Gale Jr M (2015) Innate immunity against HIV-1 infection. Nat Immunol 16:554–562. doi: 10.1038/ni.3157

Amara A, Gall SL, Schwartz O, Salamero J, Montes M, Loetscher P, Baggiolini M, Virelizier JL and Arenzana-Seisdedos F (1997) HIV coreceptor downregulation as antiviral principle: SDF-1alpha-dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. J Exp Med 186:139–46.

An P, Li R, Wang JM, Yoshimura T, Takahashi M, Samudralal R, O'Brien SJ, Phair J, Goedert JJ, Kirk GD et al. (2011) Role of exonic variation in chemokine receptor genes on AIDS: CCRL2 F167Y association with pneumocystis pneumonia. PLoS Genet 7:e1002328. doi: 10.1371/journal.pgen.1002328

An P and Winkler C a (2010) Host genes associated with HIV/AIDS: advances in gene discovery. Trends Genet 26:119–131. doi: 10.1016/j.tig.2010.01.002

Arenzana-Seisdedos F (2015) SDF-1/CXCL12: A Chemokine in the Life Cycle of HIV. Front Immunol 6:10–13. doi: 10.3389/fimmu.2015.00256

Arnold KB, Burgener A, Birse K, Romas L, Dunphy LJ, Shahabi K, Abou M, Westmacott GR, McCorrister S, Kwatampora J et al. (2015) Increased levels of inflammatory cytokines in the female reproductive tract are associated with altered expression of proteases, mucosal barrier proteins, and an influx of HIV-susceptible target cells. Mucosal Immunol 9:1–12. doi: 10.1038/mi.2015.51

Aziz N, Detels R, Chang LC and Butch AW (2016) Macrophage Inflammatory Protein-3 Alpha (MIP-3α)/CCL20 in HIV-1-Infected Individuals. J AIDS Clin Res. doi: 10.4172/2155-6113.1000587

Bansal A, Sterrett S, Erdmann N, Westfall AO, Dionne-Odom J, Overton ET and Goepfert PA (2015) Normal T-cell activation in elite controllers with preserved CD4+ T-cell counts. AIDS 29:2245–54. doi: 10.1097/QAD.0000000000000860

Bartlett J a, Fath MJ, Demasi R, Hermes A, Quinn J, Mondou E and Rousseau F (2006) An updated systematic overview of triple combination therapy in antiretroviral-naive HIV-infected adults. AIDS 20:2051–64. doi: 10.1097/01.aids.0000247578.08449.ff

Becker Y (2004) The changes in the T helper 1 (Th1) and T helper 2 (Th2) cytokine balance during HIV-1 infection are indicative of an allergic response to viral proteins that may be reversed by Th2 cytokine inhibitors and immune response modifiers--a review and hypothesi. Virus Genes 28:5–18. doi: 10.1023/B:VIRU.0000012260.32578.72

Björndal A, Deng H, Jansson M, Fiore JR, Colognesi C, Karlsson A, Albert J, Scarlatti G, Littman DR and Fenyö EM (1997) Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype. J Virol 71:7478–87.

BRASIL (1996) Lei n° 9313. 122864.

Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, Scheinberg P, Price DA, Hage CA, Kholi LM et al. (2008) Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood 112:2826–35. doi: 10.1182/blood-2008-05-159301

Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT et al. (2004) CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 200:749–59. doi: 10.1084/jem.20040874

Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, Rosenberg ES, Ellis RJ and Williams KC (2011) Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. J Infect Dis 204:154–63. doi: 10.1093/infdis/jir214

Calado M, Matoso P, Santos-Costa Q, Espirito-Santo M, Machado J, Rosado L, Antunes F, Mansinho K, Lopes MM, Maltez F et al. (2010) Coreceptor usage by HIV-1 and HIV-2 primary isolates: the relevance of CCR8 chemokine receptor as an alternative coreceptor. Virology 408:174–82. doi: 10.1016/j.virol.2010.09.020

Campbell DJ and Koch MA (2011) Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nat Rev Immunol 11:119–130. doi: 10.1038/nri2916

Card CM, Ball T and Fowke KR (2013) Immune Quiescence: a model of protection against HIV infection. Retrovirology 10:141. doi: 10.1186/1742-4690-10-141

Card CM, McLaren PJ, Wachihi C, Kimani J, Plummer FA and Fowke KR (2009) Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)CD25(+)FOXP3(+) regulatory T cells. J Infect Dis 199:1318–22. doi: 10.1086/597801

Chequer P, Hearst N, Hudes ES, Castilho E, Rutherford G, Loures L and Rodrigues L (1992) Determinants of survival in adult Brazilian AIDS patients, 1982-1989. The Brazilian State AIDS Program Co-Ordinators. AIDS 6:483–7. doi: 10.1097/00002030-199205000-00007

Choi S, Lee E-K, Lee S, Chae S, Lee M, Seo G, Kim S, Yeom J and Jun C (2005)

Ulcerative Colitis is Associated with Novel Polymorphisms in the Promoter Region of MIP-3α/CCL20 Gene. Immune Netw 5:205. doi: 10.4110/in.2005.5.4.205

Cilliers T, Willey S, Sullivan WM, Patience T, Pugach P, Coetzer M, Papathanasopoulos M, Moore JP, Trkola A, Clapham P et al. (2005) Use of alternate coreceptors on primary cells by two HIV-1 isolates. Virology 339:136–44. doi: 10.1016/j.virol.2005.05.027

Clapham PR and McKnight Á (2002) Cell surface receptors, virus entry and tropism of primate lentiviruses. J Gen Virol 83:1809–1829. doi: 10.1099/0022-1317-83-8-1809

Clerici M and Shearer GM (1993) A TH1-->TH2 switch is a critical step in the etiology of HIV infection. Immunol Today 14:107–11. doi: 10.1016/0167-5699(93)90208-3

Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC and Lusso P (1995) Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science 270:1811–5.

Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, Hakim JG, Kumwenda J, Grinsztejn B, Pilotto JHS et al. (2011) Prevention of HIV-1 Infection with Early Antiretroviral Therapy. N Engl J Med 365:493–505. doi: 10.1056/NEJMoa1105243

Comerford I, Bunting M, Fenix K, Haylock-Jacobs S, Litchfield W, Harata-Lee Y, Turvey M, Brazzatti J, Gregor C, Nguyen P et al. (2010) An immune paradox: how can the same chemokine axis regulate both immune tolerance and activation?: CCR6/CCL20: a chemokine axis balancing immunological tolerance and inflammation in autoimmune disease. Bioessays 32:1067–76. doi: 10.1002/bies.201000063

Connor RI, Sheridan KE, Ceradini D, Choe S and Landau NR (1997) Change in coreceptor use correlates with disease progression in HIV-1--infected individuals. J Exp Med 185:621–8.

DaFonseca S, Niessl J, Pouvreau S, Wacleche VS, Gosselin A, Cleret-Buhot A, Bernard N, Tremblay C, Jenabian M-A, Routy J-P et al. (2015) Impaired Th17 polarization of phenotypically naive CD4+ T-cells during chronic HIV-1 infection and potential restoration with early ART. Retrovirology 12:38. doi: 10.1186/s12977-015-0164-6

Dajotoy T, Andersson P, Bjartell A, Löfdahl C-G, Tapper H and Egesten A (2004) Human eosinophils produce the T cell-attracting chemokines MIG and IP-10 upon stimulation with IFN-gamma. J Leukoc Biol 76:685–91. doi: 10.1189/jlb.0803379

Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C et al. (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443:350–4. doi: 10.1038/nature05115

de Medeiros RM, Valverde-Villegas JM, Junqueira DM, Gräf T, Lindenau JD, de Mello MG, Vianna P, Almeida SEM and Chies JAB (2016) Rapid and Slow Progressors Show

Increased IL-6 and IL-10 Levels in the Pre-AIDS Stage of HIV Infection. PLoS One 11:e0156163. doi: 10.1371/journal.pone.0156163

Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E et al. (1996) Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE. Science 273:1856–62.

Deeks SG (2004) Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood 104:942–947. doi: 10.1182/blood-2003-09-3333

Dugas N, Dereuddre-Bosquet N, Goujard C, Dormont D, Tardieu M and Delfraissy JF (2000) Role of nitric oxide in the promoting effect of HIV type 1 infection and of gp120 envelope glycoprotein on interleukin 4-induced IgE production by normal human mononuclear cells. AIDS Res Hum Retroviruses 16:251–8. doi: 10.1089/088922200309340

Duggal P, An P, Beaty TH, Strathdee S a, Farzadegan H, Markham RB, Johnson L, O'Brien SJ, Vlahov D and Winkler C a (2003) Genetic influence of CXCR6 chemokine receptor alleles on PCP-mediated AIDS progression among African Americans. Genes Immun 4:245–50. doi: 10.1038/sj.gene.6363950

Durudas A, Milush JM, Chen H-L, Engram JC, Silvestri G and Sodora DL (2009) Elevated Levels of Innate Immune Modulators in Lymph Nodes and Blood Are Associated with More-Rapid Disease Progression in Simian Immunodeficiency Virus-Infected Monkeys. J Virol 83:12229–12240. doi: 10.1128/JVI.01311-09

Epstein FH and Luster AD (1998) Chemokines — Chemotactic Cytokines That Mediate Inflammation. N Engl J Med 338:436–445. doi: 10.1056/NEJM199802123380706

Esche C, Stellato C and Beck LA (2005) Chemokines: Key players in innate and adaptive immunity. J Invest Dermatol 125:615–628. doi: 10.1111/j.0022-202X.2005.23841.x

Fontaine J, Coutlée F, Tremblay C, Routy J-P, Poudrier J, Roger M and Montreal Primary HIV Infection and Long-Term Nonprogressor Study Groups (2009) HIV infection affects blood myeloid dendritic cells after successful therapy and despite nonprogressing clinical disease. J Infect Dis 199:1007–18. doi: 10.1086/597278

Fontaine J, Poudrier J and Roger M (2011) Short Communication: Persistence of High Blood Levels of the Chemokines CCL2, CCL19, and CCL20 During the Course of HIV Infection. AIDS Res Hum Retroviruses 27:655–657. doi: 10.1089/aid.2010.0261

Frick VO (2016) Chemokine/chemokine receptor pair CCL20/CCR6 in human colorectal malignancy: An overview. World J Gastroenterol 22:833. doi: 10.3748/wjg.v22.i2.833

Fulkerson PC, Zimmermann N, Brandt EB, Muntel EE, Doepker MP, Kavanaugh JL,

Mishra A, Witte DP, Zhang H, Farber JM et al. (2004) Negative regulation of eosinophil recruitment to the lung by the chemokine monokine induced by IFN-gamma (Mig, CXCL9). Proc Natl Acad Sci U S A 101:1987–92. doi: 10.1073/pnas.0308544100

Gadelha AJ, Accacio N, Costa RLB, Galhardo MC, Cotrim MR, de Souza R V, Morgado M, Marzochi K, Lourenco MC and Rolla VC (2002) Morbidity and survival in advanced AIDS in Rio de Janeiro, Brazil. Rev Inst Med Trop Sao Paulo 44:179–86. doi: S0036-46652002000400001 [pii]

Galvin SR and Cohen MS (2004) The role of sexually transmitted diseases in HIV transmission. Nat Rev Microbiol 2:33–42. doi: 10.1038/nrmicro794

Gauvin J, Chagnon-Choquet J, Poudrier J and Roger M (2016) Fluctuations in Blood Marginal Zone B-Cell Frequencies May Reflect Migratory Patterns Associated with HIV-1 Disease Progression Status. PLoS One 11:e0155868. doi: 10.1371/journal.pone.0155868

Giorgi J V, Liu Z, Hultin LE, Cumberland WG, Hennessey K and Detels R (1993) Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J Acquir Immune Defic Syndr 6:904–12.

Goicoechea M, Smith D, May S, Mathews C and Spina C (2009) Prevalence and T-cell phenotype of slow HIV disease progressors with robust HIV replication. J Acquir Immune Defic Syndr 52:299–301. doi: 10.1097/QAI.0b013e3181b08d70

Gorenec L, Zidovec Lepej S, Grgic I, Planinic A, Iscic Bes J, Vince A and Begovac J (2016) The comparison of Th1, Th2, Th9, Th17 and Th22 cytokine profiles in acute and chronic HIV-1 infection. Microb Pathog 97:125–30. doi: 10.1016/j.micpath.2016.06.008

Gosselin A, Monteiro P, Chomont N, Diaz-Griffero F, Said E a, Fonseca S, Wacleche V, El-Far M, Boulassel M-R, Routy J-P et al. (2010) Peripheral Blood CCR4+CCR6+ and CXCR3+CCR6+ CD4+ T Cells Are Highly Permissive to HIV-1 Infection. J Immunol 184:1604–1616. doi: 10.4049/jimmunol.0903058

Graziosi C, Ganttr KR, Vaccarezza M, Demarest JF, Daucher M, Saag MS, Shaw GM, Quinn TC, Cohen OJ, Welbon CC et al. (1996) Kinetics of cytokine expression during primary human immunodeficiency virus type 1 infection. 93:4386–4391.

Grinsztejn B, Hosseinipour MC, Ribaudo HJ, Swindells S, Eron J, Chen YQ, Wang L, Ou S-S, Anderson M, McCauley M et al. (2014) Effects of early versus delayed initiation of antiretroviral treatment on clinical outcomes of HIV-1 infection: results from the phase 3 HPTN 052 randomised controlled trial. Lancet Infect Dis 14:281–290. doi: 10.1016/S1473-3099(13)70692-3

Grossman Z, Meier-Schellersheim M, Paul WE and Picker LJ (2006) Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat Med 12:289–95. doi: 10.1038/nm1380

Gurdasani D, Iles L, Dillon DG, Young EH, Olson AD, Naranbhai V, Fidler S, Gkrania-Klotsas E, Post F a, Kellam P et al. (2014) A systematic review of definitions of extreme phenotypes of HIV control and progression. AIDS 28:149–62. doi: 10.1097/QAD.0000000000000049

Han Y, Lai J, Barditch-Crovo P, Gallant JE, Williams TM, Siliciano RF and Blankson JN (2008) The role of protective HCP5 and HLA-C associated polymorphisms in the control of HIV-1 replication in a subset of elite suppressors. AIDS 22:541–544. doi: 10.1097/QAD.0b013e3282f470e4

Hardy G, Worrell S, Hayes P, Barnett CM, Glass D, Pido-Lopez J, Imami N, Aspinall R, Dutton J, Gazzard B et al. (2004) Evidence of thymic reconstitution after highly active antiretroviral therapy in HIV-1 infection. HIV Med 5:67–73.

Hirota T, Saeki H, Tomita K, Tanaka S, Ebe K, Sakashita M, Yamada T, Fujieda S, Miyatake A, Doi S et al. (2011) Variants of C-C motif chemokine 22 (CCL22) are associated with susceptibility to atopic dermatitis: case-control studies. PLoS One 6:e26987. doi: 10.1371/journal.pone.0026987

Hoenigl M, Chaillon A and Little SJ (2016) CD4/CD8 Cell Ratio in Acute HIV Infection and the Impact of Early Antiretroviral Therapy: Table 1. Clin Infect Dis 63:425–426. doi: 10.1093/cid/ciw293

Hunt PW (2009) Natural Control of HIV-1 Replication and Long-Term Nonprogression: Overlapping but Distinct Phenotypes. J Infect Dis 200:1636–1638. doi: 10.1086/646610

Hunt PW (2012) HIV and Inflammation: Mechanisms and Consequences. Curr HIV/AIDS Rep 9:139–147. doi: 10.1007/s11904-012-0118-8

Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, Hsue P, Emu B, Krone M, Lampiris H et al. (2008) Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis 197:126–33. doi: 10.1086/524143

Izquierdo-Useros N, Lorizate M, Puertas MC, Rodriguez-Plata MT, Zangger N, Erikson E, Pino M, Erkizia I, Glass B, Clotet B et al. (2012) Siglec-1 Is a Novel Dendritic Cell Receptor That Mediates HIV-1 Trans-Infection Through Recognition of Viral Membrane Gangliosides. PLoS Biol 10:e1001448. doi: 10.1371/journal.pbio.1001448

Jafarzadeh A, Bagherzadeh S, Ebrahimi HA, Hajghani H, Bazrafshani MR, Khosravimashizi A, Nemati M, Gadari F, Sabahi A, Iranmanesh F et al. (2014) Higher Circulating Levels of Chemokine CCL20 in Patients with Multiple Sclerosis: Evaluation of the Influences of Chemokine Gene Polymorphism, Gender, Treatment and Disease Pattern. J Mol Neurosci 53:500–505. doi: 10.1007/s12031-013-0214-2

Jarrin I, Pantazis N, Dalmau J, Phillips AN, Olson A, Mussini C, Boufassa F, Costagliola D, Porter K, Blanco J et al. (2015) Does rapid HIV disease progression prior to combination antiretroviral therapy hinder optimal CD4+ T-cell recovery once HIV-1

suppression is achieved? AIDS 29:2323–33. doi: 10.1097/QAD.0000000000000805

Jinno A, Shimizu N, Soda Y, Haraguchi Y, Kitamura T and Hoshino H (1998) Identification of the chemokine receptor TER1/CCR8 expressed in brain-derived cells and T cells as a new coreceptor for HIV-1 infection. Biochem Biophys Res Commun 243:497–502. doi: 10.1006/bbrc.1998.8130

Jinquan T, Jing C, Jacobi HH, Reimert CM, Millner A, Quan S, Hansen JB, Dissing S, Malling HJ, Skov PS et al. (2000) CXCR3 expression and activation of eosinophils: role of IFN-gamma-inducible protein-10 and monokine induced by IFN-gamma. J Immunol 165:1548–56. doi: ji_v165n3p1548 [pii]

Kahn JO and Walker BD (1998) Acute Human Immunodeficiency Virus Type 1 Infection. N Engl J Med 339:33–39. doi: 10.1056/NEJM199807023390107

Keet IP, Krijnen P, Koot M, Lange JM, Miedema F, Goudsmit J and Coutinho RA (1993) Predictors of rapid progression to AIDS in HIV-1 seroconverters. Aids 7:51–57.

Khalife J, Guy B, Capron M, Kieny MP, Ameisen JC, Montagnier L, Lecocq JP and Capron A (1988) Isotypic restriction of the antibody response to human immunodeficiency virus. AIDS Res Hum Retroviruses 4:3–9. doi: 10.1089/aid.1988.4.3

Kim CJ, McKinnon LR, Kovacs C, Kandel G, Huibner S, Chege D, Shahabi K, Benko E, Loutfy M, Ostrowski M et al. (2013) Mucosal Th17 cell function is altered during HIV infection and is an independent predictor of systemic immune activation. J Immunol 191:2164–73. doi: 10.4049/jimmunol.1300829

Kim S-H, Jeong H-H, Cho B-Y, Kim M, Lee H-Y, Lee J, Wee K and Park H-S (2008) Association of Four-locus Gene Interaction with Aspirin-intolerant Asthma in Korean Asthmatics. J Clin Immunol 28:336–342. doi: 10.1007/s10875-008-9190-7

Langford SE, Ananworanich J and Cooper DA (2007) Predictors of disease progression in HIV infection: a review. AIDS Res Ther 4:11. doi: 10.1186/1742-6405-4-11

Lee AYS, Phan TK, Hulett MD and Körner H (2015) The relationship between CCR6 and its binding partners: does the CCR6-CCL20 axis have to be extended? Cytokine 72:97–101. doi: 10.1016/j.cyto.2014.11.029

Lepej SZ, Begovac J and Vince A (2006) Changes in T-cell subpopulations during four years of suppression of HIV-1 replication in patients with advanced disease. FEMS Immunol Med Microbiol 46:351–9. doi: 10.1111/j.1574-695X.2005.00034.x

Leung V, Gillis J, Raboud J, Cooper C, Hogg RS, Loutfy MR, Machouf N, Montaner JSG, Rourke SB, Tsoukas C et al. (2013) Predictors of CD4:CD8 ratio normalization and its effect on health outcomes in the era of combination antiretroviral therapy. PLoS One 8:e77665. doi: 10.1371/journal.pone.0077665

Levy JA (1993) Pathogenesis of human immunodeficiency virus infection. Microbiol Rev

57:183–289.

Levy JA (2010) HIV e a Patogenia da Aids.

Li D, Chen J, Jia M, Hong K, Ruan Y, Liang H, Liu S, Zhang X, Zhao H, Peng H et al. (2011) Loss of balance between T helper type 17 and regulatory T cells in chronic human immunodeficiency virus infection. Clin Exp Immunol 165:363–71. doi: 10.1111/j.1365-2249.2011.04435.x

Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, Southern PJ, Reilly CS, Peterson ML, Schultz-Darken N, Brunner KG et al. (2009) Glycerol monolaurate prevents mucosal SIV transmission. Nature 458:1034–8. doi: 10.1038/nature07831

Liovat A-S, Rey-Cuillé M-A, Lécuroux C, Jacquelin B, Girault I, Petitjean G, Zitoun Y, Venet A, Barré-Sinoussi F, Lebon P et al. (2012) Acute Plasma Biomarkers of T Cell Activation Set-Point Levels and of Disease Progression in HIV-1 Infection. PLoS One 7:e46143. doi: 10.1371/journal.pone.0046143

Liu B, Jia Y, Ma J, Wu S, Jiang H, Cao Y, Sun X, Yin X, Yan S, Shang M et al. (2016) Tumor-associated macrophage-derived CCL20 enhances the growth and metastasis of pancreatic cancer. Acta Biochim Biophys Sin (Shanghai) 48:1067–1074. doi: 10.1093/abbs/gmw101

Loetscher P, Pellegrino A, Gong JH, Mattioli I, Loetscher M, Bardi G, Baggiolini M and Clark-Lewis I (2001) The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. J Biol Chem 276:2986–91. doi: 10.1074/jbc.M005652200

Mackay CR (2001) Chemokines: immunology's high impact factors. Nat Immunol 2:95–101. doi: 10.1038/84298

Maggi E, Mazzetti M, Ravina A, Annunziato F, de Carli M, Piccinni MP, Manetti R, Carbonari M, Pesce AM and del Prete G (1994) Ability of HIV to promote a TH1 to TH0 shift and to replicate preferentially in TH2 and TH0 cells. Science 265:244–8.

Mahnke YD, Fletez-Brant K, Sereti I and Roederer M (2016) Reconstitution of Peripheral T Cells by Tissue-Derived CCR4+ Central Memory Cells Following HIV-1 Antiretroviral Therapy. Pathog Immun 1:260. doi: 10.20411/pai.v1i2.129

Marchetti G, Cozzi-Lepri A, Merlini E, Bellistrì GM, Castagna A, Galli M, Verucchi G, Antinori A, Costantini A, Giacometti A et al. (2011) Microbial translocation predicts disease progression of HIV-infected antiretroviral-naive patients with high CD4+ cell count. AIDS 25:1385–94. doi: 10.1097/QAD.0b013e3283471d10

Marins JRP, Jamal LF, Chen SY, Barros MB, Hudes ES, Barbosa AA, Chequer P, Teixeira PR and Hearst N (2003) Dramatic improvement in survival among adult Brazilian AIDS patients. AIDS 17:1675–82. doi: 10.1097/01.aids.0000072649.21517.80

Matte M (2012) Influência dos genes HLA classe I na progressão para a Aids em indivíduos HIV positivos.

McDermott DH, Beecroft MJ, Kleeberger CA, Al-Sharif FM, Ollier WE, Zimmerman PA, Boatin BA, Leitman SF, Detels R, Hajeer AH et al. (2000) Chemokine RANTES promoter polymorphism affects risk of both HIV infection and disease progression in the Multicenter AIDS Cohort Study. AIDS 14:2671–8.

McKinnon LR, Nyanga B, Kim CJ, Izulla P, Kwatampora J, Kimani M, Shahabi K, Mugo N, Smith JS, Anzala AO et al. (2015) Early HIV-1 Infection Is Associated With Reduced Frequencies of Cervical Th17 Cells. JAIDS J Acquir Immune Defic Syndr 68:6–12. doi: 10.1097/QAI.0000000000000389

McLaren PJ, Ball TB, Wachihi C, Jaoko W, Kelvin DJ, Danesh A, Kimani J, Plummer FA and Fowke KR (2010) HIV-exposed seronegative commercial sex workers show a quiescent phenotype in the CD4+ T cell compartment and reduced expression of HIV-dependent host factors. J Infect Dis 202 Suppl:S339-44. doi: 10.1086/655968

McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N and Haynes BF (2010) The immune response during acute HIV-1 infection: clues for vaccine development. Nat Rev Immunol 10:11–23. doi: 10.1038/nri2674

Medeiros R (2012) Avaliação De Polimorfismos Em Genes Envolvidos Na Resposta Imune Inata De Pacientes Infectados Com Hiv-1 E Sua Influência Na Progressão À Aids.

Medeiros RM De (2016) Citocinas e a modulação da resposta imune durante a infecção pelo HIV – suscetibilidade à infecção e progressão para a aids. Universidade Federal do Rio Grande do Sul

Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, Hallahan CW, Selig SM, Schwartz D, Sullivan J et al. (2000) HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc Natl Acad Sci U S A 97:2709–14. doi: 10.1073/pnas.050567397

Miguez-Burbano MJ, Shor-Posner G, Fletcher MA, Lu Y, Moreno JN, Carcamo C, Page B, Quesada J, Sauberlich H and Baum MK (1995) Immunoglobulin E levels in relationship to HIV-1 disease, route of infection, and vitamin E status. Allergy 50:157–61. doi: 10.1111/j.1398-9995.1995.tb05073.x

Ministério da Saúde. (2016) Boletim Epidemiológico - Aids e DST.

Ministério da Saúde. (1997) Consenso Sobre Terapia Anti-Retroviral Para Adultos e Adolescentes Infectados Pelo HIV 1997.

Modi WS, Goedert JJ, Strathdee S, Buchbinder S, Detels R, Donfield S, O'Brien SJ and Winkler C (2003) MCP-1-MCP-3-Eotaxin gene cluster influences HIV-1 transmission. AIDS 17:2357–65. doi: 10.1097/01.aids.0000076359.20434.44

Modi WS, Lautenberger J, An P, Scott K, Goedert JJ, Kirk GD, Buchbinder S, Phair J, Donfield S, Brien SJO et al. (2006) Genetic Variation in the CCL18-CCL3-CCL4 Chemokine Gene Cluster Influences HIV Type 1 Transmission and AIDS Disease Progression. 79:120–128.

Moore JH, Gilbert JC, Tsai C-T, Chiang F-T, Holden T, Barney N and White BC (2006) A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. J Theor Biol 241:252–261. doi: 10.1016/j.jtbi.2005.11.036

Nedellec R, Coetzer M, Shimizu N, Hoshino H, Polonis VR, Morris L, Martensson UEA, Binley J, Overbaugh J and Mosier DE (2009) Virus Entry via the Alternative Coreceptors CCR3 and FPRL1 Differs by Human Immunodeficiency Virus Type 1 Subtype. J Virol 83:8353–8363. doi: 10.1128/JVI.00780-09

Noack M and Miossec P (2014) Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev 13:668–77. doi: 10.1016/j.autrev.2013.12.004

Okulicz JF, Marconi VC, Landrum ML, Wegner S, Weintrob A, Ganesan A, Hale B, Crum-Cianflone N, Delmar J, Barthel V et al. (2009) Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US Department of Defense HIV natural history study. J Infect Dis 200:1714–23. doi: 10.1086/646609

Passam AM, Sourvinos G, Krambovitis E, Miyakis S, Stavrianeas N, Zagoreos I, Spandidos DA, K TC, In C, Cr-vi CX et al. (2007) Polymorphisms of Cx 3 CR1 and CXCR6 Receptors in Relation to HAART Therapy of HIV Type 1 Patients. 23:1026–1032. doi: 10.1089/aid.2006.0248

Pedersen C, Lindhardt BO, Jensen BL, Lauritzen E, Gerstoft J, Dickmeiss E, Gaub J, Scheibel E and Karlsmark T (1989) Clinical course of primary HIV infection: consequences for subsequent course of infection. BMJ 299:154–7. doi: doi: 10.1136/bmj.299.6692.154

Penna G, Vulcano M, Sozzani S and Adorini L (2002) Differential migration behavior and chemokine production by myeloid and plasmacytoid dendritic cells. Hum Immunol 63:1164–71.

Poropatich K and Sullivan DJ (2011) Human immunodeficiency virus type 1 long-term non-progressors: the viral, genetic and immunological basis for disease non-progression. J Gen Virol 92:247–68. doi: 10.1099/vir.0.027102-0

Proudfoot AEI (2002) Chemokine receptors: multifaceted therapeutic targets. Nat Rev Immunol 2:106–15. doi: 10.1038/nri722

Rafaels NM, Beck LA, Gao PS, Hand T, Boguniewicz M, R. T, Hata, Schneider L, Hanifin JM, Gallo RL et al. (2009) Variations in the CCL20 and CCR6 Genes are Associated with Atopic Dermatitis and Eczema Herpeticum in Populations of European and African descent.

Romagnani S, Del Prete G, Manetti R, Ravina A, Annunziato F, De Carli M, Mazzetti M, Piccinni MP, D'Elios MM and Parronchi P (1994) Role of TH1/TH2 cytokines in HIV infection. Immunol Rev 140:73–92.

Sainz T, Serrano-Villar S, Díaz L, González Tomé MI, Gurbindo MD, de José MI, Mellado MJ, Ramos JT, Zamora J, Moreno S et al. (2013) The CD4/CD8 ratio as a marker T-cell activation, senescence and activation/exhaustion in treated HIV-infected children and young adults. AIDS 27:1513–6. doi: 10.1097/QAD.0b013e32835faa72

Sallusto F, Lanzavecchia A and MacKay CR (1998a) Chemokines and chemokine receptors in T-cell priming and Th1/Th2- mediated responses. Immunol Today 19:568–574. doi: 10.1016/S0167-5699(98)01346-2

Sallusto F, Lenig D, Mackay CR and Lanzavecchia A (1998b) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 187:875–83. doi: 10.1084/jem.187.6.875

Sallusto F, Mackay CR and Lanzavecchia A (1997) Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science 277:2005–7. doi: 10.1126/science.277.5334.2005

Sallusto F, Mackay CR and Lanzavecchia A (2000) The Role of Chemokine Receptors in Primary, Effector, and Memory Immune Responses. Annu Rev Immunol 18:593–620. doi: 10.1146/annurev.immunol.18.1.593

Sankaran S, Guadalupe M, Reay E, George MD, Flamm J, Prindiville T and Dandekar S (2005) Gut mucosal T cell responses and gene expression correlate with protection against disease in long-term HIV-1-infected nonprogressors. Proc Natl Acad Sci U S A 102:9860–5. doi: 10.1073/pnas.0503463102

Saulle I, Biasin M, Gnudi F, Rainone V, Ibba SV, Lo Caputo S, Mazzotta F, Trabattoni D and Clerici M (2016) Short Communication: Immune Activation Is Present in HIV-1-Exposed Seronegative Individuals and Is Independent of Microbial Translocation. AIDS Res Hum Retroviruses 32:129–33. doi: 10.1089/AID.2015.0019

Schechter MT, Craib KJ, Le TN, Montaner JS, Douglas B, Sestak P, Willoughby B and O'Shaughnessy M V (1990) Susceptibility to AIDS progression appears early in HIV infection. AIDS 4:185–90.

Schutyser E, Struyf S and Van Damme J (2003) The CC chemokine CCL20 and its receptor CCR6. Cytokine Growth Factor Rev 14:409–26. doi: 10.1016/S1359-6101(03)00049-2

Secord EA, Kleiner GI, Auci DL, Smith-Norowitz T, Chice S, Finkielstein A, Nowakowski M, Fikrig S and Durkin HG (1996) IgE against HIV proteins in clinically healthy children with HIV disease. J Allergy Clin Immunol 98:979–84.

Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, Shacklett BL, Ferre AL, Hayes

TL, Somsouk M, Hsue PY et al. (2014) HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. PLoS Pathog 10:e1004078. doi: 10.1371/journal.ppat.1004078

Shan L and Siliciano RF (2014) Unraveling the relationship between microbial translocation and systemic immune activation in HIV infection. J Clin Invest 124:2368–2371. doi: 10.1172/JCI75799

Shimizu N, Tanaka A, Jinno-Oue A, Mori T, Ohtsuki T and Hoshino H (2010) Short Communication: Identification of the Conformational Requirement for the Specificities of Coreceptors for Human and Simian Immunodeficiency Viruses. AIDS Res Hum Retroviruses 26:321–328. doi: 10.1089/aid.2009.0161

Shimizu N, Tanaka A, Oue A, Mori T, Ohtsuki T, Apichartpiyakul C, Uchiumi H, Nojima Y and Hoshino H (2009) Broad usage spectrum of G protein-coupled receptors as coreceptors by primary isolates of HIV. AIDS 27:761–769. doi: 10.1097/QAD.0b013e328326cc0d

Signoret N, Rosenkilde MM, Klasse PJ, Schwartz TW, Malim MH, Hoxie JA and Marsh M (1998) Differential regulation of CXCR4 and CCR5 endocytosis. J Cell Sci 111 (Pt 1:2819–30. doi: 9718374

Signorini DJHP, Codeço CT, Carvalho MS, Campos DP, Monteiro MCM, Andrade M de FC de, Pinto JF da C and Sá CAM de (2005) Effect of sociodemographic, clinical-prophylactic and therapeutic procedures on survival of AIDS patients assisted in a Brazilian outpatient clinic. Rev Bras Epidemiol 8:503–10. doi: 10.1590/S1415-790X2005000300007

Smith M.W.; Carrington, M.; Winkler, C.; Lomb, D.; Dean, M.; Huttley, G. & O'Brien SJ (1997) CCR2 chemokine receptor and AIDS progression. Nat Med 3:1052–1053.

Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, Lebedeva M, DeCamp A, Li D, Grove D et al. (2009) Induction of a Striking Systemic Cytokine Cascade prior to Peak Viremia in Acute Human Immunodeficiency Virus Type 1 Infection, in Contrast to More Modest and Delayed Responses in Acute Hepatitis B and C Virus Infections. J Virol 83:3719–3733. doi: 10.1128/JVI.01844-08

Suy A, Castro P, Nomdedeu M, García F, López A, Fumero E, Gallart T, Lopalco L, Coll O, Gatell JM et al. (2007) Immunological Profile of Heterosexual Highly HIV-Exposed Uninfected Individuals: Predominant Role of CD4 and CD8 T-Cell Activation. J Infect Dis 196:1191–1201. doi: 10.1086/521193

Valverde-Villegas JM, Matte MCC, Medeiros RM De and Chies JAB (2015) New Insights about Treg and Th17 Cells in HIV Infection and Disease Progression. J Immunol Res 2015:1–14. doi: 10.1155/2015/647916

Vila-Coro AJ, Mellado M, Martín de Ana A, Lucas P, del Real G, Martínez-A C and

Rodríguez-Frade JM (2000) HIV-1 infection through the CCR5 receptor is blocked by receptor dimerization. Proc Natl Acad Sci U S A 97:3388–93. doi: 10.1073/pnas.050457797

Wada NI, Jacobson LP, Margolick JB, Breen EC, Macatangay B, Penugonda S, Martínez-Maza O and Bream JH (2015) The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS 29:463–71. doi: 10.1097/QAD.0000000000000545

Wang C, Kang SG, Lee J, Sun Z and Kim CH (2009) The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. Mucosal Immunol 2:173–83. doi: 10.1038/mi.2008.84

Wu L and KewalRamani VN (2006) Dendritic-cell interactions with HIV: infection and viral dissemination. Nat Rev Immunol 6:859–68. doi: 10.1038/nri1960

Yamazaki T, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B, Martin-Orozco N, Kang HS, Ma L, Panopoulos AD et al. (2008) CCR6 Regulates the Migration of Inflammatory and Regulatory T Cells. J Immunol 181:8391–8401. doi: 10.4049/jimmunol.181.12.8391

Yokobayashi H, Sugaya M, Miyagaki T, Kai H, Suga H, Yamada D, Minatani Y, Watanabe K, Kikuchi Y, Tamaki T et al. (2013) Analysis of serum chemokine levels in patients with HIV-associated eosinophilic folliculitis. J Eur Acad Dermatology Venereol 27:e212–e216. doi: 10.1111/j.1468-3083.2012.04592.x

Zhao K, Ishida Y, Oleksyk TK, Winkler C a and Roca AL (2012) Evidence for selection at HIV host susceptibility genes in a West Central African human population. BMC Evol Biol 12:237. doi: 10.1186/1471-2148-12-237

Zhao L, Xia J, Wang X and Xu F (2014) Transcriptional regulation of CCL20 expression. Microbes Infect 16:864–70. doi: 10.1016/j.micinf.2014.08.005

Zinkernagel RM (2000) On immunological memory. Philos Trans R Soc Lond B Biol Sci 355:369–71. doi: 10.1098/rstb.2000.0576

ANEXO A:

Artigo de revisão publicado:

"New Insights about Treg and Th17 Cells in HIV Infection and Disease Progression"

JacquelineMaría Valverde-Villegas, Maria Cristina Cotta Matte,
Rúbia Marília de Medeiros, and José Artur Bogo Chies

Artigo publicado na revista *Journal of Immunology Research* em Julho de 2015

Hindawi Publishing Corporation
Journal of Immunology Research
Article ID 647916

Review Article

New Insights about Treg and Th17 Cells in HIV Infection and Disease Progression

Jacqueline María Valverde-Villegas, Maria Cristina Cotta Matte, Rúbia Marília de Medeiros, and José Artur Bogo Chies

Immunogenetics Laboratory, Genetics Department, Genetics and Molecular Biology Post-Graduate Program, Institute of Biosciences, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves 9500, Prédio 43323, 91501-970 Porto Alegre, RS, Brazil

Correspondence should be addressed to José Artur Bogo Chies; jabchies@terra.com.br

Received 15 May 2015; Accepted 26 July 2015

Academic Editor: Andrew D. Badley

Treg and Th17 cell subsets are characterized by the expression of specific transcriptional factors and chemokine receptor as well as by secretion of specific cytokine and chemokines. These subsets are important to the differentiation, expansion, homing capacity, and recruitment of several different immune cell populations to the site of infection. Whereas Treg cells maintain self-tolerance and control the activation and expansion of autoreactive $CD4^+$ T effector cells through an anti-inflammatory response, Th17 cells, in an exacerbated unregulated proinflammatory response, can promote autoimmunity. Despite such apparently opposite functions, Th17 and Treg cells share common characteristics, and their differentiation pathways are interconnected. Recent studies have revealed quite intricate relations between Treg and Th17 cells in HIV infection and progression to AIDS. Considering Treg cells, different subsets were already investigated in the context of HIV infection, indicating a fluctuation in the total number and frequency throughout the disease course. This review focuses on the recent findings regarding the role of regulatory T and Th17 cells in the context of HIV infection, highlighting the importance of the balance between these two subsets on disease progression.

1. Introduction

One of the major hallmarks of HIV infection is the immune activation that prompt viral replication and $CD4^+$ T cells loss with disease progression, also leading to an impaired immune competence and consequently to AIDS development. It is still discussed if the loss of immune competence is caused by persistent immune activation, by a suppression of immune cells proliferation or by both phenomena [1].

The $CD4^+$ T cells exert a central role in immune response and represent the preferential target of HIV infection. The most extensive studied $CD4^+$ T cells lineages so far are Th1 and Th2, albeit HIV research now focuses on the immune balance and function of other cellular immune subsets, such as regulatory T cells (Tregs), T helper 17 (Th17), T helper 9 (Th9), and T helper 22 (Th22), where Treg/Th17 cells balance a relevant target of these studies [2, 3]. Treg cells, characterized by Forkhead Box Protein 3 ($FoxP3^+$) expression, represent an important subset that control the proliferation of different immune cell subsets [4]. Meanwhile, T helper 17 most remarkable characteristic is IL-17 production that drives the capacity to these cells to exert an important proinflammatory function against extracellular pathogens [5]. Also, it is known that both subset phenotypes (Treg and Th17) are characterized by specific transcriptional factors and chemokine receptor expressions as well as by secreting specific cytokines and chemokines. Together, all these factors are important to the differentiation, expansion, homing capacity, and immunological cell recruitment into the site of infection or to the injured tissue for restraining the inflammation and dissecting the fine balance between Th17/Treg cells [6, 7].

Natural history of HIV infection involves a variable time of progression to AIDS. HIV long-term nonprogressors (LTNP) are characterized by long periods (>10 years) of AIDS-free symptoms even without antiretroviral treatment and maintain low levels of viremia and elevated $CD4^+$ T cells counts. In contrast, rapid progressor (RP) HIV-1 subjects succumb to AIDS after a few years of infection [8].

Table 1: Immunophenotyping of Treg and Th17 cells and their precursors in different studies.

Subset cells	Markers used	References
Naive Tregs	$CD45RA^+CCR7^+CD25^{high}CD127^-Foxp3^+CD4^+$	DaFonseca et al. [99]
	$CD45RA^+CD45RO^-CCR7^+CD25^+Foxp3^+CD4^+$	Valmori et al. [107]
	$CD45RA^+CD25^+CD127^{low}Foxp3^+CD4^+$	Valmori et al. [108]
	$CD45RA^+CD45RO^-CD25^{high}CD127^{low}Foxp3^+CD4^+$	Duhen et al. [18]
	$CD45RO^-CD25^+CD127^{low}CD4^+$	Tenorio et al. [17]
Memory Tregs	$CD45RA^-CD25^{high}CD127^{low}Foxp3^+CD4^+$	Canavan et al. [133]
	$CD45RA^-CCR7^{+/-}CD25^{high}CD127^-Foxp3^+CD4^+$	DaFonseca et al. [99]
	$CD45RO^+CD25^{high}CD127^{low}Foxp3^+CD4^+$	Duhen et al. [18]
	$CD45RA^-CD25^{high}Foxp3^{high}CD4^+$	Zhou et al. [134]
	$CD45RO^+CD25^+CD127^{low}CD4^+$	Tenorio et al. [17]
Memory Th17	$CD45RA^-CCR6^+CCR4^+CXCR3^-CD4^+$	Gosselin et al. [122]; Becattini et al. [135]; Acosta-Rodriguez et al. [120]
	$CD45RA^-CCR6^+CD26^+CD161$	DaFonseca et al. [99]

Elite controllers (EC) are a particular group of LTNP, because they show persistent undetectable viremia (<50 RNA copies/mL) without treatment, although they represent less than 1% of all HIV-positive population [9]. Recent studies have focused the attention to elucidate the mechanisms involved in the variability of AIDS progression. Several components including viral factors and the host genetic diversity (e.g., the CCR5Δ32 variant and specific HLAs alleles) were already described as important factors that modulate HIV infection [10]. Nevertheless little is known about the cellular immune mechanisms involved in HIV progression and their role in immune molecular signaling, homing regulation, and cell-cell interactions. A better knowledge about these mechanisms could provide additional pieces to the complex puzzle of HIV pathogenesis. This review will focus on the recent findings regarding the role of regulatory T and Th17 cells in the context of HIV infection, highlighting the importance of the balance between these two subsets on disease progression.

2. The Role of Treg Cells on HIV Infection

2.1. Regulatory T Cells: Features and Functions. Regulatory T cells constitute a specialized subpopulation of $CD4^+$ T lymphocytes in the immune system that exerts pivotal roles on establishing and maintaining self-tolerance and immune homeostasis. These specific functions are derived from the regulation of different immune cells proliferation [11]. Based on this, it is expected that Treg cells may participate in the immune regulation in human autoimmune diseases, cancer, allograft rejections, and virus infection [12–15].

As a definition, Treg cells express high amounts of CD4, CD25 (IL-2Rα) and low CD127 (IL-7α) levels on the cell surface, although the Forkhead Box Protein 3 (FoxP3) is characterized as the gold standard marker for natural Treg cells (nTregs or tTregs, from thymic-derived regulatory T cells). IL-2Rα and FoxP3 expression (mediated by STAT5) are critical for Treg cells survival and suppressive function [14, 16]. The limitation to the use of FoxP3 as a marker for Treg is that viable cells cannot be isolated after intracellular staining. In addition, FoxP3 expression is not always indicative of a regulatory status within human $CD4^+$ T cells. A suggested alternative is the combined identification of the cell surface markers CD25 and CD127 ($CD25^{high}$, $CD127^{low/-}$) [17, 18]. In recent years, several studies have proposed a consensus panel of the markers to Treg immunophenotyping (Table 1). Another studied marker, CD39 (an ectonucleotidase involved in the hydrolysis of extracellular ATP into adenosine), identifies a bulk of human T cell regulatory population associated with high FoxP3 expression and inhibits T cell proliferation and cytokine secretion [19–21].

The suppressive capacity of Treg cells is widely dependent and influenced by several factors, such as IL-2, inhibitory cytokines (IL-10, TGF-β, or IL-35), CD152 (CTL-associated antigen 4, CTLA-4), and GITR (glucocorticoid-induced tumor necrosis factor receptor) [21]. IL-2 and, in a lesser degree, IL-7 and IL-15 cytokines are required for the correct differentiation of tTreg cells and the survival of tTreg cells and peripherally Treg cells (pTregs). Also, TGF-β seems to be an important cytokine involved on pTreg cells differentiation and homeostasis, although IL-2 is also required for TGF-β-mediated induction of FoxP3 [22]. Since several cytokines play a pivotal role on Treg cells function and differentiation, recent studies are investigating and suggesting their use on different conditions. The administration of IL-2 has been associated to increase in circulating Treg cells number and activation [23, 24]. IL-7 did not affect Treg cells proliferation but suppresses Treg cells capacity *in vitro* and *in vivo*. Also, IL-7 exerts a synergistic effect through downmodulation of the ectoenzyme CD39, favoring Th17 conversion [25]. In addition, expression of the enzyme indoleamine 2,3-dioxygenase (IDO), a tryptophan-degrading enzyme, represents another mechanism for immunosuppressive function [26].

2.2. The Role of Treg Cells in HIV Infection and Progression to AIDS: Friend or Foe? Persistent immune activation is considered a reliable predictor for HIV disease progression and may lead to erosion, depletion, and exhaustion of the $CD4^+$ T cell repertoire [27]. One of the immune mechanisms capable of controlling the activation and expansion of immune cells is the suppressive function exerted by Treg cells [28]. The role of Treg cells on HIV infection is still inconclusive since these cells can be involved both in the promotion as well

as in the prevention of disease progression. Some findings point to a beneficial effect through suppression of chronic immune activation and inhibition of activated $CD4^+$ T cells and consequent control of viral replication. On the other hand, a detrimental role is observed since the inhibition of specific HIV immune response through suppressive potential can promote viral persistence at the host [29, 30].

Considering that Treg cells express on their surface the chemokine receptor 4 (CXCR4) and chemokine receptor 5 (CCR5) molecules, these cells can potentially be susceptible to HIV R5-tropic and X4-tropic infection [31]. Some studies reported that HIV-infected Treg cells have its function and phenotype profile altered; however, opposite results have already been described [31–33]. Recently, Angin et al. [34] successfully isolated and *in vitro* expanded CD4 regulatory T cells from (HIV-positive) subjects. Expansion of functional Treg cells from blood and lymphoid tissues of HIV-infected subjects allied with its preserved suppressive capacity possibly indicates that these cells are not intrinsically defective in the context of HIV infection [34].

However, another study demonstrated that HIV-1 infection disrupts Treg cells function and its genes expression [35]. Treg cells infected with HIV-1 seem to be less potent in suppressing autologous $CD8^+$ and $CD4^+$ cell proliferation as compared to uninfected Treg cells. This impairment on Treg cells function can lead to HIV-associated generalized immune activation and inflammation [35]. According to this, infection of Treg cells with HIV X4-tropic strain results in a decrease of FoxP3 expression and decreased suppressive capacity [36]. Also, reduction in the expression of IL-2Rα in Treg cells was observed in HIV-infected subjects with high viral load. This alteration could result in reduced Treg cells capacity function in these individuals, considering that the homeostatic role of this cells depends on IL-2 and the expression of IL-2Rα at the cell surface [37].

Treg cells seems to be a major contributor to the immune activation observed during chronic HIV infection, since a strong relationship between Treg cells depletion and $CD4^+$ T cell activation was observed [38, 39]. It is important to carefully observe that, in chronic HIV infection, a gradual increase of Treg cells (in terms of percentage) and a decrease of its absolute numbers, during progression of the disease, have already been described [39–43]. The opposite results regarding Treg cells relative and absolute frequencies are related to the fact that these cells are preferentially preserved compared to conventional $CD4^+$ T cells [30]. Moreover, it is important to point that the discrepancy observed about Treg cells frequency on HIV infection can be attributed, at least in part, to (i) different surface markers used to characterize/isolate Treg cells; (ii) differential clinical stages of HIV disease, (iii) differences on sample analysed (blood or lymphoid tissues); and (iv) Treg subpopulations.

Human Treg cells have been subdivided according to their activation state: $CD45RA^+$ are defined as naïve Treg cells and $CD45RO^+$ defined as effector/memory Tregs cells in humans. Different cell subsets were already investigated in the context of HIV infection. When approaching the relative frequency (percentage), an increase of memory Treg cells and a decrease of naive Treg cells were observed as $CD4^+$ T cells decline. The level of HIV viremia inversely correlates with memory and naive Treg absolute cell numbers. In addition, immune activation was inversely correlated with lower memory and naive Treg absolute cells numbers [17]. A distinct Treg cells phenotype was already identified on HIV infection. These cells express HLA-G on their surface but do not express FoxP3 or CD25 and are distinct in their profile and function from the classical regulatory T cells. However, these Treg cells ($HLA\text{-}G^+$) seem to be diminished in progressive HIV-1 infection and may contribute to immune overactivation during disease progression [44].

It is noteworthy that Treg cells have an important role in immune homeostasis, and different evidences indicate that these cell repertoires can be disrupted in HIV infection. A better understanding of the Treg cells repertoire frequency and function in HIV-infected subjects with different patterns of progression to AIDS may help to elucidate the mechanisms affected by such cells on HIV pathogenesis and consequently their future therapeutic use.

There is an increasing number of studies approaching the role of Treg cells on different HIV progression groups, although the results are still conflicting. For instance, some of them suggest that low immune activation contributes to a slower disease progression [45]. Chase et al. [46] observed that Treg cells frequency and function were preserved among elite suppressor subjects (elite controllers), which may be a mechanism to limit immune activation. In the same line, Jiao et al. [47] observed a decrease of Treg cells absolute counts during HIV disease progression in the typical progressors group but not in LTNP subjects. One of the main reasons for the differences in Treg cells loss among distinct clinical progression groups would be that Treg cells migrate to lymphoid tissues in the typical progressors, but not in LTNP, which may contribute to Treg cells preservation on this last group and elite controller group [48]. According to this, lower levels of $FOXP3^+$, CTLA-4, and TGF-β, but not IL-10, were observed in the tonsils of HIV-infected subjects classified in the nonprogression group compared to HIV typical progressors [33], indicating that the accumulation of Treg cells within lymphoid tissues is a feature of chronic progression. More recently, it was shown that viremic slow progressors subject has lower Treg cells numbers associated with $CD4^+$ T cell decreased proliferation and surprisingly mucosal T cell activation. In this study, the low Treg cells numbers in the rectal mucosa may contribute to immune activation although they may also support stronger anti-HIV immune responses and a preserved Treg/Th17 cells balance [45].

Although some studies support the evidence of preserved Treg cells frequency and function in slow progressors subjects, there is no consensus in the literature since no differences among Treg cells frequency of slow progressors compared to HIV-infection acute disease and seronegative individuals have been described. Gaardbo et al. [49] showed no alteration on Treg cells numbers among LTNP, EC, viremic controllers, typical progressors, and HIV-seronegative individuals both in blood and in lymphoid tissues. However, activated Treg cells were elevated in LTNP and elite controllers compared to typical progressors and HIV-seronegative

controls, whereas resting Treg cells were diminished, suggesting an important role of different Treg cells subsets on HIV pathogenesis [49]. In this same direction, Brandt et al. [50] observed a lower frequency of Treg cells in EC compared to viremic individuals (HIV-seropositive HAART-naïve), and the frequency was correlated with T cell proliferating and activation.

2.3. Treg Cells in Animal Models: Investigating Treg Cells on SIV Infection. Similar to HIV infection, the exact mechanism of regulatory T cells function as well as its frequency during Simian Immunodeficiency Virus (SIV) infection is unclear. Li et al. [51] observed a higher absolute and relative number of Treg cells in Chinese *Rhesus macaques* in the early stages after SIV infection. No alteration on Treg cells suppressive capacity after infection was described. Estes et al. [52] observed an important regulatory response (mediated by $FoxP3^+$ and TGF-β^+ cells) after SIV exposure that may be involved in immune suppression of antiviral response and favor viral persistence. Although the majority of studies evaluate peripheral blood, Treg cells accumulation in lymphoid tissues was also described [53]. In addition to this, Tregs cells can potentially influence disease progression since lower FoxP3 mRNA levels were observed in an SIV nonprogressors model when compared to SIV progressors [33].

A study performed by Pereira et al. [54] investigated the frequency of Treg cells on two animal models with distinct profiles of SIV progression: African primate *Sooty mangabeys* (SM) (that do not develop immunodeficiency or disease) and Asian *Rhesus macaques* (RM) (a disease progression model). A decrease in Treg cells numbers was observed in chronically SIV-infected RM compared to uninfected animals. In longitudinal analysis, the SIVmac239-infected RM showed a transient increased Treg cells frequency in the acute phase of infection [54]. After the acute phase, a progressive decrease in the frequency and number of Treg cells was observed and correlated with high viral load. Antiretroviral treatment promoted an increase in the frequency and absolute count of Treg cells. None of these differences was observed on the SM model [54]. Another strategy used to investigate the role of Treg cells in HIV infection was to block Treg cells with an anti-CTLA-4 blocking antibody. CTLA-4 blockage in chronically SIV-infected ART-treated macaques was associated with lower IDO and TGF-β levels, as well as decreased viral RNA levels in lymph nodes and an increased immune specific response, suggesting a potentially therapeutic approach on HIV treatment [55].

2.4. The Impact of Highly Active Antiretroviral Therapy (HAART) on Tregs. Highly active antiretroviral therapy can significantly influence Treg cells numbers in HIV-infected subjects, decreasing or even normalizing its frequency at similar numbers to that of healthy controls [56, 57]. Some studies report that lower Treg cells numbers were found in blood and lymphoid tissues of treated compared to untreated subjects [58, 59].

Additionally, it has been hypothesized that Treg cells may contribute to the complete success of the treatment since subjects that do not respond to HAART seem to show higher Treg cells numbers as compared to responders [58, 60, 61]. Gaardbo et al. [62] also demonstrated that subjects with suboptimal immunological recovery had higher percentages of Treg cells and activated Treg cells, as well as lower resting Treg cells frequency in blood. In this same direction, higher levels of Treg cells in blood and lymphoid tissues predict a higher immunological reconstitution in individuals with low $CD4^+$ T cell counts [62]. In a study performed by Jiao et al. [47], HAART increased peripheral Treg cells counts and induced a decrease in the immune activation and $CD8^+$ T cell apoptosis in complete responders but not in nonresponders subjects. In conclusion, considering the important role of Treg cells in the balance between immune activation and/or suppression during HIV progression as well as its influence on HAART response, these cells may be useful as therapeutic targets or for prognostic monitoring in the future.

3. The Role of Th17 on HIV Infection

3.1. Th17 Cells: Features and Functions. Subpopulations of Th17 T helper lymphocytes were recently described and characterized by its involvement in mucosal immune inflammatory response, being its major function to protect the host against extracellular bacterial and fungal infections [5]. Th17 cells can be found under homeostatic conditions, particularly in the lamina propria of the small intestine [63]. However, during infection or under inflammatory conditions, Th17 cells can be induced in other tissues. This cellular lineage is responsible for the release of several cytokines that will act in nearby cells, inducing the production of chemokines able to recruit neutrophils and macrophages to the site of infection [64]. Further, Th17 cells can induce the expression of antimicrobial peptides, as lipocalin-2, Reg3γ, β-defensins, and calprotectin [65].

Th17 human cells are characterized by the expression of the transcription factor RORc and by the surface markers CD161, IL-23R, CCR6, and CCR4 [66, 67]. Moreover, the expression of CCR5 seems to be tissue-specific, with Th17 cells in the peripheral blood being predominantly CCR5-negative although they are CCR5-positive at the gastrointestinal tract [68]. The induction of RORc is dependent on STAT3, preferentially activated by IL-6, IL-21, and IL-23 in the presence of low amounts of TGF-β [69, 70]. Additionally, a balance between IL-6 and TGF-β concentrations has a pivotal role in driving Th17 immune responses, as will be better discussed later [71, 72].

Stimulated Th17 effector cells express several proinflammatory cytokines, such as IL-17, IL-21, IL-22, and IL-26, and chemokines as CXCL-6, CXCL-7, CXCL-8, and CCL20 [73], which contribute to the expansion of the inflammatory response through cells recruitment and activation and induction of antimicrobial peptides production. IL-17 leads to inflammation through NF-$\kappa\beta$ and MAPKs and the induction of genes that code for matrix metalloproteinases, growth factors, other proinflammatory cytokines, and chemokines that attract neutrophils [74].

A balance of proinflammatory and anti-inflammatory or suppressive cytokines in the cellular microenvironment seems to be determinant to the differentiation of the Th17 cells population in specific subsets: Th17 cells expressing both Th17-Th1 and Th17-Th2 surface markers were found in response to the presence of IL-6, IL17, and IL-1β and addition of IL-12 or IL-4, respectively [75]. Another subset, Th17-Treg cells, seems to involve a more complex signalling context [76].

3.2. The Role of Th17 Cells in HIV Infection and Progression to AIDS. Th17 cells are constitutively observed throughout the intestinal lamina propria and in gut-associated lymphoid tissues (GALT). Approximately 80–90% of the $CD4^+$ T cells present in GALT are able to secrete IL-17 [66]. Furthermore, it is recognized that GALT is the main region for HIV replication and massive $CD4^+$ T cells depletion in early infection is observed in this compartment [77]. Indeed, extreme permissiveness of Th17 cells to HIV-1 infection can be explained based on the fact that mucosal $CD4^+$ T cells present a $CD45RO^+$ memory phenotype and express CCR5 and/or CXCR4 [78]. Therefore, the loss of Th17 cells during the HIV infection affects the intestinal mucosal barrier as well as local innate and adaptive immune functions [78].

The presence of HIV-specific Th17 cells in HIV-infected individuals during early infection was already reported; however, this response was not detectable during chronic or nonprogressive stages of the infection disease [79]. Conversely, Brenchley et al. [80] demonstrated that, in HIV-infected and uninfected individuals, Th17 cells respond to bacterial and fungal antigens; nevertheless, Th17 cells response was not specific for viral antigens, including HIV. However many studies found that massive infection of $CD4^+$ T cells in GALT is directly associated with inflammation of the mucosal tissues and a breakdown of the mucosal integrity, resulting in microbial translocation from the lumen of the gut into peripheral blood [81, 82].

As has been suggested by some authors, Th17 cells may have dual impact on HIV infection due to the functional capacity in the mucosal tissue. In the acute phase of infection, in an inflammatory environment, Th17 cells could promote cell migration to the gut and create conditions for viral replication [83–85]. Nevertheless, in the chronic phase of infection, the reduced number of Th17 cells in the gut has been associated with a decrease in mucosal restoration and increase of microbial translocation and immune hyperactivation, which would contribute to exacerbation of the infection [80, 86].

Initial studies evaluating Th17 populations in HIV infected subjects demonstrated that Th17 cells were depleted in the gut-associated lymphoid tissue [39, 87]. In two subsequent studies, Salgado et al. [88] and Ciccone et al. [89] evaluated the numbers of Th17 cells in LTNP and typical progressor subjects. They reported similar results, suggesting that the number of Th17 cells in LTNP is greater than in typical progressor subjects. Furthermore, Salgado et al. [88] also observed a negative correlation between plasma HIV-RNA levels and Th17 cell number and with $CD4^+$ $IL7R^+$ cell number: HIV infected with higher of viral load showed the lowest numbers of Th17 cells and $IL7R^+CD4^+$ cells. These authors suggest that increased numbers of Th17 cells in LTNP subjects could better preserve the immune response against bacterial infections. Thus, low microbial translocation could explain the reduced activation and slower progression of the disease in LTNP subjects. Supporting these results, Singh et al. [90] showed that extensive elimination of $CD4^+$ T lymphocytes in the GALT in the early stages of HIV-1 infection affects the intestinal homeostasis and significantly decreases the effector and regulatory functions of Th17 cells.

3.3. Th17 Cells in Animal Models: Investigating Th17 Cells on SIV Infection. Since Th17 cells of the SIV host have the same phenotype and general functions of the human Th17 cells, these cellular lineages have been investigated in different animal models, as *Sooty mangabeys* (SM) (that do not develop immunodeficiency or disease) and Asian *Rhesus macaques* (RM) (a disease progression model). The Th17 cells studies in SM can be highlighted because, in spite of severe depletion of $CD4^+$ T cells in the mucosal tissues during acute SIV infection, even in the face of high viral replication similar to infections by HIV-human and SIV-RM, they do not progress to AIDS [91].

Raffatellu et al. [92] showed the inability of SIV-infected macaques to assemble an inflammatory GALT response against *S. typhimurium* due to an overall $CD4^+$ T cells depletion in this tissue. Also, a significant systemic spread *S. typhimurium* after the loss of Th17 cells was observed. Another important study, by Paiardini et al. [93], revealed that, after nonpathogenic SIV infection, SM are able to maintain or increase the levels of Th17 cytokines due to the recovery of $CD4^+$ T cells supported by the bone marrow and that this recovery contributes to the resistance against progression to AIDS. Other studies identified significant differences in the mucosal barrier integrity in models of HIV and SIV infection [91]. According to Brenchley et al. [94], Th17 cells are preferentially depleted in the mucosa of HIV^+ humans and SIV^+ *Rhesus macaque* pathogenic infections, but these cells were preserved in SM-SIV infections.

Recent studies have correlated the expression of CCR6 in Th17 cells and preservation of the gut mucosal barrier. This fact can be highlighted by the maintenance of Th17 cells in the gut and the reduced microbial translocation in SIV-infected RM treated with IL-21, a key cytokine in the activation of Th17 response [95]. Also, there are in the human Th17 repertoire, especially prevalent in the GALT, cells expressing high levels of CCR5, which would be a target of a preferential and rapid depletion [96].

SIV replication in the infected RM is restricted by the size of the preexisting Th17 cells compartment: animals with a high representation of such cells in blood and in the intestinal tissue previously to infection experienced peak and set-point viral loads about one log unit lower than those with a lower representation of Th17 cells [97]. Reciprocally, treatment of macaques with IL-2 and G-CSF before infection led to the depletion of Th17 cells, reduction of the ratio between Th17 and Treg cells, and higher viral loads for 6 months after infection [97]. These results suggest that the host immune

system pool previous to the infection has an influence on the disease course after infection and provides a new framework for understanding interindividual variation in response to HIV-infection.

3.4. The Impact of Highly Active Antiretroviral Therapy on Th17 Cells. In the HIV infection, Th17 cells seem to be preferentially depleted in the intestinal mucosa and to a lesser extent in peripheral blood [77]. In the acute phase, the low levels of $CD4^+$ T cells can be restored with the viral load reduction mediated by HAART. Macal et al. [98] showed that the highest level of $CD4^+$ T cells restoration during HAART correlates with a substantial increase in mucosal Th17 cells and a decrease in inflammation markers. However, it is unclear why HAART cannot restore Th17 cells in the intestinal mucosa of some individuals: this same study observed that in some HIV-infected subjects a low level of immune activation persists in GALT despite long-term therapy. A possible explanation is that as Th17 cells are highly susceptible to HIV infection, this subset would be depleted early in HIV infection, leading to nonrestoration of the Th17 cells in spite of HAART. On the other hand, there are evidences showing that the paucity of the Th17-lineage committed precursor cells coincides with the Th17 polarization deficit in HIV chronically infected on HAART individuals versus HIV-negative controls [99]. Therefore, it can be suggested that the initial exhaustion of the precursor Th17 cell subsets in early stages, in some HIV-infected individuals, could be correlated with the Th17 restoration deficit despite an undetectable viral load. These studies are discussed in more detail further in this review (see What about the Balance between Th17 and Treg Cells in HIV Infection?).

Ndhlovu et al. [100] reported that healthy children exhibit a higher frequency of Th17 cells in the peripheral blood than HIV-infected children. Also, infected children with viral load greater than 50 copies/mL had a greater decrease in the frequency of these cells compared to children with undetectable viral load, suggesting that a preservation of Th17 cells depends on viral suppression [100]. Recently Pilakka-Kanthikeel et al. [101] comparing virologic responders and virologic failures HIV-infected children to uninfected pediatric subjects showed that microbial translocation persisted after 44 weeks in both responders and failures HIV-infected groups. A study by Alvarez et al. [102] performed *in vitro* demonstrated that virus replication can be suppressed by 3TC therapy, but the restoration of Th17 response observed in non-infected controllers was only achieved with the combination of 3TC and a "cocktail" of Th17 cytokines (IL-6, IL-1β, TGF-β, and IL-23). Taking into consideration that it was possible to restore Th17 response, it will be interesting to conduct more studies with such potential therapy.

4. Th17 and Treg Balance

The Th17/Treg balance is defined as "a state of equilibrium of the immune system that permits accurate and rapid protective responses against pathogens but curtails potential for causing harm to the host through targeting of 'self' and provoking overexuberant inflammatory processes" [6]. It is known that Th17 and Treg cells have opposite roles in the development and outcomes of autoimmune/inflammatory diseases. Whereas Th17 cells can promote autoimmunity due to a proinflammatory response, Treg cells maintain self-tolerance and controls activation and expansion of autoreactive $CD4^+$ T effector cells through an anti-inflammatory response [7]. However, Th17 and Treg cells share common characteristics, and their differentiation pathways are interconnected.

Recent reports demonstrated that Treg and Th17 cells have a high grade of plasticity due the fact that their initial differentiation is not an endpoint of T cell development [75, 76]. This plasticity allows a functional adaptation to various physiological situations during an immune response and might also be a critical disturbing factor for the Th17/Treg balance, leading to the immunopathogenesis of autoimmune/inflammatory diseases [75].

The maintenance of a Th17/Treg balance mainly depends on environmental factors and genetic predisposition. Besides, the plasticity of both cell subsets is highly dependent on the cytokine milieu and in the inflammatory context. Importantly, the commensal microbiota composition has a particularly significant influence in the immune system regulation and an imbalance in the gut microbiome could lead to alterations of immune responses in both GALT and periphery [6]. Of note, there are mechanisms of peripheral tolerance, achieved in large part through the action of Treg cells.

TGF-β is a critical factor for both Th17 and Treg cells, essential for inducing both RORc and FoxP3 [72, 103]. $CD4^+FoxP3^+RORc^+$ cells represent a transient population, able to give rising to either Th17 or Treg cells depending on the local conditions. If sensing a proinflammatory environment, TGF-β induces RORc expression and Th17 cells differentiation [6, 104]. In the absence of an inflammation, TGF-β promotes FoxP3 expression and in combination with IL-2 promotes differentiation, expansion, and survival of Treg cells that maintain immune tolerance. This fact is due to a FoxP3-mediated inhibition of the activity of RORc and RORα, resulting in abrogation of IL-17 and IL-23 expression [105].

It was observed that Treg cells can acquire a Th17-like phenotype. They are able to release IL-17 and express RORc and high levels of CCR6 but can retain a suppressive capacity (although this capacity is rapidly lost upon strong activation in the presence of IL-1β and IL-6) and FoxP3 expression (Th17Treg profile) [75]. Other studies focused on naïve cells as precursor population of Tregs and Th17 cells and observed that both subsets have a common precursor. It was observed that natural Tregs differentiate from $CD25^+$ naïve T cells (NTregs) [106, 107]. Valmori et al. [108] reported that polarization of human Th17 cells preferentially occurs from $FoxP3^+$ naïve Treg cells in the presence of IL-2 and IL-1β and is increased by IL-23 and TGF-β. Recently, Mercer et al. [109] named these Th17-like phenotype Treg cells as $IL\text{-}17^+$ Tregs cells, due to the fact that this subset produces IL-17, and observed that naïve Treg cells (TNreg) expressing CCR6 have

a predetermined capacity to differentiate into IL-17$^+$ Treg cells with suppressive activity *in vitro*. They also observed that a small portion of naïve Treg cells expressing CCR6 have the propensity to polarize into Th17 cells. CCR6 is expressed by both Treg and Th17 cells and plays a significant role in Treg-mediated suppression and in the migration of Th17 cells to inflammatory sites [110].

Other important factors can influence the Th17/Treg balance. The fine-tuning of Treg cells upregulate chemokine and cytokine receptors in a pattern matching that of the immune T effector cells, whereas chemokine receptors such as CCR6 and CXCR3 facilitate the spatial proximity of suppressive Treg and inflammatory effector cells and cytokine receptors (e.g., IL-1R, IL-6R) that may compete for important factors, thus, limiting the activation or differentiation of T effector cells [6, 104].

Also, the stability of Treg cells has been questioned. It was observed that adoptive transfer of FoxP3$^+$Treg cells into lymphopenic hosts leads to loss of FoxP3 expression in these cells and their differentiation into follicular T helper cells (Tfh) in Peyer's patches [111]. In contrast, in another study, CD25$^+$CD4$^+$ T cells were stable and did not lose FoxP3 upon adoptive transfer into lymphopenic hosts, whereas a relatively minor fraction of CD25$^-$ or CD25^{low} FoxP3$^+$ cells can lose FoxP3 expression and divert into effector T cell lineages [112].

Studies observed a reduction in Treg cell numbers and/or a loss of Treg function in animal models and human autoimmune diseases. Nevertheless, it is important to highlight that an increased number of Treg cells in autoimmune disease do not necessarily mean that these cells are able to control the immune response. As aforementioned, Treg cells have a certain degree of plasticity and can lose their suppressive function, especially under inflammatory conditions. Furthermore, data on peripheral Treg cell numbers and function in human autoimmune/inflammatory diseases are contradictory and remain subject to debate.

4.1. What about the Balance between Th17 and Treg Cells in HIV Infection? Several studies were carried out to investigate the Th17/Treg balance state in typical progressors treated or untreated, EC, slow progressors, HIV-infected subjects, and SIV infection model [113]. Since Treg cells are developmentally linked to Th17 cells, the ratio of Th17 to Treg cells is used as an index of the relative balance between these two cell subsets. An impaired Th17/Treg balance in HIV-1 infection has a deleterious effect on gut mucosal immunity and fuels immune activation by enhancing microbial translocation [3, 80].

The importance of the Th17/Treg balance maintenance was highlighted by experiments performed in animal models using SIV infection. For instance, a loss of the Th17/Treg balance was observed in pathogenic SIV infection in Pig-tailed Macaques (PTs) when compared with nonpathogenic infection in African Green Monkeys (AGMs). SIV-infected PTs, but not SIV-infected AGMs, rapidly developed systemic immune activation and a selective depletion of Th17 cells, suggesting that loss of the Th17/Treg balance is related to SIV disease progression [113].

Li et al. [114] observed a continuous loss of Th17 cells which was accompanied by a concomitant rise in the frequency of Treg cells, resulting in a Th17/Treg cells imbalance during the HIV-1 progression disease in untreated chronic HIV-1 infected followed up for more than 1 year. This study included a small group of EC and remarkably, Th17/Treg cells ratios in those elite controllers remained comparable with ratios observed for HIV-seronegative controls. Complementary to these data, in an Indian HIV-infected cohort, Th17 cells from peripheral blood were significantly more depleted in late stage infected as compared to early stage infected and slow progressor subjects. In this same study, Treg cells frequencies in the subjects with slow progression HIV-1 infection were comparable to the HIV-seronegative controls [115]. Another study performed by Brandt et al. [50] also observed that Th17/Treg cells ratio was similar in EC and HIV-seronegative controls. Taken together, these results suggest that the maintenance of the equilibrium between Th17 and Treg cells would correlate with a "better prognosis" in terms of disease course. In addition, in this study, in untreated viremic and treated HIV-infected subjects, the Th17/Treg cells ratio was lower compared with HIV-seronegative controls. Interestingly, a study followed up HIV/AIDS subjects before and after HAART and observed that the Th17/Treg cells ratio was significantly decreased before treatment, while HAART partially normalized the Th17/Treg cells ratio [116], suggesting that the HAART treatment can restore the Th17/Treg cells balance.

It has been recently shown that IDO induced tryptophan (Trp) catabolism promotes T cell differentiation into Treg cells through FoxP3 overexpression and suppresses the expression of RORc and the generation of Th17 cells [26]. Also, enhanced IDO activity was associated with HIV disease progression, and such activity leads to a Th17/Treg imbalance in the peripheral blood [117]. This chronic activation by IDO pathway diminishes the host's capacity to generate Th17 cells affecting the mucosal immune barrier critically dependent upon Th17 cells [3]. However, a recent study observed that IDO induced Trp catabolism into kynurenine that induces a harmful effect on the Th17/Treg cells ratio that may subsequently contribute to enhanced microbial translocation during HIV-1 infection. Importantly, EC compared to ART successfully treated and healthy subjects displayed a distinctive Trp catabolism characterized by similar Kyn/Trp ratios and preserved IDO expression and Th17/Treg cells ratios [118]. Thereby, efforts to prevent an imbalance (or restore a balance) of Th17/Treg in HIV-infected individuals could be envisaged as a potential treatment alternative.

Studies are showing that precursor populations of Treg and Th17 cells are target of HIV infection, and this phenomenon perturbs the Treg and Th17 cell polarization and consequently the balancing of these subsets. Mercer et al. [109] observed that IL17$^+$Treg cells (derived from naïve Treg cells) are selectively reduced in number in HIV-infected subjects with suppressed viral loads through HAART. Then, DaFonseca et al. [99] showed that a Th17 polarization is impaired and this deficit coincided with the paucity of CD25^{high}CD127$^-$FoxP3$^+$ (naïve Tregs or nTregs) and CD25^{high}CD127$^+$FoxP3$^-$ (called double positive) subset

cells in chronically HIV-infected aviremic subjects under HAART. In this study, the nTreg cells from recently infected untreated viremic subjects harbored higher levels of integrated/unintegrated HIV-DNA when compared with the same cells from chronically HIV-infected aviremic subjects under HAART. Finally, both recent studies suggest the requirement for new therapeutic strategies designed to the preservation of IL17$^+$Tregs- and Th17-lineage committed naïve precursors.

5. Role of Chemokine Receptors on Th17 and Treg Cells: Implications for HIV Pathogenesis

5.1. Characterization of Th17 Cells by Chemokine Receptors and HIV Infection. Chemokine receptors have an important role in the phenotypic characterization of memory T cell subsets with distinct migration capacities and effector functions. The ligands for these receptors are inflammatory chemokines and chemoattractants, which are expressed in inflamed tissues and mediate the selective recruitment of different types of effector cells [119].

Memory CD4$^+$ T cells are highly heterogeneous in its potential homing and effector functions against a specific pathogen. Studies associate the commitment of lineage and antigenic specificity of CD4$^+$ T memory subsets with chemokine receptors expression [120–122]. It was observed that CCR4$^+$CCR6$^+$CD4$^+$ T cells subsets produce IL-17 and express the transcription factor ROR$\gamma\tau$ (Th17 profile) and were specific for *Candida albicans*, whilst CXCR3$^+$CCR6$^+$CD4$^+$ T cells subsets produce IL-17 and IFN-γ and express the transcription factors ROR$\gamma\tau$ and T-bet (Th1Th17 profile) and were specific for *Mycobacterium tuberculosis* [120].

There is emerging interest in the knowledge of the phenotype of HIV-infected CD4$^+$ T cells, with several studies demonstrating that HIV is very selective in choosing its cellular targets. It is well established that memory CD4$^+$ T cells are more permissive to HIV compared with naive T cells [77]. Thereby, it was observed that CCR4$^+$CCR6$^+$ CD4$^+$ T and CXCR3$^+$CCR6$^+$CD4$^+$ T cell subsets in peripheral blood were highly permissive to replication of both R5 and X4 HIV strains. Interestingly, these CD4$^+$ T cell subsets showed a persistent decline during chronic infection despite antiretroviral therapy [84, 122]. More recently, it was observed that *Tetanus toxoid* and *C. albicans* specific CD4$^+$ T cells with a Th17 profile (and high expression of CCR6 and its CCL20 ligand) were permissive to HIV infection, whereas CMV-specific CD4$^+$ T cells with a Th17 profile were highly resistant to both R5 and X4 HIV strains [121]. These results show a preferential infection of peripheral CCR6$^+$CD4$^+$ T cells by HIV and the importance of different CD4$^+$ T cell subsets against specific opportunistic pathogens that are depleted at different rates [123].

Studies observed that memory and effector Th17 cells are present in a subset of CCR6$^+$ cells in both peripheral blood and inflamed tissues and are preferential target to HIV-1 infection [124]. Even though Th17 cells express more than one trafficking receptor in a tissue-specific manner, CCR6 is the unique receptor that is uniformly expressed by all subsets of Th17 cells [125]. CCR6 is a gut homing chemokine receptor and has a critical role in cell migration into Peyer's patches of the distal small intestine where CCL20 (MIP-3α) is expressed [125]. During normal development and immune homeostasis, CCL20 selectively attract CCR6-expressing lymphocytes and DCs to the mucosal surfaces, organizing lymphoid tissues, such as Peyer's patches, mesenteric lymph nodes, and GALT [126]. Th17 cells subsets express CCR6 and produce CCL20. The production of CCL20 from Th17 cells is regulated similarly as IL-17 (induced by TGF-β along with IL-6) [126]. In contrast, an *in vitro* study observed that CCR6 expression on Th17 cells is coordinately regulated by TGF-β and IL-2. TGF-β, but not IL-6, was able to induce CCR6 on T cells; conversely, IL-2 effectively suppressed the expression of CCR6 on Th17 cells [72, 125]. Th17 cells, by producing CCL20, could also attract other Th17 cells via CCR6, meaning that the production of CCL20 can lead to further recruitment of other CCR6-expressing Th17 cells and sustained chronic inflammation [126].

The high susceptibility of Th17 cells to HIV *in vitro* is reflected by their *in vivo* depletion in the peripheral blood of HIV-infected individuals receiving treatment, compared with HIV-uninfected subjects [83]. It was suggested that CCR4$^+$CCR6$^+$ CD4$^+$ and CXCR3$^+$CCR6$^+$ CD4$^+$ cell subsets could have the potential to be recruited to the intestinal and vaginal mucosa through a CCR6$^-$CCL20 dependent mechanism significantly contributing to HIV dissemination and persistence in cells, also attracting other CCR6$^+$ CD4$^+$ T cells to viral replication sites, *in vivo* [84, 122, 124]. Also, the $\alpha4\beta7$ integrin identifies a subset of Th17 cells that is preferentially infected and depleted during acute SIV infection [125]. Accordingly, a study observed that the loss of peripheral $\alpha4^+\beta7^+$ memory CD4$^+$ T cells correlates with the loss of CD4$^+$ T cells in GALT during pathogenic SIV/HIV infection [127]. Taken together, these studies indicates that the ability of Th17 cells subsets to migrate into the GALT and other infection sites (e.g., periphery) depends on the imprinting for homing which is mediated by a combination of adhesion molecules and chemokine receptors (Figure 1).

5.2. Characterization of Tregs by Chemokine Receptors and HIV Infection. As aforementioned, homing and trafficking of effector cells are mainly facilitated by chemokines and expression of their chemokine receptors on distinct T cell subsets, and Treg cells are no exception. It was observed that CD45RA$^-$ FoxP3$^+$ T cells from peripheral blood express the CCR4, CCR5, CCR6, CXCR3, and CXCR6, chemokine receptors, which are commonly expressed by memory/effector T cells [128]. CCR4 and, even more, CCR8 have already been reported to be important for regulatory human CD25$^+$CD4$^+$ T cells [129]. Of note, mature dendritic cells preferentially attract Treg cells that express CCR4 and CCR8 through CCL17, CCL22, and CCL1 chemokine secretion (Figure 1). Thereby, it was suggested that CCR4 and/or CCR8 may guide Treg cells to inflamed areas and sites of antigen presentation in secondary lymphoid tissues in order to attenuate T cell

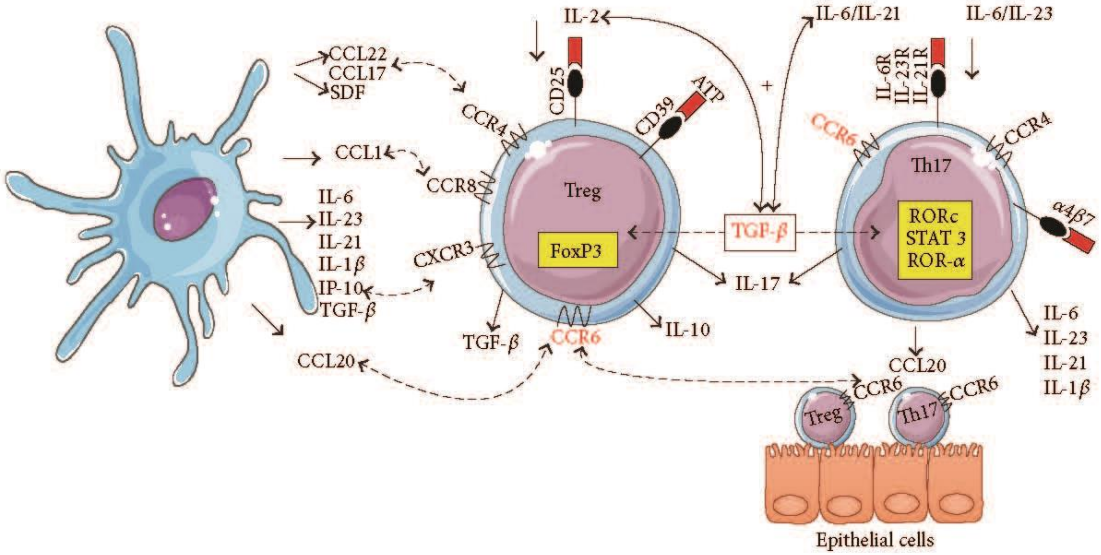


FIGURE 1: The interaction network between transcriptional factors, cytokines, chemokines, and their receptors in Th17 and Treg cells. The fine-tuning of Th17/Treg balance is regulated by expression of transcription factors that are activated by cytokines milieu and their receptors. TGF-β along with mainly IL-6 induces RORc, ROR-α, or STAT3 expression to differentiate Th17 cells while that in combination with IL-2 induces FoxP3 expression to differentiate Treg cells, while homing and immunological cells recruitment of both cell subsets are powerful mechanism mediated by chemokines and their chemokine receptors such as CCR6, CCR4, or CXCR3 which facilitates the recruitment of suppressive Treg and inflammatory effector Th17 cells (e.g., by means of CCR6-CCL20) into the site infection or injured tissue. Of note, other immunological cells, as dendritic cells, influence this balance because they produce cytokines, chemokines, and other molecules that participate in this interaction network.

activation or inhibit APC function [129]. This scenario suggests that chemokines secreted by APCs and chemokine receptors expressed on T cell subsets regulate the competition of T cells for access to antigen-bearing APCs.

Regarding CCR6, an important receptor expressed on Th17 cells, $CCR6^+$ Treg cells exhibit a phenotype of activation, memory, and expansion that are typical for an effector memory function [110]. Unlike Th17, Treg cells do not produce CCL20 [126]. However, it was observed that Treg cells migrate towards to CCL20-producing Th17 cells *in vitro* in a completely CCR6 dependent manner (migratory response was completely abolished in CCR6-deficient Th17 and Treg cells) [126]. In this study, it is proposed that Th17 cells produce CCL20 that attract other $CCR6^+$ Th17 cells as well as $CCR6^+$ Tregs through CCR6.

In the context of viral infections, Qin et al. [130] observed that a simultaneous antagonism of CCR4 by increased CXCR3 ligand expression (CXCL11) and loss of CCR4 ligand expression contributed to reducing homing of $FoxP3^+$ Treg cells to lymph node and intestinal tissues during SIV infection. In this study, the increment of IFN-γ as an upstream regulator of CXCR3 ligand expression and the decrease in TGF-β as an upstream regulator of IFN-γ expression revealed a complex set of interrelationship that control multiple positive and negative feedback system [130]. In the early stage of HIV infection, plasma IP-10 (CXCR3 ligand) levels were predictive of rapid progression than viremia or $CD4^+$ T cells levels [131]. Regarding CCL20 (CCR6 ligand), saliva was shown to increase significantly CCL20 secretion. Thus, it suggests that saliva could facilitate HIV entry and other pathogens through the genital mucosa during sexual intercourse [132].

Nevertheless, little is currently known about how chemokines and chemokine receptors regulate the homing and trafficking of Treg cells in HIV infection. Differential profiles of Treg homing receptors could be critical in the control of the inflammatory response against HIV. Also, interactions between chemokines and their receptors, such as CCL20/CCR6-mediated signals, can be strongly induced by proinflammatory stimuli. Future studies approaching how Treg cell subsets interact with each other and with the remaining cells by means of their chemokine receptors would certainly help in the understanding of the HIV infection pathogenesis.

6. Conclusions

HIV-1 infection is characterized by a gradual decrease of the immunological competence and a massive depletion of

$CD4^+$ T cells, particularly in GALT, which leads to microbial translocation, contributing to immune hyperactivation, an important pathogenic mechanism HIV-1 infection. Th17 cells are proinflammatory $CD4^+$ T cell subsets and play a pivotal role in host defense, mainly in the gastrointestinal tissue. Currently, most evidence suggests that Th17 cells have a beneficial role in HIV infection by promoting gut mucosa recovery, preventing microbial translocation and decreasing immune hyperactivation. However, a pathogenic role of these cells, particularly the induction of an increase in viral replication through the production of inflammatory cytokines, should not be ruled out. The role of Treg cells in regulating T cell activation during immune responses to pathogens such as HIV-1 is a subject of great interest. Their effects can be beneficial or detrimental depending on the balance between attenuating HIV-induced immune hyperactivation and mounting an immune response to HIV-1 and opportunistic pathogens.

The interaction between the cytokines milieu, chemokines, and chemokine receptors and the acquisition of tissue-specific homing form a complex network that is influenced mainly by the plasticity of T cells, genetic host, and environmental factors. Recent studies prompted that this network can disturb the Th17/Treg balance during HIV-1 infection. However, the mechanisms underlying this interaction are still not completely understood, and more studies need to be carried out in that direction. Finally, new findings about Th17/Treg outcomes and the understanding of interindividual variability in HIV infection will be crucial to the development of new treatment strategies and vaccines.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this review.

Authors' Contribution

Jacqueline María Valverde-Villegas, Maria Cristina Cotta Matte, and Rúbia Marília de Medeiros contributed equally to this paper. José Artur Bogo Chies reviewed the final paper.

References

[1] M. D. Hazenberg, S. A. Otto, B. H. B. van Benthem et al., "Persistent immune activation in HIV-1 infection is associated with progression to AIDS," *AIDS*, vol. 17, no. 13, pp. 1881–1888, 2003.

[2] J. Zhu, H. Yamane, and W. E. Paul, "Differentiation of effector CD4 T cell populations," *Annual Review of Immunology*, vol. 28, no. 1, pp. 445–489, 2010.

[3] B. Kanwar, D. Favre, and J. M. McCune, "Th17 and regulatory T cells: implications for AIDS pathogenesis," *Current Opinion in HIV and AIDS*, vol. 5, no. 2, pp. 151–157, 2010.

[4] A. Y. Rudensky, "Regulatory T cells and Foxp3," *Immunological Reviews*, vol. 241, no. 1, pp. 260–268, 2011.

[5] T. Korn, E. Bettelli, M. Oukka, and V. K. Kuchroo, "IL-17 and Th17 cells," *Annual Review of Immunology*, vol. 27, no. 1, pp. 485–517, 2009.

[6] D. R. Littman and A. Y. Rudensky, "Th17 and regulatory T cells in mediating and restraining inflammation," *Cell*, vol. 140, no. 6, pp. 845–858, 2010.

[7] M. Noack and P. Miossec, "Th17 and regulatory T cell balance in autoimmune and inflammatory diseases," *Autoimmunity Reviews*, vol. 13, no. 6, pp. 668–677, 2014.

[8] K. Poropatich and D. J. Sullivan Jr., "Human immunodeficiency virus type 1 long-term non-progressors: the viral, genetic and immunological basis for disease non-progression," *Journal of General Virology*, vol. 92, no. 2, pp. 247–268, 2011.

[9] K. A. O'Connell, J. R. Bailey, and J. N. Blankson, "Elucidating the elite: mechanisms of control in HIV-1 infection," *Trends in Pharmacological Sciences*, vol. 30, no. 12, pp. 631–637, 2009.

[10] P. An and C. A. Winkler, "Host genes associated with HIV/AIDS: advances in gene discovery," *Trends in Genetics*, vol. 26, no. 3, pp. 119–131, 2010.

[11] S. Sakaguchi, T. Yamaguchi, T. Nomura, and M. Ono, "Regulatory T Cells and Immune Tolerance," *Cell*, vol. 133, no. 5, pp. 775–787, 2008.

[12] F. A. H. Cooles, J. D. Isaacs, and A. E. Anderson, "Treg cells in rheumatoid arthritis: an update," *Current Rheumatology Reports*, vol. 15, no. 9, article 352, 2013.

[13] M. Beyer and J. L. Schultze, "Regulatory T cells in cancer," *Blood*, vol. 108, no. 3, pp. 804–811, 2006.

[14] Q. Tang and J. A. Bluestone, "Regulatory T-cell therapy in transplantation: moving to the clinic," *Cold Spring Harbor Perspectives in Medicine*, vol. 3, no. 11, 2013.

[15] T. Veiga-Parga, S. Sehrawat, and B. T. Rouse, "Role of regulatory T cells during virus infection," *Immunological Reviews*, vol. 255, no. 1, pp. 182–196, 2013.

[16] S. Sakaguchi, M. Miyara, C. M. Costantino, and D. A. Hafler, "$FOXP3^+$ regulatory T cells in the human immune system," *Nature Reviews Immunology*, vol. 10, no. 7, pp. 490–500, 2010.

[17] A. R. Tenorio, J. Martinson, D. Pollard, L. Baum, and A. Landay, "The relationship of T-regulatory cell subsets to disease stage, immune activation, and pathogen-specific immunity in HIV infection," *Journal of Acquired Immune Deficiency Syndromes*, vol. 48, no. 5, pp. 577–580, 2008.

[18] T. Duhen, R. Duhen, A. Lanzavecchia, F. Sallusto, and D. J. Campbell, "Functionally distinct subsets of human $FOXP3^+$ treg cells that phenotypically mirror effector Th cells," *Blood*, vol. 119, no. 19, pp. 4430–4440, 2012.

[19] S. Z. Josefowicz, L.-F. Lu, and A. Y. Rudensky, "Regulatory T cells: mechanisms of differentiation and function," *Annual Review of Immunology*, vol. 30, no. 1, pp. 531–564, 2012.

[20] S. Deaglio, K. M. Dwyer, W. Gao et al., "Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression," *The Journal of Experimental Medicine*, vol. 204, no. 6, pp. 1257–1265, 2007.

[21] N. Seddiki, L. Cook, D. C. Hsu et al., "Human antigen-specific $CD4^+CD25^+CD134^+CD39^+$ T cells are enriched for regulatory T cells and comprise a substantial proportion of recall responses," *European Journal of Immunology*, vol. 44, no. 6, pp. 1644–1661, 2014.

[22] J. D. Goldstein, L. Pérol, B. Zaragoza, A. Baeyens, G. Marodon, and E. Piaggio, "Role of cytokines in thymus- versus peripherally derived-regulatory T cell differentiation and function," *Frontiers in Immunology*, vol. 4, no. 155, Article ID Article 155, pp. 1–10, 2013.

[23] S. Brandenburg, T. Takahashi, M. de la Rosa et al., "IL-2 induces in vivo suppression by $CD4^+CD25^+Foxp3^+$ regulatory T cells,"

European Journal of Immunology, vol. 38, no. 6, pp. 1643–1653, 2008.

[24] H. Zhang, K. S. Chua, M. Guimond et al., "Lymphopenia and interleukin-2 therapy alter homeostasis of $CD4^+CD25^+$ regulatory T cells," *Nature Medicine*, vol. 11, no. 11, pp. 1238–1243, 2005.

[25] M. Younas, S. Hue, C. Lacabaratz et al., "IL-7 modulates in vitro and in vivo human memory t regulatory cell functions through the CD39/ATP axis," *The Journal of Immunology*, vol. 191, no. 6, pp. 3161–3168, 2013.

[26] B. Baban, P. R. Chandler, M. D. Sharma et al., "IDO activates regulatory T cells and blocks their conversion into Th17-like T cells," *The Journal of Immunology*, vol. 183, no. 4, pp. 2475–2483, 2009.

[27] A. E. Sousa, J. Carneiro, M. Meier-Schellersheim, Z. Grossman, and R. M. M. Victorino, "CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load," *The Journal of Immunology*, vol. 169, no. 6, pp. 3400–3406, 2002.

[28] M. F. Chevalier and L. Weiss, "The split personality of regulatory T cells in HIV infection," *Blood*, vol. 121, no. 1, pp. 29–37, 2013.

[29] B. F. de St Groth and A. L. Landay, "Regulatory T cells in HIV infection: pathogenic or protective participants in the immune response?" *AIDS*, vol. 22, no. 6, pp. 671–683, 2008.

[30] F. Simonetta and C. Bourgeois, "CD4+FOXP3+ regulatory T-cell subsets in human immunodeficiency virus infection," *Frontiers in Immunology*, vol. 4, article 215, 12 pages, 2013.

[31] M. E. Moreno-Fernandez, W. Zapata, J. T. Blackard, G. Franchini, and C. A. Chougnet, "Human regulatory T cells are targets for human immunodeficiency virus (HIV) infection, and their susceptibility differs depending on the HIV type 1 strain," *Journal of Virology*, vol. 83, no. 24, pp. 12925–12933, 2009.

[32] M. Angin, D. S. Kwon, H. Streeck et al., "Preserved function of regulatory T cells in chronic HIV-1 infection despite decreased numbers in blood and tissue," *Journal of Infectious Diseases*, vol. 205, no. 10, pp. 1495–1500, 2012.

[33] J. Nilsson, A. Boasso, P. A. Velilla et al., "HIV-1-driven regulatory T-cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS," *Blood*, vol. 108, no. 12, pp. 3808–3817, 2006.

[34] M. Angin, P. L. Klarenbeek, M. King et al., "Regulatory T cells expanded from HIV-1-infected individuals maintain phenotype, TCR repertoire and suppressive capacity," *PLoS ONE*, vol. 9, no. 2, Article ID e86920, 2014.

[35] M. Angin, S. Sharma, M. King et al., "HIV-1 infection impairs regulatory T-cell suppressive capacity on a per-cell basis," *Journal of Infectious Diseases*, vol. 210, no. 6, pp. 899–903, 2014.

[36] M. Pion, D. Jaramillo-Ruiz, A. Martínez, M. A. Muñoz-Fernández, and R. Correa-Rocha, "HIV infection of human regulatory T cells downregulates Foxp3 expression by increasing DNMT3b levels and DNA methylation in the FOXP3 gene," *AIDS*, vol. 27, no. 13, pp. 2019–2029, 2013.

[37] G. Méndez-Lagares, D. Jaramillo-Ruiz, M. Pion et al., "HIV infection deregulates the balance between regulatory T cells and IL-2-producing CD4 T cells by decreasing the expression of the IL-2 receptor in Treg," *Journal of Acquired Immune Deficiency Syndromes*, vol. 65, no. 3, pp. 278–282, 2014.

[38] M. P. Eggena, B. Barugahare, N. Jones et al., "Depletion of regulatory T cells in HIV infection is associated with immune activation," *The Journal of Immunology*, vol. 174, no. 7, pp. 4407–4414, 2005.

[39] A. Prendergast, J. G. Prado, Y.-H. Kang et al., "HIV-1 infection is characterized by profound depletion of CD161+ Th17 cells and gradual decline in regulatory T cells," *AIDS*, vol. 24, no. 4, pp. 491–502, 2010.

[40] S. Tsunemi, T. Iwasaki, T. Imado et al., "Relationship of CD4+CD25+ regulatory T cells to immune status in HIV-infected patients," *AIDS*, vol. 19, no. 9, pp. 879–886, 2005.

[41] C. A. R. Baker, R. Clark, F. Ventura et al., "Peripheral CD4 loss of regulatory T cells is associated with persistent viraemia in chronic HIV infection," *Clinical and Experimental Immunology*, vol. 147, no. 3, pp. 533–539, 2007.

[42] P. W. Hunt, A. L. Landay, E. Sinclair et al., "A low T regulatory cell response may contribute to both viral control and generalized immune activation in HIV controllers," *PLoS ONE*, vol. 6, no. 1, Article ID e15924, 2011.

[43] C. Mauricio Rueda Rios, "Chronically HIV-1 infected patients exhibit low frequencies of CD25+ regulatory T cells," *The Open Virology Journal*, vol. 6, no. 1, pp. 49–58, 2012.

[44] C. Li, I. Toth, J. Schulze zur Wiesch et al., "Functional characterization of HLA-G+ regulatory T cells in HIV-1 infection," *PLoS Pathogens*, vol. 9, no. 1, Article ID e1003140, 2013.

[45] J. M. Shaw, P. W. Hunt, J. W. Critchfield et al., "Short communication: HIV^+ viremic slow progressors maintain low regulatory t cell numbers in rectal mucosa but exhibit high t cell activation," *AIDS Research and Human Retroviruses*, vol. 29, no. 1, pp. 172–177, 2013.

[46] A. J. Chase, H.-C. Yang, H. Zhang, J. N. Blankson, and R. F. Siliciano, "Preservation of $FoxP3^+$ regulatory T cells in the peripheral blood of human immunodeficiency virus type 1-infected elite suppressors correlates with low $CD4^+$ T-cell activation," *Journal of Virology*, vol. 82, no. 17, pp. 8307–8315, 2008.

[47] Y. Jiao, J. Fu, S. Xing et al., "The decrease of regulatory T cells correlates with excessive activation and apoptosis of $CD8^+$ T cells in HIV-1-infected typical progressors, but not in long-term non-progressors," *Immunology*, vol. 128, no. 1, pp. e366–e375, 2009.

[48] J. M. Shaw, P. W. Hunt, J. W. Critchfield et al., "Increased frequency of regulatory t cells accompanies increased immune activation in rectal mucosae of HIV-positive noncontrollers," *Journal of Virology*, vol. 85, no. 21, pp. 11422–11434, 2011.

[49] J. C. Gaardbo, A. Ronit, H. J. Hartling et al., "Immunoregulatory t cells may be involved in preserving CD4 T cell counts in hiv-infected long-term nonprogressors and controllers," *Journal of Acquired Immune Deficiency Syndromes*, vol. 65, no. 1, pp. 10–18, 2014.

[50] L. Brandt, T. Benfield, H. Mens et al., "Low level of regulatory T cells and maintenance of balance between regulatory T cells and TH17 cells in HIV-1-infected elite controllers," *Journal of Acquired Immune Deficiency Syndromes*, vol. 57, no. 2, pp. 101–108, 2011.

[51] S.-Y. Li, H.-J. Xia, Z.-X. Dai et al., "Dynamics and functions of $CD4^+CD25^{high}$ regulatory T lymphocytes in Chinese rhesus macaques during the early stage of infection with SIVmac239," *Archives of Virology*, vol. 157, no. 5, pp. 961–967, 2012.

[52] J. D. Estes, Q. Li, M. R. Reynolds et al., "Premature induction of an immunosuppressive regulatory T cell response during acute simian immunodeficiency virus infection," *The Journal of Infectious Diseases*, vol. 193, no. 5, pp. 703–712, 2006.

[53] K. Allers, C. Loddenkemper, J. Hofmann et al., "Gut mucosal $FOXP3^+$ regulatory $CD4^+$ T cells and nonregulatory $CD4^+$ T

cells are differentially affected by simian immunodeficiency virus infection in rhesus macaques," *Journal of Virology*, vol. 84, no. 7, pp. 3259–3269, 2010.

[54] L. E. Pereira, F. Villinger, N. Onlamoon et al., "Simian immunodeficiency virus (SIV) infection influences the level and function of regulatory T cells in SIV-infected rhesus macaques but not SIV-infected sooty mangabeys," *Journal of Virology*, vol. 81, no. 9, pp. 4445–4456, 2007.

[55] A. Hryniewicz, A. Boasso, Y. Edghill-Smith et al., "CTLA-4 blockade decreases TGF-beta, IDO, and viral RNA expression in tissues of SIVmac251-infected macaques," *Blood*, vol. 108, no. 12, pp. 3834–3842, 2006.

[56] M. Montes, C. Sanchez, D. E. Lewis et al., "Normalization of FoxP3+ regulatory T cells in response to effective antiretroviral therapy," *Journal of Infectious Diseases*, vol. 203, no. 4, pp. 496–499, 2011.

[57] M. M. Pozo-Balado, M. Martínez-Bonet, I. Rosado et al., "Maraviroc reduces the regulatory T-cell frequency in antiretroviral-naive HIV-infected subjects," *Journal of Infectious Diseases*, vol. 210, no. 6, pp. 890–898, 2014.

[58] C. M. Rueda, P. A. Velilla, C. A. Chougnet, and M. T. Rugeles, "Incomplete normalization of regulatory T-cell frequency in the gut mucosa of Colombian HIV-infected patients receiving long-term antiretroviral treatment," *PLoS ONE*, vol. 8, no. 8, Article ID e71062, 2013.

[59] J. Andersson, A. Boasso, J. Nilsson et al., "Cutting edge: the prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients," *Journal of Immunology*, vol. 174, no. 6, pp. 3143–3147, 2005.

[60] G. Mendez-Lagares, M. M. Pozo-Balado, M. Genebat, A. Garcia-Perganeda, M. Leal, and Y. M. Pacheco, "Severe immune dysregulation affects $CD4^+CD25(hi)FoxP3^+$ regulatory T cells in HIV-infected patients with low-level CD4 T-cell repopulation despite suppressive highly active antiretroviral therapy," *Journal of Infectious Diseases*, vol. 205, no. 10, pp. 1501–1509, 2012.

[61] S. Piconi, D. Trabattoni, A. Gori et al., "Immune activation, apoptosis, and treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy," *AIDS*, vol. 24, no. 13, pp. 1991–2000, 2010.

[62] J. C. Gaardbo, H. J. Hartling, A. Ronit et al., "Regulatory T cells in HIV-infected immunological nonresponders are increased in blood but depleted in lymphoid tissue and predict immunological reconstitution," *Journal of Acquired Immune Deficiency Syndromes*, vol. 66, no. 4, pp. 349–357, 2014.

[63] R. S. B. Smith, D. Phillip, and T. T. MacDonald, "Lymphocyte populations within the lamina propria," in *Principles of Mucosal Immunology*, chapter 7, pp. 87–101, Garland Science, 2012.

[64] M. Miyamoto, O. Prause, M. Sjöstrand, M. Laan, J. Lötvall, and A. Lindén, "Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways," *The Journal of Immunology*, vol. 170, no. 9, pp. 4665–4672, 2003.

[65] S. C. Liang, X.-Y. Tan, D. P. Luxenberg et al., "Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides," *The Journal of Experimental Medicine*, vol. 203, no. 10, pp. 2271–2279, 2006.

[66] I. I. Ivanov, B. S. McKenzie, L. Zhou et al., "The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells," *Cell*, vol. 126, no. 6, pp. 1121–1133, 2006.

[67] L. Maggi, V. Santarlasci, M. Capone et al., "CD161 is a marker of all human IL-17-producing T-cell subsets and is induced by RORC," *European Journal of Immunology*, vol. 40, no. 8, pp. 2174–2181, 2010.

[68] W. Sato, T. Aranami, and T. Yamamura, "Cutting edge: human Th17 cells are identified as bearing $CCR2^+CCR5^-$ phenotype," *The Journal of Immunology*, vol. 178, no. 12, pp. 7525–7529, 2007.

[69] X. O. Yang, A. D. Panopoulos, R. Nurieva et al., "STAT3 regulates cytokine-mediated generation of inflammatory helper T cells," *The Journal of Biological Chemistry*, vol. 282, no. 13, pp. 9358–9363, 2007.

[70] L. Zhou, I. I. Ivanov, R. Spolski et al., "IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways," *Nature Immunology*, vol. 8, no. 9, pp. 967–974, 2007.

[71] E. V. Acosta-Rodriguez, G. Napolitani, A. Lanzavecchia, and F. Sallusto, "Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells," *Nature Immunology*, vol. 8, no. 9, pp. 942–949, 2007.

[72] J. E. Konkel and W. Chen, "Balancing acts: the role of TGF-β in the mucosal immune system," *Trends in Molecular Medicine*, vol. 17, no. 11, pp. 668–676, 2011.

[73] R. M. Onishi and S. L. Gaffen, "Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease," *Immunology*, vol. 129, no. 3, pp. 311–321, 2010.

[74] J. K. Kolls and A. Lindén, "Interleukin-17 family members and inflammation," *Immunity*, vol. 21, no. 4, pp. 467–476, 2004.

[75] M. Kleinewietfeld and D. A. Hafler, "The plasticity of human Treg and Th17 cells and its role in autoimmunity," *Seminars in Immunology*, vol. 25, no. 4, pp. 305–312, 2013.

[76] N. Komatsu, K. Okamoto, S. Sawa et al., "Pathogenic conversion of $Foxp3^+$ T cells into TH17 cells in autoimmune arthritis," *Nature Medicine*, vol. 20, no. 1, pp. 62–68, 2014.

[77] J. M. Brenchley, B. J. Hill, D. R. Ambrozak et al., "T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: implications for HIV pathogenesis," *Journal of Virology*, vol. 78, no. 3, pp. 1160–1168, 2004.

[78] M. Paiardini, B. Cervasi, E. Reyes-Aviles et al., "Low levels of SIV infection in sooty mangabey central memory $CD4^+$ T cells are associated with limited CCR5 expression," *Nature Medicine*, vol. 17, no. 7, pp. 830–836, 2011.

[79] F. Y. Yue, A. Merchant, C. M. Kovacs, M. Loutfy, D. Persad, and M. A. Ostrowski, "Virus-specific interleukin-17-producing $CD4^+$ T cells are detectable in early human immunodeficiency virus type 1 infection," *Journal of Virology*, vol. 82, no. 13, pp. 6767–6771, 2008.

[80] J. M. Brenchley, D. A. Price, T. W. Schacker et al., "Microbial translocation is a cause of systemic immune activation in chronic HIV infection," *Nature Medicine*, vol. 12, no. 12, pp. 1365–1371, 2006.

[81] Q. Li, L. Dua, J. D. Estes et al., "Peak SIV replication in resting memory $CD4^+$ T cells depletes gut lamina propria $CD4^+$ T cells," *Nature*, vol. 434, no. 7037, pp. 1148–1152, 2005.

[82] M. Guadalupe, E. Reay, S. Sankaran et al., "Severe $CD4^+$ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy," *Journal of Virology*, vol. 77, no. 21, pp. 11708–11717, 2003.

[83] A. El Hed, A. Khaitan, L. Kozhaya et al., "Susceptibility of human Th17 cells to human immunodeficiency virus and their perturbation during infection," *The Journal of Infectious Diseases*, vol. 201, no. 6, pp. 843–854, 2010.

[84] P. Ancuta, P. Monteiro, and R.-P. Sekaly, "Th17 lineage commitment and HIV-1 pathogenesis," *Current Opinion in HIV and AIDS*, vol. 5, no. 2, pp. 158–165, 2010.

[85] N. R. Klatt and J. M. Brenchley, "Th17 cell dynamics in HIV infection," *Current Opinion in HIV and AIDS*, vol. 5, no. 2, pp. 135–140, 2010.

[86] J. M. Brenchley and D. C. Douek, "HIV infection and the gastrointestinal immune system," *Mucosal Immunology*, vol. 1, no. 1, pp. 23–30, 2008.

[87] W. Maek-A-Nantawat, S. Buranapraditkun, J. Klaewsongkram, and K. Ruxrungthum, "Increased interleukin-17 production both in helper T cell subset Th17 and CD4-negative T cells in human immunodeficiency virus infection," *Viral Immunology*, vol. 20, no. 1, pp. 66–75, 2007.

[88] M. Salgado, N. I. Rallón, B. Rodés, M. López, V. Soriano, and J. M. Benito, "Long-term non-progressors display a greater number of Th17 cells than HIV-infected typical progressors," *Clinical Immunology*, vol. 139, no. 2, pp. 110–114, 2011.

[89] E. J. Ciccone, J. H. Greenwald, P. I. Lee et al., "CD4+ T cells, including Th17 and cycling subsets, are intact in the gut mucosa of HIV-1-infected long-term nonprogressors," *Journal of Virology*, vol. 85, no. 12, pp. 5880–5888, 2011.

[90] A. Singh, M. Vajpayee, S. A. Ali, K. Mojumdar, N. K. Chauhan, and R. Singh, "HIV-1 diseases progression associated with loss of Th17 cells in subtype 'C' infection," *Cytokine*, vol. 60, no. 1, pp. 55–63, 2012.

[91] M. Paiardini, "Th17 cells in natural SIV hosts," *Current Opinion in HIV and AIDS*, vol. 5, no. 2, pp. 166–172, 2010.

[92] M. Raffatellu, R. L. Santos, D. E. Verhoeven et al., "Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut," *Nature Medicine*, vol. 14, no. 4, pp. 421–428, 2008.

[93] M. Paiardini, B. Cervasi, J. C. Engram et al., "Bone marrow-based homeostatic proliferation of mature T cells in nonhuman primates: Implications for AIDS pathogenesis," *Blood*, vol. 113, no. 3, pp. 612–621, 2009.

[94] J. M. Brenchley, M. Paiardini, K. S. Knox et al., "Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections," *Blood*, vol. 112, no. 7, pp. 2826–2835, 2008.

[95] S. Pallikkuth, L. Micci, Z. S. Ende et al., "Maintenance of intestinal Th17 cells and reduced microbial translocation in SIV-infected rhesus macaques treated with interleukin (IL)-21," *PLoS Pathogens*, vol. 9, no. 7, Article ID e1003471, 2013.

[96] Y. Alvarez, M. Tuen, G. Shen et al., "Preferential HIV infection of $CCR6^+$ Th17 cells is associated with higher levels of virus receptor expression and lack of CCR5 ligands," *Journal of Virology*, vol. 87, no. 19, pp. 10843–10854, 2013.

[97] D. J. Hartigan-O'Connor, K. Abel, K. K. A. Van Rompay, B. Kanwar, and J. M. McCune, "SIV replication in the infected rhesus macaque is limited by the size of the preexisting TH17 cell compartment," *Science Translational Medicine*, vol. 4, no. 136, Article ID 136ra69, 2012.

[98] M. Macal, S. Sankaran, T.-W. Chun et al., "Effective CD4+ T-cell restoration in gut-associated lymphoid tissue of HIV-infected patients is associated with enhanced Th17 cells and polyfunctional HIV-specific T-cell responses," *Mucosal Immunology*, vol. 1, no. 6, pp. 475–488, 2008.

[99] S. DaFonseca, J. Niessl, S. Pouvreau et al., "Impaired Th17 polarization of phenotypically naive CD4+ T-cells during chronic HIV-1 infection and potential restoration with early ART," *Retrovirology*, vol. 12, no. 1, p. 38, 2015.

[100] L. C. Ndhlovu, J. M. Chapman, A. R. Jha et al., "Suppression of HIV-1 plasma viral load below detection preserves IL-17 producing T cells in HIV-1 infection," *AIDS*, vol. 22, no. 8, pp. 990–992, 2008.

[101] S. Pilakka-Kanthikeel, S. Huang, T. Fenton, W. Borkowsky, C. K. Cunningham, and S. Pahwa, "Increased gut microbial translocation in HIV-infected children persists in virologic responders and virologic failures after antiretroviral therapy," *The Pediatric Infectious Disease Journal*, vol. 31, no. 6, pp. 583–591, 2012.

[102] Y. Alvarez, M. Tuen, A. Nàdas, and C. E. Hioe, "*In Vitro* restoration of Th17 response during HIV infection with an antiretroviral drug and Th17 differentiation cytokines," *AIDS Research and Human Retroviruses*, vol. 28, no. 8, pp. 823–834, 2012.

[103] E. Bettelli, Y. Carrier, W. Gao et al., "Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells," *Nature*, vol. 441, no. 7090, pp. 235–238, 2006.

[104] A. Chaudhry, D. Rudra, P. Treuting et al., "$CD4^+$ regulatory T cells control TH17 responses in a stat3-dependent manner," *Science*, vol. 326, no. 5955, pp. 986–991, 2009.

[105] L. Zhou, J. E. Lopes, M. M. W. Chong et al., "TGF-β-induced Foxp3 inhibits T_H17 cell differentiation by antagonizing RORγt function," *Nature*, vol. 453, no. 7192, pp. 236–240, 2008.

[106] A. K. Antons, R. Wang, K. Oswald-Richter et al., "Naive precursors of human regulatory T cells require FoxP3 for suppression and are susceptible to HIV infection," *The Journal of Immunology*, vol. 180, no. 2, pp. 764–773, 2008.

[107] D. Valmori, A. Merlo, N. E. Souleimanian, C. S. Hesdorffer, and M. Ayyoub, "A peripheral circulating compartment of natural naive CD4+ Tregs," *The Journal of Clinical Investigation*, vol. 115, no. 7, pp. 1953–1962, 2005.

[108] D. Valmori, C. Raffin, I. Raimbaud, and M. Ayyoub, "Human $ROR\gamma t^+$ TH17 cells preferentially differentiate from naive $FOXP3^+$Treg in the presence of lineagespecific polarizing factors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 45, pp. 19402–19407, 2010.

[109] F. Mercer, A. Khaitan, L. Kozhaya, J. A. Aberg, and D. Unutmaz, "Differentiation of IL-17-producing effector and regulatory human T cells from lineage-committed naive precursors," *Journal of Immunology*, vol. 193, no. 3, pp. 1047–1054, 2014.

[110] M. Kleinewietfeld, F. Puentes, G. Borsellino, L. Battistini, O. Rötzschke, and K. Falk, "CCR6 expression defines regulatory effector/memory-like cells within the CD25+CD4+ T-cell subset," *Blood*, vol. 105, no. 7, pp. 2877–2886, 2005.

[111] M. Tsuji, N. Komatsu, S. Kawamoto et al., "Preferential generation of follicular B helper T cells from $Foxp3^+$ T cells in gut Peyer's patches," *Science*, vol. 323, no. 5920, pp. 1488–1492, 2009.

[112] N. Komatsu, M. E. Mariotti-Ferrandiz, Y. Wang, B. Malissen, H. Waldmann, and S. Hori, "Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 6, pp. 1903–1908, 2009.

[113] D. Favre, S. Lederer, B. Kanwar et al., "Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection," *PLoS Pathogens*, vol. 5, no. 2, Article ID e1000295, 2009.

[114] D. Li, J. Chen, M. Jia et al., "Loss of balance between T helper type 17 and regulatory T cells in chronic human immunodeficiency virus infection," *Clinical and Experimental Immunology*, vol. 165, no. 3, pp. 363–371, 2011.

[115] A. Singh, M. Vajpayee, S. A. Ali, and N. K. Chauhan, "Cellular interplay among Th17, Th1, and Treg cells in HIV-1 subtype 'C' infection," *Journal of Medical Virology*, vol. 86, no. 3, pp. 372–384, 2014.

[116] Y. He, J. Li, Y. Zheng et al., "A randomized case-control study of dynamic changes in peripheral blood Th17/Treg cell balance and interleukin-17 levels in highly active antiretroviral-treated HIV type 1/AIDS patients," *AIDS Research and Human Retroviruses*, vol. 28, no. 4, pp. 339–345, 2012.

[117] D. Favre, J. Mold, P. W. Hunt et al., "Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of T_H17 to regulatory T cells in HIV disease," *Science Translational Medicine*, vol. 2, no. 32, Article ID 32ra36, 2010.

[118] M.-A. Jenabian, M. Patel, I. Kema et al., "Distinct tryptophan catabolism and Th17/Treg balance in HIV progressors and elite controllers," *PLoS ONE*, vol. 8, no. 10, Article ID e78146, 2013.

[119] F. Sallusto, C. R. Mackay, and A. Lanzavecchia, "The role of chemokine receptors in primary, effector, and memory immune responses," *Annual Review of Immunology*, vol. 18, no. 1, pp. 593–620, 2000.

[120] E. V. Acosta-Rodriguez, L. Rivino, J. Geginat et al., "Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells," *Nature Immunology*, vol. 8, no. 6, pp. 639–646, 2007.

[121] H. Hu, M. Nau, P. Ehrenberg et al., "Distinct gene-expression profiles associated with the susceptibility of pathogen-specific CD4 T cells to HIV-1 infection," *Blood*, vol. 121, no. 7, pp. 1136–1144, 2013.

[122] A. Gosselin, P. Monteiro, N. Chomont et al., "Peripheral blood $CCR4^+CCR6^+$ and $CXCR3^+CCR6^+$ $CD4^+$ T cells are highly permissive to HIV-1 infection," *The Journal of Immunology*, vol. 184, no. 3, pp. 1604–1616, 2010.

[123] C. Geldmacher, A. Schuetz, N. Ngwenyama et al., "Early depletion of *Mycobacterium tuberculosis*-specific T helper 1 cell responses after HIV-1 infection," *The Journal of Infectious Diseases*, vol. 198, no. 11, pp. 1590–1598, 2008.

[124] P. Monteiro, A. Gosselin, V. S. Wacleche et al., "Memory $CCR6^+CD4^+$ T cells are preferential targets for productive HIV type 1 infection regardless of their expression of integrin β_7," *The Journal of Immunology*, vol. 186, no. 8, pp. 4618–4630, 2011.

[125] M. Kader, X. Wang, M. Piatak et al., "$\alpha4^+\beta7^{hi}CD4^+$ memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection," *Mucosal Immunology*, vol. 2, no. 5, pp. 439–449, 2009.

[126] T. Yamazaki, X. O. Yang, Y. Chung et al., "CCR6 regulates the migration of inflammatory and regulatory T cells," *The Journal of Immunology*, vol. 181, no. 12, pp. 8391–8401, 2008.

[127] X. Wang, H. Xu, A. F. Gill et al., "Monitoring $\alpha4\beta7$ integrin expression on circulating CD4+ T cells as a surrogate marker for tracking intestinal CD4+ T-cell loss in SIV infection," *Mucosal Immunology*, vol. 2, no. 6, pp. 518–526, 2009.

[128] H. W. Lim, H. E. Broxmeyer, and C. H. Kim, "Regulation of trafficking receptor expression in human Forkhead Box $P3^+$ regulatory T cells," *Journal of Immunology*, vol. 177, no. 2, pp. 840–851, 2006.

[129] A. Iellem, M. Mariani, R. Lang et al., "Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by $CD4^+CD25^+$ regulatory T cells," *Journal of Experimental Medicine*, vol. 194, no. 6, pp. 847–853, 2001.

[130] S. Qin, Y. Sui, A. C. Soloff et al., "Chemokine and cytokine mediated loss of regulatory T cells in lymph nodes during pathogenic simian immunodeficiency virus infection," *The Journal of Immunology*, vol. 180, no. 8, pp. 5530–5536, 2008.

[131] A.-S. Liovat, M.-A. Rey-Cuillé, C. Lécuroux et al., "Acute plasma biomarkers of T cell activation set-point levels and of disease progression in HIV-1 infection," *PLoS ONE*, vol. 7, no. 10, Article ID e46143, 2012.

[132] A. G. Lourenço, M. C. Komesu, A. A. Machado, T. Bourlet, B. Pozzetto, and O. Delézay, "Potential contribution of saliva to the sexual transmission of HIV through the secretion of CCL20 by genital epithelial cells," *Journal of Medical Virology*, vol. 86, no. 1, pp. 58–63, 2014.

[133] J. B. Canavan, C. Scotta, A. Vossenkamper et al., "Developing in vitro expanded $CD45RA^+$ regulatory T cells as an adoptive cell therapy for Crohn's disease," *Gut*, 2015.

[134] H. Zhou, H. Zhao, Y. Hao et al., "Excessive conversion and impaired thymic output contribute to disturbed regulatory T-cell homeostasis in AIDS patients with low CD4 cell counts," *AIDS*, vol. 27, no. 7, pp. 1059–1069, 2013.

[135] S. Becattini, D. Latorre, F. Mele et al., "Functional heterogeneity of human memory $CD4^+$ T cell clones primed by pathogens or vaccines," *Science*, vol. 347, no. 6220, pp. 400–406, 2015.

ANEXO B:

Artigo de dados publicado:

“Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background”

Jacqueline M. Valverde-Villegas, Bruno Paiva dos Santos, Rúbia Marília de Medeiros, Vanessa Suñé Mattevi, Rosmeri Kuhmmer Lazzaretti, Eduardo Sprinz, Regina Kuhmmer, José Artur Bogo Chies

Artigo publicado na revista *Human Immunology* em Janeiro de 2017

Human Immunology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

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

Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background

Jacqueline M. Valverde-Villegas [a], Bruno Paiva dos Santos [b,e], Rúbia Marília de Medeiros [a], Vanessa Suñé Mattevi [c], Rosmeri Kuhmmer Lazzaretti [d], Eduardo Sprinz [d], Regina Kuhmmer [d], José Artur Bogo Chies [a,*]

[a] Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil
[b] Universidade Luterana do Brasil (ULBRA), Canoas, Rio Grande do Sul, Brazil
[c] Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, Rio Grande do Sul, Brazil
[d] Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Rio Grande do Sul, Brazil
[e] INSERM U1026, Tissue Bioengineering, Université de Bordeaux, Bordeaux F-33076, France

ARTICLE INFO

Article history:
Received 21 March 2016
Revised 19 December 2016
Accepted 2 January 2017
Available online xxxx

Keywords:
Ethnic background
TLRs polymorphisms
HIV-1
HCV
Susceptibility

ABSTRACT

The genetic background of human populations can influence the susceptibility and outcome of infection diseases. Toll-like receptors (TLRs) have been previously associated with susceptibility to human immunodeficiency virus (HIV) infection, disease progression and hepatitis C, virus (HCV) co-infection in different populations, although mostly in Europeans. In this study, we investigated the genetic role of endosomal TLRs on susceptibility to HIV infection and HCV co-infection through the analysis of *TLR7* rs179008, *TLR8* rs3764880, *TLR9* rs5743836 and *TLR9* rs352140 polymorphisms in 789 Brazilian individuals (374 HIV+ and 415 HIV−), taking into account their ethnic background. Amongst the 357 HIV+ individuals with available data concerning HCV infection, 98 were positive. In European descendants, the *TLR9* rs5743836 C carriers displayed a higher susceptibility to HIV infection [dominant, Odds Ratio (OR) = 1.53; 95% CI: 1.05–2.23; $P = 0.027$]. In African descendants, *TLR9* rs5743836 CT genotype was associated with protection to HIV infection (codominant, OR = 0.51; 95% CI: 0.30–0.87; $P = 0.013$). Also, the *TLR9* rs352140 AA variant genotype was associated with susceptibility to HIV+/HCV+ co-infection in African descendants (recessive, OR = 2.92; 95% CI: 1.22–6.98, $P = 0.016$). These results are discussed in the context of the different ethnic background of the studied individuals highlighting the influence of this genetic/ethnic background on the susceptibility to HIV infection and HIV/HCV co-infection in Brazilian individuals.

1. Introduction

It is well established that host genetic factors are of considerable interest to the field of HIV/AIDS and can account for some of the differences regarding HIV-1 susceptibility and progression [1]. Among the genetic factors considered to be important in the initial detection and efficiency of the response against HIV-1, those coding for endosomal toll-like receptors (TLRs), are of special interest [2].

TLR7/8/9 recognize nucleic acids from viruses and bacteria and are exclusively expressed within endocytic compartments [3]. In the context of HIV infection, it was reported that a guanine-uridine-rich single strand RNA derived from HIV-1 is recognized by TLR7/8 and stimulates dendritic cells and macrophages to secrete interferon alpha (IFN-α) and proinflammatory cytokines [4,5]. Also, a direct interaction of HIV-1 gp120 with plasmacytoid dendritic cells (pDCs) inhibits TLR9-mediated responses, including pDC activation, IFN-α secretion and the cytolytic activity of Natural Killer (NK) cells [6], suggesting that HIV-1 can evade immune

* Corresponding author at: Laboratory of Immunogenetics, Institute of Biosciences, Department of Genetics, UFRGS, Av. Bento Gonçalves – 9500, Campus do Vale, 91501970, PO Box 15053, Porto Alegre, RS, Brazil.
E-mail address: jabchies@terra.com.br (J.A.B. Chies).

http://dx.doi.org/10.1016/j.humimm.2017.01.001
0198-8859/

responses through the inhibition of innate immune components [7].

Some studies observed the role of endosomal TLRs on HCV and outcome [8,9]. For instance, HCV infection, in the same way as HIV, triggers IFN-α secretion in pDCs via TLR7 or TLR9 [2,10]. Of note, HIV+/HCV co-infected subjects with chronic HCV infections have higher HCV viral load when compared with individuals infected with only HCV. Moreover, studies have observed a relationship of endosomal TLRs and HIV/HCV co-infection [11,12].

Functional genetic polymorphisms as *TLR7* rs179008, *TLR8* rs3764880, *TLR9* rs5743836 and *TLR9* rs352140 have been observed to influence HIV acquisition [13,14] and AIDS progression [15–17] and also the susceptibility to HIV/HCV co-infection and outcome [11,18]. For instance, the *TLR7* rs179008 T variant, associated to low IFN-α production was also associated with accelerated disease progression [15], the *TLR8* rs3764880 G variant, lead to a slower progression of the disease in HIV-1+ subjects [17]. Regarding the *TLR9* rs5743836 and rs352140 polymorphisms, they have been linked to viral load, CD4+ T cell counts and disease progression in HIV-1 infected individuals and mother-to-child HIV-1 transmission [13,14,16]. Genotypic frequencies and haplotype distribution of endosomal TLRs in healthy populations vary according to ethnicity and even within the same ethnic group [19,20]. This highlights the necessity to studies considering ethnic ancestries to the comprehension of how the genetic background influences susceptibility to these diseases.

Taken together, the aim of this study is to evaluated the frequency of *TLR7* rs179008, *TLR8* rs3764880, *TLR9* rs5743836, and *TLR9* rs352140 polymorphisms in HIV+ (and HCV co-infected) Brazilian individuals classified according to their ancestry. Our results evidenced distinct ethnic backgrounds associated to different endosomal TLR gene polymorphisms and different outcomes. In the present manuscript, we will discuss these results and the potential influence of the genetic/ethnic background on the susceptibility to HIV infection and HIV/HCV co-infection in those individuals.

Table 1
Demographic and clinical characteristics of HIV+ subjects and HIV− controls.

Demographic	No. of subjects (%)[a]		
	HIV+ subjects (n = 374)	HIV− controls (n = 415)	HIV+ vs. HIV− *P*-value
Median age ± SD	42.98 ± 9.42	44.34 ± 8.99	NS
Sex[*]			
Female	166 (0.45)	190 (0.46)	NS
Male	203 (0.55)	224 (0.54)	
Ethnicity			
European descendants	212 (0.57)	310 (0.75)	0.000002
African descendants	162 (0.43)	105 (0.25)	
Clinical			
HCV co-infection (yes)[b]	98/357 (0.27)	NA	–
European descendants	41/98 (0.42)	NA	
African descendants	57/98 (0.58)	NA	
HAART (yes)	374 (1.00)	NA	–

SD, standard deviation; NA, not applicable; NS, not significant.
[a] Percentages are based on known data.
[b] HCV test available for 357 HIV+ patients.
[*] Missing data.

2. Material and methods

2.1. HIV+ subjects and controls

Table 1 summarizes demographic and clinical data of the 789 individuals. 374 were HIV-1+ subjects (212 European descendants and 162 African descendants) from the South Brazilian HIV-1 Cohort (SOBRHIV) at the HIV/AIDS outpatient clinic at Hospital de Clínicas de Porto Alegre [21]. Of them, 357 had data on HCV infection and 98 were HIV+/HCV+ co-infected. All HIV+ subjects were adults on highly active antiretroviral therapy (HAART) for at least one year with viral load <50 copies per milliliter. The control group was composed of 415 HIV− adult individuals (310 European descendants and 105 African descendants) collected amongst healthy blood donors (that are systematically screened for fever, cold, flu, pregnancy, hepatitis, Chagas disease and malaria) resident at the same urban center as HIV+ subjects. The majority of HIV+ and HIV− subjects were European descendants and sex and age were similar in both European and African groups.

Issues regarding the skin color-based classification criteria that are used in Brazil are well documented [22]. Of note, different geographic regions in Brazil present distinct admixture levels that reflect the country occupancy and history. In this sense, the north of Brazil presents high levels of admixture, with a high frequency of the three major ancestral groups (meaning: Native South Americans, Africans and Europeans). Conversely, the Southern region has a lower degree of admixture, with a major component of European-descendants and low admixture with Native South-American groups.

The study protocol was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre and informed consent according to the Declaration of Helsinki was signed by all participants.

2.2. Genotyping

DNA was isolated from whole blood using the salting-out method [23] and stored at -20°C. The *TLR7* rs179008, *TLR8* rs3764880, and *TLR9* rs352140 polymorphisms were amplified by PCR-RFLP using *Apo*I, *Nla*III and *Bst*UI restriction endonucleases, respectively, according to the protocol described by Cheng et al. (2007) [24]. The *TLR7* rs179008 and *TLR9* rs352140 cleavages were visualized in 6% polyacrylamide gel and *TLR8* rs3764880 was visualized in 8% polyacrylamide gel, both silver nitrate stained. *TLR9* rs5743836 was genotyped by Bidirectional PCR amplification of specific alleles (BI-PASA) as described by Carvalho et al. (2007) [20] with modifications by dos Santos et al. (2012) [25] and visualized in a 2% agarose gel stained with ethidium bromide. Positive controls for each genotype (wild-type homozygous, heterozygous and variant-type homozygous) were included in all experiments. If any doubt about genotyping arouse during procedures, all samples of this specific amplification group were re-evaluated. Randomly selected samples were sequenced to confirm the genotypes when protocols were standardized.

2.3. Statistical analysis

The Hardy–Weinberg equilibrium test was performed in cases and controls using the chi-square test (χ^2). Clinical and demographic data were compared among the groups using T student $P < 0.001$. Genetic models (codominant, dominant and recessive) were tested to ensure that interesting findings are not missed [26]. Odds Ratio (OR) with 95% confidence intervals (CI) were calculated for each model using binomial logistic regression or chi-square test (Mid-P exact). We stratified our data by ethnic

Please cite this article in press as: J.M. Valverde-Villegas et al., Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background, Hum. Immunol. (2017), http://dx.doi.org/10.1016/j.humimm.2017.01.001

background since genotypic frequencies of endosomal TLRs are different among human populations. Frequencies for *TLR7* and *TLR8* genes are presented by sex since these genes are located on the X chromosome. Haplotype frequencies, D′ and r^2 were estimated using MLocus program [27]. A two-tailed value of $P < 0.05$ was used to indicate statistical significance. Statistical analyses were performed using SPSS 18.0 and WinPepi version 11.1 softwares.

3. Results

3.1. Allelic and genotypic frequencies distribution stratified by ethnicity

All polymorphisms were in Hardy-Weinberg equilibrium in HIV+ individuals and controls when stratified by ethnicity. Table 2 shows allelic and genotypic frequencies in HIV+ individuals and controls according to the ethnicity. According to the dominant model (TC + CC vs TT), the *TLR9* rs5743836 C allele carrier subjects (TC or CC) are more susceptible to HIV-1 infection (OR = 1.53; 95%; CI: 1.05–2.23; $P = 0.027$) in individuals of European ancestry. However, in individuals of African ancestry, according the codominant model, HIV+ subjects with the *TLR9* rs5743836 TC genotype are protected against HIV-1 infection (OR = 0.51, CI 95%: 0.30–087, $P = 0.013$). Concerning the *TLR7* rs179008, *TLR8* rs3764880 and *TLR9* rs352140, no association between these polymorphisms with HIV-1 infection was observed.

3.2. Allelic and genotypic frequencies in HIV/HCV co-infected subjects

Table 3 shows the comparison among HIV-1+ HCV+ co-infected subjects or not co-infected stratified by ethnicity. According the recessive genetic model, the *TLR9* rs352140 variant AA genotype frequency was higher in the HIV+/HCV+ group when compared with the HIV+/HCV− group (0.29 vs. 0.13; $P = 0.016$, OR = 2.92; 95% CI: 1.22–6.98) in African descendants. No differences in allelic and genotypic frequencies were found for the other polymorphisms.

3.3. Haplotype frequencies distribution of TLR9 SNPs

In the analysis of linkage disequilibrium (LD) for *TLR9* polymorphisms, the D′ values between rs5743836 and rs352140 were shown to be in modest disequilibrium in European descendants (D′ = 0.70). While the D′ values in African descendants were shown to be in low disequilibrium (D′ < 0.7). Table S1 shows haplotypic frequencies for *TLR9* polymorphisms stratified by ethnicity. Comparing HIV+ subjects and controls for both ethnic groups, no statistical differences were observed in the four haplotypes distribution. Nevertheless, differences due to the ethnic background become quite evident when both ethnic groups were compared among HIV+ subjects and controls.

4. Discussion

In this study, in European descendants, the minor C allele carriers (TC or CC) of *TLR9* rs5743836 have a higher susceptibility to HIV-1 infection according to the dominant model. In accordance with our data, Pine et al., (2009) described an association of the C allele with high viral load and rapid AIDS progression, although these associations were not significant after corrections [16]. Of note, in the study of Pine et al., the cohort was restricted to white individuals from the United States of America. In marked contrast, our results in individuals of African ancestry indicated *TLR9* rs5743836 TC or CC genotypes as protective against HIV-1 infection both according to codominant model. In addition, our group also observed an association of the *TLR9* rs5743836 C allele carriers with protection to HIV-1 infection in another Brazilian cohort of African descendants, classified according to rapid or slow

Table 2
Binomial logistic regression of genetic models of *TLR7/8/9* SNPs in HIV+ subjects and HIV− controls stratified by ethnicity.

Gene	SNP	European descendants						African descendants					
		Model	Genotypes	HIV+ n (%)	HIV− n (%)	OR (95% CI)	*P*-value	Model	Genotypes	HIV+ n (%)	HIV− n (%)	OR (95% CI)	*P*-value
TLR9	rs5743836	Dominant	TT	136 (0.64)	227 (0.73)	1	**0.027**	Codominant	TT	90 (0.58)	47 (0.46)	1	**0.013**
									TC	50 (0.33)	51 (0.49)	**0.51 (0.30–0.87)**	
			TC + CC	76 (0.36)	83 (0.27)	**1.53 (1.05–2.23)**			CC	14 (0.09)	5 (0.05)		
	rs352140	Recessive	GG + GA	138 (0.70)	227 (0.74)	1	0.372	Dominant	GG	45 (0.32)	43 (0.41)	1	0.124
			AA	59 (0.30)	81 (0.26)	1.20 (0.81–1.78)			GA + AA	98 (0.68)	62 (0.59)	1.51 (0.89–2.55)	
TLR8	rs3764880	Dominant	AA♀	47 (0.49)	69 (0.47)	1	0.743	Dominant	AA♀	24 (0.41)	21 (0.48)	1	0.486
			AG + GG♀	48 (0.51)	77 (0.53)	0.92 (0.54–1.54)			AG + GG♀	34 (0.59)	23 (0.52)	1.29 (0.58–2.87)	
			A♂	52 (0.55)	97 (0.65)				A♂	57 (0.68)	43 (0.73)		
			G♂	42 (0.45)	52 (0.35)				G♂	27 (0.32)	16 (0.27)		
TLR7	rs179008	Dominant	AA♀	70 (0.69)	102 (0.71)	1	0.833	Dominant	AA♀	46 (0.71)	29 (0.67)	1	0.753
			AT + TT♀	31 (0.31)	42 (0.29)	1.08 (0.61–1.88)			AT + TT♀	19 (0.29)	14 (0.33)	0.86 (0.37–2.00)	
			A♂	91 (0.86)	136 (0.86)				A♂	78 (0.80)	52 (0.85)		
			T♂	15 (0.14)	23 (0.14)				T♂	19 (0.20)	9 (0.15)		

OR: Odds Ratio; CI: Confidence interval; SNP, single nucleotide polymorphism.
Bold value indicates statistical significance of univariate logistic regression ($P < 0.05$).

Please cite this article in press as: J.M. Valverde-Villegas et al., Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background, Hum. Immunol. (2017), http://dx.doi.org/10.1016/j.humimm.2017.01.001

Table 3
Binomial logistic regression of genetic models of *TLR7/8/9* SNPs in HIV+/HCV− and HIV+/HCV+ subjects stratified by ethnicity.

Gene	SNP	European descendants						African descendants				
		Model	Genotypes	HIV+/HCV− n (%)	HIV+/HCV+ n (%)	OR (95% CI)	*P*-value	Genotypes	HIV+/HCV− n (%)	HIV+/HCV+ n (%)	OR (95% CI)	*P*-value
TLR9	rs5743836	Dominant	TT	101 (0.62)	28 (0.68)	1	0.480	TT	58 (0.60)	29 (0.55)	1	0.547
			TC + CC	61 (0.38)	13 (0.31)	0.77 (0.37–1.60)		TC + CC	39 (0.40)	24 (0.45)	1.23 (0.63–2.42)	
	rs352140	Recessive	GG + GA	105 (0.70)	27 (0.69)	1	0.926	GG + GA	77 (0.87)	36 (0.71)	1	**0.016**
			AA	45 (0.30)	12 (0.31)	1.04 (048–2.37)		AA	11 (0.13)	15 (0.29)	**2.92 (1.22–6.98)**	
TLR8	rs3764880	Dominant	AA♀	37 (0.49)	8 (0.53)	1	0.784	AA♀	18 (0.44)	6 (0.38)	1	0.769
			AG + GG♀	39 (0.51)	7 (0.47)	1.10 (0.61–1.97)		AG + GG♀	23 (0.56)	10 (0.62)	0.89 (0.56–1.43)	
			A♂	32 (0.48)	15 (0.68)			A♂	33 (0.67)	22 (0.69)		
			G♂	35 (0.52)	7 (0.32)			G♂	16 (0.33)	10 (0.31)		
TLR7	rs179008	Dominant	AA♀	57 (0.70)	10 (0.63)	1	0.562	AA♀	33 (0.73)	12 (0.63)	1	0.550
			AT + TT♀	24 (0.30)	6 (0.37)	0.79 (0.39–1.62)		AT + TT♀	12 (0.27)	7 (0.37)	0.724 (0.34–1.55)	
			A♂	64 (0.83)	22 (0.92)			A♂	43 (0.77)	32 (0.84)		
			T♂	13 (0.17)	2 (0.08)			T♂	13 (0.23)	6 (0.16)		

OR: Odds Ratio; CI: Confidence interval; SNP, single nucleotide polymorphism.
Bold value indicates statistical significance of univariate logistic regression ($P < 0.05$).

progression to AIDS (de Medeiros RM, personal communication). No association was observed in our study concerning *TLR9* rs352140 and HIV infection, differently from some other studies [14,28,29].

One important role of TLR9 concerns the dendritic cells, which produce proinflammatory cytokines via NF-kappaB in response to their activation [3]. An *in vitro* functional study indicated that peripheral blood mononuclear cells carrying the *TLR9* rs5743836 TC genotype expresses high levels of TLR9 in response to synthetic CpG oligodeoxynucleotides (CpG ODN) and also induces increased B cell proliferation and IL-6 production, as compared to TT carrying cells [30]. The activation of memory B cells by TLR9 may play a significant role in memory B cell homeostasis and a sustained production of antibodies. Jiang et al., (2008) observed that purified B cells from HIV-1+ subjects had a decreased TLR9 mRNA expression as compared to those from HIV-1 negative controls, in response to CpG ODN; suggesting that decreased TLR9 expression could contribute to the functional defects of B cells in HIV infection [31].

A comparative analysis between HIV+ co-infected subjects or not with HCV was also performed. In African descendants, the *TLR9* rs352140 variant AA frequency was higher in HIV+/HCV+ when compared to HIV+/HCV− individuals (OR = 2.92, 95% CI: 1.22–6.98; $P = 0.016$), suggesting that AA genotype carriers have higher susceptibility to HIV+/HCV+ co-infection. Of note, Clausen et al. (2014) reported that A allele carriers of *TLR9* rs352140 were associated with spontaneous HCV resolution in a Caucasian cohort, but in the validation cohort this association did not reach statistical significance [32]. More studies are needed to clarify the role of the *TLR9* rs352140 variant on HIV/HCV co-infection.

Regarding TLR9 haplotype frequencies, no significant differences were observed between HIV+ subjects and controls. However, different haplotypic frequencies between individuals from distinct ethnic origins become evident. For instance, controls of African ancestry have a higher overall CG haplotypic frequency than controls of European ancestry (0.18 vs 0.02). Indeed, a previous study had already observed a higher frequency of the CG haplotype among African Americans [33]. These different frequencies seem to be related to the ethnic origin of our population, being independent of the disease. A study from our group observed a similar haplotype distribution for these *TLR* SNPs in individuals with systemic lupus erythematosus [25].

Previous studies in our group approached genetic aspects of HIV + (and HCV co-infected) individuals, classified according to their ethnic origin. Concerning the *human leukocyte antigen-G* (HLA-G) gene, genotypic frequencies were distinct according to the ethnic background evaluated [34]. In the case of *mannose binding-lectine* (MBL) polymorphisms, it was possible to observe a protective effect against HIV infection or HCV co-infection only in individuals of African ancestry [35]. In the present study, once more, the influence of the ethnic background was evidenced on susceptibility to HIV infection and HCV co-infection.

To emphasize the importance of the ethnic background, we compared the genotypic frequencies obtained on this study with European and African populations available at the HapMap database (Table S2). The controls of European ancestry from the present study were quite similar to the Europeans from the database set. However, there was a considerable difference between the African descendants from our study and the African individuals from the HapMap database. This result must reflect the Brazilian history, characterized by extensive, although asymmetrical, admixture [22]. Besides, it is possible to observe quite similar frequencies amongst the *TLR9* rs5743836 and rs352140 allelic frequencies from our data and other studies on African descendant admixed populations [36,37].

There are robust evidences linking genetic differences between ethnic groups and the pool of pathogens associated to a given human population. An interesting study by Kai Zhao et al. (2012) observed a higher frequency of HIV protective alleles of different immune-related genes in Biaka Western Pygmies compared to other sub-Saharan Africans. This work hypothesized that immunodeficiency viruses may have first infected humans, such as the Biaka Western Pygmies, that historically resided in communities within the forest range of the common chimpanzee (the natural host of SIV, from who HIV originated). SIV probably crossed species barrier more than once, and this would generate a selection pressure for resistance, which could be reflected in the characteristic genomic signature of the descendants of these affected populations [38]. A study of the evolution and natural selection of *TLRs* in different ethnic groups described a higher genetic diversity of *TLR9* in African populations when compared with the European and Asian populations [39]. Nonetheless, further replication studies with other HIV+, HCV+ and HIV+/HCV+ admixed cohorts allied to

Please cite this article in press as: J.M. Valverde-Villegas et al., Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background, Hum. Immunol. (2017), http://dx.doi.org/10.1016/j.humimm.2017.01.001

evolutionary studies need to be done to fully explain the ethnically related differences observed.

5. Conclusion

We suggest that the *TLR9* rs5743836 polymorphism has a significant role in HIV-1 infection depending on the ethnic background, since it was associated with susceptibility to HIV infection in HIV+ individuals of European ancestry and protection to HIV infection in African descendants. The effect of this variant, although small, is statistically significant when assessed without correction for multiple comparisons and therefore needs to be replicated in populations stratified by ethnicity. Also, the *TLR9* rs352140 polymorphism was associated with susceptibility to HIV+/HCV+ co-infection in African descendants. Our data reinforces the importance of considering the ethnic background in human population studies, and highlights the need for replication studies in other admixed HIV-1+ cohorts.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We thank Sidia Callegari-Jacques for the statistical support. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [grant numbers 190760/2010-9 and 306349/2011-6] and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS) [Grant numbers 10/1516-6 and 12/2151-2]. Jacqueline M. Valverde-Villegas received a scholarship by the Programa Estudantes-Convênio de Pós-Graduação (PEC-PG).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2017.01.001.

References

[1] D. van Manen, A.B. van't Wout, H. Schuitemaker, Genome-wide association studies on HIV susceptibility, pathogenesis and pharmacogenomics, Retrovirology 9 (2012) 70, http://dx.doi.org/10.1186/1742-4690-9-70.
[2] T.H. Mogensen, J. Melchjorsen, C.S. Larsen, S.R. Paludan, Innate immune recognition and activation during HIV infection, Retrovirology 7 (2010) 54, http://dx.doi.org/10.1186/1742-4690-7-54.
[3] A.L. Blasius, B. Beutler, Intracellular Toll-like receptors, Immunity 32 (2010) 305–315, http://dx.doi.org/10.1016/j.immuni.2010.03.012.
[4] F. Heil, H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, et al., Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8, Science 303 (2004) 1526–1529, http://dx.doi.org/10.1126/science.1093620.
[5] E. Schlaepfer, R.F. Speck, Anti-HIV activity mediated by natural killer and CD8+ cells after toll-like receptor 7/8 triggering, PLoS ONE 3 (2008) e1999, http://dx.doi.org/10.1371/journal.pone.0001999.
[6] E. Martinelli, C. Cicala, D. Van Ryk, D.J. Goode, K. Macleod, J. Arthos, et al., HIV-1 gp120 inhibits TLR9-mediated activation and IFN-{alpha} secretion in plasmacytoid dendritic cells, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 3396–3401, http://dx.doi.org/10.1073/pnas.0611353104.
[7] G. Kaur, N. Mehra, Genetic determinants of HIV-1 infection and progression to AIDS: susceptibility to HIV infection, Tissue Antigens 73 (2009) 289–301, http://dx.doi.org/10.1111/j.1399-0039.2009.01220.x.
[8] E. Askar, G. Ramadori, S. Mihm, Toll-like receptor 7 rs179008/Gln11Leu gene variants in chronic hepatitis C virus infection, J. Med. Virol. 82 (2010) 1859–1868, http://dx.doi.org/10.1002/jmv.21893.
[9] E. Schott, H. Witt, K. Neumann, A. Bergk, J. Halangk, V. Weich, et al., Association of TLR7 single nucleotide polymorphisms with chronic HCV-infection and response to interferon-a-based therapy, J. Viral Hepat. 15 (2008) 71–78, http://dx.doi.org/10.1111/j.1365-2893.2007.00898.x.
[10] Y.-L. Zhang, Y.-J. Guo, Bin Li, S.-H. Sun, Hepatitis C virus single-stranded RNA induces innate immunity via Toll-like receptor 7, J. Hepatol. 51 (2009) 29–38, http://dx.doi.org/10.1016/j.jhep.2009.03.012.
[11] A. Fernández-Rodríguez, J. Berenguer, M.A. Jiménez-Sousa, M. García-Álvarez, T. Aldámiz-Echevarría, D. Pineda-Tenor, et al., Toll-like receptor 8 (TLR8) polymorphisms are associated with non-progression of chronic hepatitis C in HIV/HCV coinfected patients, Infect. Genet. Evol. 36 (2015) 339–344, http://dx.doi.org/10.1016/j.meegid.2015.10.006.
[12] V.D. Gonzalez, A.L. Landay, J.K. Sandberg, Innate immunity and chronic immune activation in HCV/HIV-1 co-infection, Clin. Immunol. 135 (2010) 12–25, http://dx.doi.org/10.1016/j.clim.2009.12.005.
[13] E. Ricci, S. Malacrida, M. Zanchetta, I. Mosconi, M. Montagna, C. Giaquinto, et al., Toll-like receptor 9 polymorphisms influence mother-to-child transmission of human immunodeficiency virus type 1, J. Transl. Med. 8 (2010) 49, http://dx.doi.org/10.1186/1479-5876-8-49.
[14] E.A. Said, F. Al-Yafei, F. Zadjali, S.S. Hasson, M.S. Al-Balushi, S. Al-Mahruqi, et al., Association of single-nucleotide polymorphisms in TLR7 (Gln11Leu) and TLR9 (1635A/G) with a higher CD4T cell count during HIV infection, Immunol. Lett. 160 (2014) 58–64, http://dx.doi.org/10.1016/j.imlet.2014.04.005.
[15] D.-Y. Oh, K. Baumann, O. Hamouda, J.K. Eckert, K. Neumann, C. Kücherer, et al., A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression, AIDS 23 (2009) 297–307, http://dx.doi.org/10.1097/QAD.0b013e32831fb540.
[16] S.O. Pine, M.J. McElrath, P. Bochud, Polymorphisms in toll-like receptor 4 and toll-like receptor 9 influence viral load in a seroincident cohort of HIV-1-infected individuals, AIDS 23 (2009) 2387–2395, http://dx.doi.org/10.1097/QAD.0b013e328330b489.
[17] D.-Y. Oh, S. Taube, O. Hamouda, C. Kücherer, G. Poggensee, H. Jessen, et al., A functional toll-like receptor 8 variant is associated with HIV disease restriction, J. Infect. Dis. 198 (2008) 701–709, http://dx.doi.org/10.1086/590431.
[18] I. Mozer-Lisewska, J. Sikora, A. Kowala-Piaskowska, M. Kaczmarek, G. Dworacki, J. Zeromski, The incidence and significance of pattern-recognition receptors in chronic viral hepatitis types B and C in man, Arch. Immunol. Ther. Exp. (Warsz) 58 (2010) 295–302, http://dx.doi.org/10.1007/s00005-010-0087-9.
[19] E. Martínez-Robles, M. Yebra-Bango, S. Mellor-Pita, P. Tutor-Ureta, J.A. Vargas, M.J. Citores, Genotypic distribution of common variants of endosomal toll like receptors in healthy Spanish women. A comparative study with other populations, Gene 578 (2016) 32–37, http://dx.doi.org/10.1016/j.gene.2015.12.004.
[20] A. Carvalho, A. Marques, P. Maciel, F. Rodrigues, Study of disease-relevant polymorphisms in the TLR4 and TLR9 genes: a novel method applied to the analysis of the Portuguese population, Mol. Cell. Probes 21 (2007) 316–320, http://dx.doi.org/10.1016/j.mcp.2007.03.005.
[21] F. Dabis, E. Balestre, P. Braitstein, P. Miotti, W.G.M. Brinkhof, M. Schneider, et al., Cohort Profile: Antiretroviral therapy in lower income countries (ART-LINC): international collaboration of treatment cohorts, Int. J. Epidemiol. 34 (2005) 979–986, http://dx.doi.org/10.1093/ije/dyi164.
[22] S.D.J. Pena, G. di Pietro, M. Fuchshuber-Moraes, J.P. Genro, M.H. Hutz, F.D.S.G. Kehdy, et al., The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected, PLoS ONE 6 (2011), http://dx.doi.org/10.1371/journal.pone.0017063.
[23] D.K. Lahiri, J.I. Nurnberger, A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies, Nucleic Acids Res. 19 (1991) 5444, http://dx.doi.org/10.1093/nar/19.19.5444.
[24] P.-L. Cheng, H.-L. Eng, M.-H. Chou, H.-L. You, T.-M. Lin, Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population, Transl. Res. 150 (2007) 311–318, http://dx.doi.org/10.1016/j.trsl.2007.03.010.
[25] B.P. dos Santos, J.V. Valverde, P. Rohr, O.A. Monticielo, J.C.T. Brenol, R.M. Xavier, TLR7/8/9 polymorphisms and their associations in systemic lupus erythematosus patients from southern Brazil, Lupus 21 (2012) 302–309, http://dx.doi.org/10.1177/0961203311425522.
[26] C.M. Lewis, J. Knight, Introduction to genetic association studies, Cold Spring Harb. Protoc. 7 (2012) 297–306, http://dx.doi.org/10.1101/pdb.top068163.
[27] J.C. Long, R.C. Williams, M. Urbanek, An E-M algorithm and testing strategy for multiple-locus haplotypes, Am. J. Hum. Genet. 56 (1995) 799–810.
[28] N. Soriano-Sarabia, A. Vallejo, R. Ramírez-Lorca, M. del M. Rodríguez, A. Salinas, I. Pulido, Influence of the Toll-like receptor 9 1635A/G polymorphism on the CD4 count, HIV viral load, and clinical progression, J. Acquir. Immune Defic. Syndr. 49 (2008) 128–135, http://dx.doi.org/10.1097/QAI.0b013e318184fb41.
[29] R.D. Mackelprang, A.W. Bigham, C. Celum, G. de Bruyn, K. Beima-Sofie, G. John-Stewart, et al., Toll-like receptor polymorphism associations with HIV-1 outcomes among sub-Saharan Africans, J. Infect. Dis. 209 (2014) 1623–1627, http://dx.doi.org/10.1093/infdis/jit807.
[30] A. Carvalho, N.S. Osório, M. Saraiva, C. Cunha, A.J. Almeida, M. Teixeira-Coelho, et al., The C allele of rs5743836 polymorphism in the human TLR9 promoter links IL-6 and TLR9 up-regulation and confers increased B-cell proliferation, PLoS ONE 6 (2011) e28256, http://dx.doi.org/10.1371/journal.pone.0028256.
[31] W. Jiang, M.M. Lederman, R.J. Mohner, B. Rodriguez, T.M. Nedrich, C.V. Harding, et al., Impaired naive and memory B-cell responsiveness to TLR9 stimulation in human immunodeficiency virus infection, J. Virol. 82 (2008) 7837–7845, http://dx.doi.org/10.1128/JVI.00660-08.
[32] L.N. Clausen, S. Ladelund, N. Weis, J. Bukh, T. Benfield, Genetic variation in toll-like receptors and retinoic acid-inducible gene I and outcome of hepatitis C

Please cite this article in press as: J.M. Valverde-Villegas et al., Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background, Hum. Immunol. (2017), http://dx.doi.org/10.1016/j.humimm.2017.01.001

virus infection: a candidate gene association study, J. Viral Hepat. 21 (2014) 578–584, http://dx.doi.org/10.1111/jvh.12188.

[33] R. Lazarus, W.T. Klimecki, B.A. Raby, D. Vercelli, L.J. Palmer, D.J. Kwiatkowski, et al., Single-nucleotide polymorphisms in the Toll-like receptor 9 gene (TLR9): frequencies, pairwise linkage disequilibrium, and haplotypes in three U.S. ethnic groups and exploratory case–control disease association studies, Genomics 81 (2003) 85–91, http://dx.doi.org/10.1016/S0888-7543(02)00022-8.

[34] G.K. da Silva, P. Vianna, T.D. Veit, S. Crovella, E. Catamo, E.A.A. Cordero, et al., Influence of HLA-G polymorphisms in human immunodeficiency virus infection and hepatitis C virus co-infection in Brazilian and Italian individuals, Infect. Genet. Evol. 21 (2014) 418–423, http://dx.doi.org/10.1016/j.meegid.2013.12.013.

[35] G.K. da Silva, R. Guimarães, V.S. Mattevi, R.K. Lazzaretti, E. Sprinz, R. Kuhmmer, et al., The role of mannose-binding lectin gene polymorphisms in susceptibility to HIV-1 infection in Southern Brazilian patients, AIDS 25 (2011) 411–418, http://dx.doi.org/10.1097/QAD.0b013e328342fef1.

[36] B. Willie, N.B. Hall, C.M. Stein, R.J. Jurevic, A. Weinberg, R.K. Mehlotra, et al., Association of Toll-like receptor polymorphisms with HIV status in North Americans, Genes Immun. 15 (2014) 569–577, http://dx.doi.org/10.1038/gene.2014.54.

[37] D.R. Velez, C. Wejse, M.E. Stryjewski, E. Abbate, W.F. Hulme, J.L. Myers, et al., Variants in toll-like receptors 2 and 9 influence susceptibility to pulmonary tuberculosis in Caucasians, African-Americans, and West Africans, Hum. Genet. 127 (2010) 65–73, http://dx.doi.org/10.1007/s00439-009-0741-7.

[38] K. Zhao, Y. Ishida, T.K. Oleksyk, C.A. Winkler, A.L. Roca, Evidence for selection at HIV host susceptibility genes in a West Central African human population, BMC Evol. Biol. 12 (2012) 237, http://dx.doi.org/10.1186/1471-2148-12-237.

[39] L.B. Barreiro, M. Ben-Ali, H. Quach, G. Laval, E. Patin, J.K. Pickrell, et al., Evolutionary dynamics of human toll-like receptors and their different contributions to host defense, PLoS Genet. 5 (2009), http://dx.doi.org/10.1371/journal.pgen.1000562.

Please cite this article in press as: J.M. Valverde-Villegas et al., Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background, Hum. Immunol. (2017), http://dx.doi.org/10.1016/j.humimm.2017.01.001

ANEXO C:

Artigo de revisão aceito para publicação:

“La era -ómica de la inmunología: La inmunogenética de enfermedades infecciosas, el VIH como modelo”

“Omics era of the immunology: Immunogenetic of infectious diseases, the HIV as model”

MSc. Jacqueline María Valverde-Villegas

Artigo aceito para publicação na Revista Peruana de Divulgación Científica en Genética y Biología Molecular em Janeiro de 2017

Artículo de revisión

La era -ómica de la inmunología: La inmunogenética de enfermedades infecciosas, el VIH como modelo

Omics era of the immunology: Immunogenetic of infectious diseases, the HIV as model

MSc. Jacqueline María Valverde-Villegas[1,2]

[1] Laboratorio de Inmunogenética, Universidad Federal de Rio Grande del Sur (UFRGS), Brasil

[2] Programa de Post-graduación en Genética y Biología Molecular (PPGGBM, UFRGS)

Autor de correspondencia:

MSc. Jacqueline María Valverde-Villegas

Email: jacquelin0203@gmail.com

Laboratorio de Inmunogenética. Instituto de Biociencias. Departamento de Genética. Universidad Federal de Rio Grande del Sur.

Av. Bento Gonçalves – 9500, Campus do Vale. 91501970

Porto Alegre, Rio Grande del Sur-Brasil.

Caja postal 15053

Teléfono: +55 51 3308 6740; Fax: +55 51 3308 7311

Abstract

Case-control genetic association studies on infectious diseases are increasingly challenged by the genetic diversity of both hosts and pathogens. In the case of Human Immunodeficiency Virus (HIV) infection, through molecular genotyping and Genome-Wide Association Studies (GWAS), polymorphisms on genes of the immune system have extensively been associated with susceptibility, resistance, progression and response to treatment. In this sense, the search of clinical phenotypes predisposing or protecting individuals to infection diseases have been a main area of interest in the immunogenetic studies during the last years. These studies have revealed that the ethnic origin of a given individual (and therefore its genetic background as a whole) is a pivotal factor to be considered in the interpretation of the results. In this review, genetic association studies involving candidate genes from innate and adaptive immune response are approached in the HIV context, taking it as an infection model. Advances and limitations of the genetic association studies are discussed and some examples highlighting the potential application of immunogenetics in the medical/clinical field.

Keywords: immunogenetics, infectious diseases, ethnicity, HIV

Resumen

Los estudios de asociación genética caso-control en enfermedades infecciosas son cada vez más desafiantes debido a la diversidad genética del hospedero y la diversidad de los patógenos. A través de las técnicas moleculares de genotipificación de variantes genéticas candidatas y el estudio de asociación del genoma completo (en inglés GWAS, Genome-Wide Association Studies), polimorfismos en genes del sistema inmunológico han sido asociados con la susceptibilidad, resistencia, progresión de la infección y respuesta al tratamiento. Así, los diferentes fenotipos clínicos entre los individuos frente a una infección han sido blanco de estudio en la inmunogenética durante los últimos años. Tales estudios han señalado que la etnia de las poblaciones es un factor importante a ser considerado en la interpretación de los resultados. En este artículo de revisión se describen algunos estudios de asociación genética en genes candidatos del sistema inmune innato y adaptativo tomando como modelo la infección por el Virus de la Inmunodeficiencia Humana (VIH). Avances y limitaciones en los estudios de asociación genética también son discutidos, así también son destacados algunos ejemplos de la aplicación de la inmunogenética en el campo de la medicina.

Palabras claves: Inmunogenética, enfermedades infecciosas, etnia, VIH

Introducción

Los estudios de asociación genética caso-control analizan la influencia de variantes genéticas del genoma humano sobre una determinada enfermedad. La mayoría de estas variaciones analizadas son polimorfismos de base única (en inglés SNPs, *single nucleotide polymorphisms*), además de deleciones e inserciones. Aunque los genes que codifican los antígenos leucocitarios humanos (en inglés HLA, *human leucocyte antigen*) sean los alelos más comúnmente evaluados en estos estudios, un amplio espectro de otros genes también es objeto de análisis. De esta manera, aquellos estudios que analizan la diversidad de genes del sistema inmunológico corresponden al área de la inmunogenética. Dicha área ha contribuido enormemente al entendimiento de la base genética del sistema inmune. Sin embargo este entendimiento se hace aún más complejo cuando se trata de enfermedades multifactoriales, como son las infecciosas, debido a la existencia de miles de genes que actúan en diferentes vías frente a una respuesta específica del sistema inmunológico. Además, estudios de evolución molecular han demostrado que ante esa complejidad se suma la influencia del origen étnico de las poblaciones y la diversidad genética de los patógenos (1).

Con el auge del desarrollo de las técnicas moleculares para la genotipificación de variantes genéticas, los estudios independientes de asociación genética caso-control se incrementaron en el campo de las enfermedades infecciosas. Además, estudios de asociación del genoma completo (en inglés GWAS, *Genome-Wide Association Studies*) han permitido replicar asociaciones ya reportadas por estudios independientes e identificar nuevas variaciones. Estos estudios han puesto en evidencia que variaciones en genes del sistema inmune innato y adaptativo influencian en la susceptibilidad, resistencia, progresión de la infección y respuesta al tratamiento en varias enfermedades, incluyendo la infección por VIH (2–4). Sin embargo, se ha observado una falta de reproducibilidad de las asociaciones genéticas reportadas y esto puede deberse a varios factores como: el origen étnico, el tamaño de la muestra, diversidad en la caracterización de la progresión, momento de la infección, diversidad del patógeno, tratamiento, factores ambientales, entre otros.

Entre las enfermedades infecciosas que se conocen en el mundo, la infección por el Virus de la Inmunodeficiencia Humana (VIH) es la que más ha llamado la atención en el campo de la inmunogenética. Los estudios de asociación genética se incrementaron con el

descubrimiento del papel de la deleción de 32 pares de bases en el gen *CCR5* (*CCR5*Δ*32*) y su asociación con resistencia a la infección por el VIH tipo 1 y una progresión más lenta a la fase del Síndrome de la Inmunodeficiencia Adquirida (SIDA) (5–7). Posteriormente se observaron diferentes respuestas frente a la infección por el VIH-1 y fenotipos extremos entre los individuos VIH-1+ comenzaron a identificarse. Así, los progresores rápidos se caracterizan por llegar a la fase del SIDA en un periodo máximo de 3 años luego de la seroconversión (8,9). El otro grupo extremo es representado por los progresores lentos, los cuales llegan a la fase del SIDA después de convivir con el virus por más de 10 años y estar bien inmunológicamente en ausencia del tratamiento antirretroviral (9,10). Además, hay otro grupo especial, compuesto por individuos que son llamados controladores de elite, que en ausencia del tratamiento antirretroviral mantienen naturalmente niveles estables de linfocitos T CD4+ y controlan la carga viral en bajos niveles y en muchos casos dicha carga viral llega a ser indetectable (10,11). Con la clasificación de estos grupos, los investigadores comenzaron a estudiar los factores genéticos del hospedero que podrían estar influenciando en las diferentes respuestas de estos individuos frente al virus.

En este artículo se describen los genes candidatos que están siendo más comúnmente analizados en las enfermedades infecciosas, tomando como modelo la infección por el VIH. Se aborda el papel del origen étnico como factor importante a ser considerado en estudios de asociación genética. Además, se comenta sobre algunos avances y limitaciones de estos estudios y se destacan algunas aplicaciones que la inmunogenética ha venido contribuyendo en los últimos años en el campo clínico de la medicina.

1. Genes candidatos asociados a enfermedades infecciosas: El VIH como modelo

1.1 Genes del sistema inmune innato

El sistema inmune innato es la primera línea de defensa contra las infecciones. Consta de mecanismos celulares y bioquímicos que ya existen antes de la infección y que están listos para responder rápidamente a las infecciones (13). Los receptores tipo Toll (en inglés TLRs, *toll-like receptor*s) han sido bastante estudiados porque reconocen diversos patrones moleculares asociados a patógenos (en inglés PAMPs, *pathogen-associated molecular patterns*). Cuando los TLRs reconocen estos PAMPs inician una cascada de

señalización intracelular y activan leucocitos, los cuales, vía la activación y la síntesis de factores de transcripción, van a producir generalmente citocinas y quimiocinas pro-inflamatorias, como el NF-kB (factor nuclear potenciador de las cadenas ligeras kappa de las células B activadas) (14). En las infecciones virales se destacan los TLRs 3, 7, 8 y 9, que se expresan en los endosomas de las células del hospedero y reconocen ácidos nucleicos de simple y doble cadena los cuales pueden provenir de los virus que infectan las células (15). Variantes genéticas que puedan comprometer la función de los genes que codifican estos TLRs han sido analizadas en estudios de asociación genética caso-control. Por ejemplo, polimorfismos localizados en los genes *TLR3*, *TLR7*, *TLR8* y *TLR9* han sido asociados con susceptibilidad a infección por VIH, con la progresión a la fase del SIDA y con transmisión vertical del virus que se da de la madre al hijo durante la gestación, el parto o la lactancia (16–20).

En nuestro grupo de investigación hemos encontrado asociaciones genéticas de los *TLRs* endosomales con la susceptibilidad/resistencia a infección por el VIH y la progresión al SIDA. Fue encontrada una asociación genética del polimorfismo rs5743836 del gen *TLR9* con susceptibilidad a infección por el VIH-1 en individuos VIH-1+ euro-descendientes, e interesantemente esta misma variante alélica fue asociada con resistencia a infección en los individuos VIH-1+ afro-descendientes (21). Ya el polimorfismo funcional rs179008 del gen *TLR7* ha sido asociado con progresión rápida a la fase del SIDA en mujeres adultas VIH-1+ (de Medeiros RM et al. 2016, comunicación personal). Otros estudios, también relacionados con la inmunidad innata, se han centrado en polimorfismos de genes que codifican la lectina de unión a manosa (en inglés MBL, *mannose-binding lectin*). Así, da Silva GK et al. (2011) observaron una frecuencia aumentada de los genotipos del *MBL2* (el cual lleva a bajos niveles de la proteína) en individuos VIH+ cuando comparados con individuos controles VIH-, sugiriendo que individuos VIH-1+ con bajos niveles de esta proteína son más susceptibles a infección por VIH (22).

1.2 Genes del sistema inmune adaptativo

El sistema inmune adaptativo se caracteriza por reconocer moléculas distintas (propias y no propias) y su capacidad de recordar y responder con mayor intensidad en exposiciones repetidas al mismo patógeno (13). La región génica más estudiada del

sistema inmune adaptativo, y por ser la más polimorfa entre los mamíferos, corresponde al complejo mayor de histocompatibilidad (en inglés MHC, *Major Histocompatibility Complex*). En los humanos este complejo es denominado HLA (en inglés *HLA*, *human leucocyte antigen*) y los genes de esa región codifican moléculas HLA de clase I, II que participan en la presentación de antígenos propios (del individuo) y no propios (de los patógenos) a los linfocitos T CD8 (clase I) y CD4 (clase II) (13).

Los métodos actuales de secuenciamiento del ADN han permitido definir con más precisión subtipos de alelos *HLA* y sus diferencias entre los individuos, y los métodos moleculares de genotipificación han permitido realizar estudios de asociación genética de alelos específicos de *HLA* con enfermedades infecciosas. A pesar de la alta variabilidad del *HLA-B*, varios estudios de asociación genética han observado una relación de los alelos HLA-B*27 y HLA-B*57 con progresión lenta al SIDA, control de la carga viral, altos niveles de linfocitos T CD4 y ausencia de síntomas en la fase aguda de la infección (23,24). Por otro lado, el HLA-B*35 parece estar asociado con la progresión rápida al SIDA (25,26). Mientras que la molécula HLA-G, considerada como una molécula no clásica de HLA, se destaca por su papel inmunosupresor en las infecciones virales y la diversidad genética del gen *HLA-G* ha sido bastante estudiada en nuestro grupo en diferentes contextos. En la infección por el VIH, se observó la alta frecuencia del polimorfismo de 14pb inserción/deleción del gen *HLA-G* que fue asociada a susceptibilidad a infección por el VIH-1 en individuos seropositivos afro-descendientes (27) .

1.3 Genes que vinculan el sistema inmune innato y adaptativo

Citocinas, quimiocinas y sus receptores

La comunicación entre el sistema inmune innato y adaptativo es realizada por una interacción compleja entre las células del sistema inmune y proteínas plasmáticas solubles como las citocinas y quimiocinas (28). Frente a una infección o señal de tejido dañado, la primera respuesta del sistema inmunológico es la inducción de una fase aguda, que viene a ser la acumulación de leucocitos del sistema inmune innato (por ejemplo, macrófagos, neutrófilos, células dendríticas, células NK (*natural killers*)) los cuales producen citocinas y quimiocinas en el sitio de infección desencadenando un proceso inflamatorio (29). En el

caso que la infección no haya sido eliminada se da paso a la fase crónica de la infección, la cual involucra el reclutamiento, migración y activación de leucocitos del sistema inmune adaptativo (por ejemplo, monocitos y linfocitos T CD4, CD8) llevado acabo por citocinas y quimiocinas (13). Las citocinas cuando ligadas a sus receptores estimulan la proliferación y diferenciación de las células T y activan otras subpoblaciones de células (13). Ya las quimiocinas, cuando están ligadas con sus receptores, son las principales responsables del reclutamiento y migración de estas células al local de infección donde se lleva a cabo la activación de linfocitos y monocitos los cuales, a su vez, también van a producir citocinas y quimiocinas (30).

Varios estudios han observado que polimorfismos genéticos de citocinas y quimiocinas están asociados con la susceptibilidad, progresión de la infección y respuesta al tratamiento en diversas infecciones virales. En nuestro grupo hemos analizado 8 variantes en genes de citocinas (*IL-2*, *IL-4*, *IL-6*, *IL-10*, *IL-17*, *Interferón-α*) y 15 variantes en genes de quimiocinas y sus receptores (*CCR3*, *CCR4*, *CCR5*, *CCR6*, *CCR8*, *CXCR3*, *CXCR6*, *CCL20*, *CCL22 y IP-10*), en el contexto de infección por el VIH-1. Los análisis de interacción genética evidenciaron que los polimorfismos rs1800872 de *IL-10* y rs8193036 de *IL-17A* fueron asociados con susceptibilidad a infección por el VIH-1 en individuos brasileros euro-descendientes (de Medeiros RM et al. 2016, comunicación personal). Así también, los polimorfismos rs3091250 de *CCR3*, rs5606198 de *IP-10* y rs4359426 de *CCL22* fueron asociados con susceptibilidad a infección por el VIH-1 en una cohorte de individuos VIH-1+ del Sur del Brasil. Y en ese mismo trabajo, los polimorfismos rs13034664 de *CCL20* y rs4359426 de *CCL22* fueron asociados con progresión rápida al SIDA (Valverde-Villegas JM et al. 2016, artículo enviado a revista internacional para publicación).

El papel inmunoregulador de citocinas y quimiocinas se da solo cuando éstas están ligadas a sus respectivos receptores, los cuales son expresados en la superficie de las células. Los receptores de quimiocinas son usados para caracterizar diferentes fenotipos de linfocitos T CD4+ y además algunos de ellos son utilizados por los virus para entrar a la célula e infectarlas. El receptor CCR5 es utilizado preferencialmente por el VIH-1 como co-receptor para entrar a las células blanco. Entre las variaciones genéticas de estos receptores, la deleción de 32 pares de bases en el gen *CCR5* (*CCR5Δ32*) ha sido

ampliamente estudiada. Esta mutación, que hace no funcional al receptor CCR5, ha sido asociada con resistencia a infección por el VIH y con progresión lenta al desarrollo de la fase del SIDA en poblaciones europeas (5–7). Desde ese descubrimiento muchos investigadores comenzaron a analizar la frecuencia de esa mutación en diferentes poblaciones humanas con distinto origen étnico y su asociación en diferentes contextos de infección viral. A diferencia del papel protector de esta mutación en la infección por el VIH-1, un estudio de meta-análisis observó que individuos de los EEUU portadores homocigotos del alelo *CCR5del32* tienen un alto riesgo a desarrollar síntomas por la infección del virus del Nilo occidental (West Nile Virus, WNV) (31). Con ello, observamos que el papel de este polimorfismo, sea de protección o susceptibilidad a una infección, puede depender del patógeno, origen étnico de la población, del momento de la infección o factores ambientales, entre otros.

2. Influencia del origen étnico en los estudios de asociación inmunogenética

Las diferentes respuestas de los individuos frente a un mismo patógeno se deben en parte a la influencia de la diversidad genética entre las poblaciones humanas. Estudios de inmunogenética realizados en Brasil, y específicamente de nuestro grupo, demuestran la influencia del factor étnico, puesto que este país es altamente mixogenizado. Los análisis son estratificados de acuerdo a la ascendencia étnica, generalmente en euro-descendientes y afro-descendientes (22,32,33) o caso contrario los cálculos estadísticos son corregidos por la variable etnia en la regresión logística. Más interesante aún, recientemente algunos estudios han observado que esa diversidad genética puede ser diferente inclusive dentro de un mismo grupo étnico (34). Existen muchos estudios de asociación genética realizados en cohortes europeas, seguido de las asiáticas y en los últimos años se han incrementado los estudios en poblaciones de origen africana, pero son pocos los estudios de asociación genética en cohortes de origen indígena y mixogenizadas.

Los diferentes estudios que han sido referidos a lo largo de este manuscrito evidenciaron asociaciones que son específicas al origen étnico. Como se ha citado, Valverde-Villegas JM et al. (2016) observaron que el efecto del polimorfismo rs5743836 del *TLR9* dependía del origen étnico (21). Este SNP fue asociado con protección a

infección en individuos VIH-1+ de origen afro-descendiente, mientras que el mismo fue asociado a susceptibilidad a infección en los euro-descendientes VIH-1+. Comúnmente observamos que las frecuencias alélicas y genotípicas de los genes se distribuyen de una forma diferente entre las poblaciones étnicas, y polimorfismos genéticos asociados a susceptibilidad, resistencia, progresión o característica clínica de una enfermedad tendrán un papel diferente (para bien o para mal) de acuerdo a su distribución en una población específica. A pesar que cada vez más parece ser complicado clasificar a los individuos de acuerdo a su origen étnico utilizando los marcadores informativos de ancestría (en inglés, AIMs, *ancestry-informative markers*), y esto es un tema que no se aborda aquí, los esfuerzos deben continuar para refinar los paneles de los AIMs, así, como otras estrategias deben ser utilizadas para conseguir realizar esta clasificación e interpretar los resultados de asociación genética de una forma más adecuada.

3. Avances y limitaciones de los estudios de asociación inmunogenética

Con la disponibilidad de la secuencia completa del genoma humano el International SNP Consortium y el HapMap Project fueron mapeando variaciones genéticas comunes en distintas poblaciones con ancestría europea, africana y asiática usando como referencia la secuencia completa original (35,36). Esas variaciones genéticas son almacenadas en bases de datos especializadas como HapMap (35), Immuno Polymorphism Database (37), International Immunogenetics Information System (38), Ensembl (39), entre otras, las cuales son de libre disponibilidad. Con la aplicación inmediata de estas bases de datos comenzaron a surgir estudios de asociación genética con el objetivo de identificar variantes genéticas asociadas a un fenotipo, generalmente un rasgo clínico.

Los estudios independientes caso-control de asociación de genes candidatos analizan cohortes de individuos representativos de un solo lugar y como consecuencia un tamaño de muestra relativamente pequeño. Este tipo de estudio generalmente consigue analizar pocas variantes genéticas. Por otro lado, los GWAs barren todo el genoma y analizan entre miles o millones de variaciones genéticas, entre ellos las ya reportadas por los estudios independientes. Muchas veces un GWAS analiza cohortes de individuos de diferentes países llegando a obtener cientos o miles de individuos para el estudio. Estas cohortes generalmente pertenecen a consorcios, producto de las colaboraciones entre diferentes centros de investigación.

En la actualidad existen más estudios independientes de asociación genética que aquellos realizados por consorcios/GWAS. Al mismo tiempo que se incrementaron los estudios independientes también se observó una falta de reproducibilidad entre los estudios de replicación para verificar la asociación genética. Este estudio de replicación implica repetir el estudio en otra cohorte de individuos para confirmar la asociación genética del (o los) polimorfismo (s) identificado (s) en el estudio original. Sin embargo, para que este estudio de replicación sea interpretado correctamente, la muestra a ser analizada debe tener características similares a la cohorte original (40). La reproducibilidad de los estudios por GWAS también ha sido incongruente en el contexto de las enfermedades infecciosas, en comparación con otras enfermedades genéticas humanas, en donde los resultados han sido más consistentes (41). Se ha sugerido que esa falta de reproducibilidad de los estudios independientes de asociación genética y de GWAS en las enfermedades infecciosas, puede ser debida al tamaño de la muestra, origen étnico de las poblaciones humanas, diversidad genética de los patógenos, y a los diferentes criterios clínicos utilizados para definir el "caso"; por ejemplo, la edad del diagnóstico de la enfermedad o de la progresión, diferencias en la gravedad, entre otros. A ello se suma, el uso de un adecuado grupo control, el cual es altamente difícil de obtener debido a que la información de individuos expuestos al patógeno pero no infectados, es limitada (41,42).

Frente a ello, para una mejor comprensión del papel real de estas variantes genéticas en las enfermedades infecciosas, los investigadores se han visto en la necesidad de: a) aumentar el número de la muestra, b) incrementar la robustez de los análisis estadísticos, c) estandarizar el diagnóstico de la enfermedad, gravedad o fase clínica, d) montar cohortes de grupos expuestos infectados y expuestos no infectados, d) realizar estudios meta-análisis y multicéntricos. Los estudios de meta-análisis, son un método estadístico que combina los resultados de estudios independientes de asociación genética sobre un mismo tópico (aumentando, por lo tanto, el tamaño de la muestra), explora las fuentes de heterogeneidad e identifica subgrupos asociados con el factor de interés (43). Esta herramienta está demostrando ser eficaz para una mayor comprensión del papel real de los polimorfismos genéticos asociados a enfermedades complejas, como son las infecciosas.

Es importante señalar que a veces es inevitable analizar una cohorte de pacientes con un número pequeño, pues existen grupos raros de pacientes que deben ser analizados. Como ya se mencionó, por ejemplo, los controladores de elite, que son individuos que controlan naturalmente la carga del VIH y se mantienen estables inmunológicamente en ausencia de la terapia, representan apenas el 1% de la población de individuos VIH-1+ (12). A pesar de los pequeños grupos que se consiga analizar, los estudios independientes de asociación genética son necesarios para la identificación de polimorfismos con efecto pequeño o modesto, los cuales difícilmente se identificarían por los estudios de GWAS debido a la robustez de su estadística (en donde variaciones genéticas con un efecto grande generalmente son detectados por este método) (44). Con la ayuda de estudios de meta-análisis es posible corroborar el papel de estas variantes con efecto pequeño o modesto, o se identificarían asociaciones no observadas previamente. Además, nuevos métodos estadísticos también están siendo utilizados para la identificación de efectos epistáticos (interacción de variantes genéticas en donde el efecto de una variante alélica depende de la presencia o ausencia de otra variante alélica) (45) y las herramientas bioinformáticas cada vez más están siendo utilizadas para inferir el efecto de las variantes genéticas sobre el papel funcional de las proteínas.

4. Aplicaciones de la inmunogenética en el tratamiento de enfermedades infecciosas

Hay algunos ejemplos que nos demuestran la importancia y la necesidad de generar conocimientos sobre la diversidad genética del hospedero y su relación con la diversidad genética de los patógenos en las diferentes poblaciones humanas. Tales conocimientos han sido direccionados a mejorar el tratamiento de las enfermedades infecciosas. Por ejemplo, el uso del antagonista Maraviroc en el tratamiento a los individuos VIH-1+. La función de este fármaco es bloquear el receptor CCR5 (46). Como ya se dijo, este receptor es preferencialmente usado por el virus como co-receptor para entrar e infectar a la célula. Y de no haberse conocido el papel de la deleción deltaΔ32 del *CCR5* y su asociación con resistencia a la infección del VIH-1, no estaríamos hablando de Maraviroc. Sin embargo, es importante señalar que el papel natural del receptor CCR5, cuando interactúa con sus ligantes (RANTES, MIP1α y β), es de regular la quimiotaxia de leucocitos específicos ante una determinada respuesta del sistema inmune. Así, el bloqueo del CCR5, además de impedir la entrada del VIH-1 a la célula, puede perjudicar una adecuada respuesta inmune

frente a otros patógenos. Con ello también observamos que es importante realizar los test de genotipificación de esta mutación a los individuos que van a tratarse con Maraviroc, pues estos podrían ser heterocigotos del alelo deltaΔ32 y por lo tanto se tendría que evaluar el uso de este antagonista como parte del tratamiento. Por otro lado, se sabe que el VIH de tipo 2 (VIH-2) usa el CXCR4 (47) u otros receptores de quimiocinas alternativos como co-receptor de entrada para infectar a las células (48) , así el tratamiento con Maraviroc en individuos infectados con el VIH-2 tendría que evaluarse. Interesantemente, diferentes estudios han observado que los individuos infectados por el VIH-2 progresan de una forma lenta a SIDA o simplemente no llegan a la fase SIDA (49).

Entre los medicamentos utilizados en el tratamiento a individuos VIH-1+, también se incluye el Abacavir (ABC). Este es un análogo de nucleósido, inhibidor de la transcriptasa inversa, una enzima importante para la replicación del virus en la célula. Se ha observado que esta droga tiene un alto efecto adverso generando una reacción de hipersensibilidad en el 5-8% de individuos VIH-1+ euro-descendientes que lo consume. Estudios de inmunogenética han observado que la hipersensibilidad por Abacavir está fuertemente asociada a la susceptibilidad genética dada por la presencia del alelo HLA-B*5701 en las diferentes poblaciones (50–52). Con ello, observamos la importancia de conocer la frecuencia de este alelo en las poblaciones y/o el *screening* genético individual para reducir la incidencia de hipersensibilidad en los pacientes que se tratan con Abacavir.

Finalmente, otro ejemplo a destacar es uno generado por el GWAS en la respuesta al tratamiento a la infección por el virus de la hepatitis C (VHC). La variación genética rs12979860 en el locus *IL28B* (codificante del interferon-λ-3, IFN-λ-3) ha sido fuertemente asociada con una sostenida respuesta virológica al tratamiento con interferón alfa pegilado (PEG-IFN-α) y ribavirina en pacientes con infección crónica por VHC. Los individuos portadores de este polimorfismo tienen de 2 a 3 veces más posibilidades de erradicar el virus (53). Además, se observó que el efecto de este polimorfismo parece ser aún más fuerte en pacientes infectados por el subtipo G1 del VHC, pero que tal efecto depende de la ancestría (54), destacando así la importancia de la acción conjunta de factores virales y del hospedero. Hoy en día la genotipificación de esta variante en los pacientes VHC+ es una práctica común en el manejo del tratamiento contra la infección por el VHC.

Conclusiones

La inmunogenética ha puesto en evidencia que la base genética de las enfermedades infecciosas es muy compleja y que principalmente se debe a la alta diversidad de multiloci del sistema inmune innato y adaptativo. Esta alta diversidad del hospedero es influenciada por el origen étnico y la diversidad de los patógenos, y los estudios de asociación genética se ven afectados ante esta complejidad. Sin embargo, para lidiar con ello, avances como estudios de meta-análisis, GWAS, estudios funcionales, análisis de interacción génica y estudios bioinformáticos se están realizando para dilucidar el papel de las variantes genéticas sobre las enfermedades infecciosas en las diferentes poblaciones.

Agradecimientos

Un agradecimiento especial al Dr. José Artur Bogo Chies por la valiosa revisión crítica y las sugerencias realizadas en esta revisión. Así también, otro agradecimiento especial a Mariela Luján Escribano y William Valverde Espinoza por la lectura y las revisiones del español.

Referencias

1. Barreiro LB, Ben-Ali M, Quach H, Laval G, Patin E, Pickrell JK, et al. Evolutionary dynamics of human toll-like receptors and their different contributions to host defense. PLoS Genet. 2009;5(7).
2. Fellay J, Shianna K V, Ge D, Colombo S, Ledergerber B, Weale M, et al. A Whole-Genome Association Study of Major Determinants for Host Control of HIV-1. Science (80-). 2007 Aug 17;317(5840):944–7.
3. Pelak K, Goldstein DB, Walley NM, Fellay J, Ge D, Shianna K V., et al. Host determinants of HIV-1 control in African Americans. J Infect Dis. 2010 Apr 15;201(8):1141–9.
4. van Manen D, van ’t Wout AB, Schuitemaker H. Genome-wide association studies on HIV susceptibility, pathogenesis and pharmacogenomics. Retrovirology. 2012 Aug 24;9(1):70.

5. Romiti ML, Colognesi C, Cancrini C, Mas A, Berrino M, Salvatori F, et al. Prognostic value of a CCR5 defective allele in pediatric HIV-1 infection. Mol Med. 2000 Jan;6(1):28–36.

6. Rappaport J, Cho Y-Y, Hendel H, Schwartz EJ, Schachter F, Zagury J-F. 32 bp CCR-5 gene deletion and resistance to fast progression in HIV-1 infected heterozygotes. Lancet. 1997 Mar;349(9056):922–3.

7. Hendel H, Hénon N, Lebuanec H, Lachgar A, Poncelet H, Caillat-Zucman S, et al. Distinctive effects of CCR5, CCR2, and SDF1 genetic polymorphisms in AIDS progression. J Acquir Immune Defic Syndr Hum Retrovirol. 1998 Dec 1;19(4):381–6.

8. Olson AD, Guiguet M, Zangerle R, Gill J, Perez-Hoyos S, Lodi S, et al. Evaluation of rapid progressors in HIV infection as an extreme phenotype. J Acquir Immune Defic Syndr. 2014 Sep 1;67(1):15–21.

9. Langford SE, Ananworanich J, Cooper DA. Predictors of disease progression in HIV infection: a review. AIDS Res Ther. 2007 May 14;4:11.

10. Casado C, Colombo S, Rauch A, Martínez R, Günthard HF, Garcia S, et al. Host and viral genetic correlates of clinical definitions of HIV-1 disease progression. Vartanian J-P, editor. PLoS One. 2010 Jun 11;5(6):e11079.

11. Deeks SG, Walker BD. Human Immunodeficiency Virus Controllers: Mechanisms of Durable Virus Control in the Absence of Antiretroviral Therapy. Immunity. 2007 Sep;27(3):406–16.

12. Hubert JB, Burgard M, Dussaix E, Tamalet C, Deveau C, Le Chenadec J, et al. Natural history of serum HIV-1 RNA levels in 330 patients with a known date of infection. The SEROCO Study Group. AIDS. 2000 Jan 28;14(2):123–31.

13. Abbas AK, Litchman AH PS. Imunologia Celular e Molecular. Saunders. Elsevier Editora Ltda; 2012.

14. Kawai T, Akira S. TLR signaling. Cell Death Differ. 2006 May 20;13(5):816–25.

15. Takeuchi O, Akira S. Innate immunity to virus infection. Immunol Rev. 2009 Jan;227(1):75–86.

16. Oh D-Y, Baumann K, Hamouda O, Eckert JK, Neumann K, Kücherer C, et al. A

frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression. AIDS. 2009 Jan 28;23(3):297–307.

17. Oh D-Y, Taube S, Hamouda O, Kücherer C, Poggensee G, Jessen H, et al. A functional toll-like receptor 8 variant is associated with HIV disease restriction. J Infect Dis. 2008 Sep 1;198(5):701–9.

18. Ricci E, Malacrida S, Zanchetta M, Mosconi I, Montagna M, Giaquinto C, et al. Toll-like receptor 9 polymorphisms influence mother-to-child transmission of human immunodeficiency virus type 1. J Transl Med. 2010 May 25;8(1):49.

19. Soriano-Sarabia N, Vallejo A, Ramírez-Lorca R, Rodríguez M del M, Salinas A, Pulido I, et al. Influence of the Toll-like receptor 9 1635A/G polymorphism on the CD4 count, HIV viral load, and clinical progression. J Acquir Immune Defic Syndr. 2008 Oct 1;49(2):128–35.

20. Sironi M, Biasin M, Cagliani R, Forni D, De Luca M, Saulle I, et al. A common polymorphism in TLR3 confers natural resistance to HIV-1 infection. J Immunol. 2012 Jan 15;188(2):818–23.

21. Valverde-Villegas JM, dos Santos BP, de Medeiros RM, Mattevi VS, Lazzaretti RK, Sprinz E, et al. Endosomal toll-like receptor gene polymorphisms and susceptibility to HIV and HCV co-infection – Differential influence in individuals with distinct ethnic background. Hum Immunol. 2017 Jan;6–11.

22. da Silva GK, Guimarães R, Mattevi VS, Lazzaretti RK, Sprinz E, Kuhmmer R, et al. The role of mannose-binding lectin gene polymorphisms in susceptibility to HIV-1 infection in Southern Brazilian patients. AIDS. 2011 Feb;25(4):411–8.

23. Magierowska M, Theodorou I, Debré P, Sanson F, Autran B, Rivière Y, et al. Combined genotypes of CCR5, CCR2, SDF1, and HLA genes can predict the long-term nonprogressor status in human immunodeficiency virus-1-infected individuals. Blood. 1999 Feb 1;93(3):936–41.

24. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc Natl Acad Sci. 2000 Mar 14;97(6):2709–14.

471 25. Carrington M. HLA and HIV-1: Heterozygote Advantage and B*35-Cw*04
472 Disadvantage. Science (80-). 1999 Mar 12;283(5408):1748–52.

473 26. Gao X, Nelson GW, Karacki P, Martin MP, Phair J, Kaslow R, et al. Effect of a
474 single amino acid change in MHC class I molecules on the rate of progression to
475 AIDS. N Engl J Med. 2001;344(22):1668–75.

476 27. da Silva GK, Vianna P, Veit TD, Crovella S, Catamo E, Cordero EAA, et al.
477 Influence of HLA-G polymorphisms in human immunodeficiency virus infection
478 and hepatitis C virus co-infection in Brazilian and Italian individuals. Infect Genet
479 Evol. Elsevier B.V.; 2014 Jan;21(June 2012):418–23.

480 28. Altfeld M, Gale Jr M. Innate immunity against HIV-1 infection. Nat Immunol. 2015
481 May 19;16(6):554–62.

482 29. McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF. The immune
483 response during acute HIV-1 infection: clues for vaccine development. Nat Rev
484 Immunol. Nature Publishing Group; 2010 Jan 11;10(1):11–23.

485 30. Luther S a, Cyster JG. Chemokines as regulators of T cell differentiation. Nat
486 Immunol. 2001 Feb 1;2(2):102–7.

487 31. Lim JK, Louie CY, Glaser C, Jean C, Johnson B, Johnson H, et al. Genetic
488 deficiency of chemokine receptor CCR5 is a strong risk factor for symptomatic
489 West Nile virus infection: a meta-analysis of 4 cohorts in the US epidemic. J Infect
490 Dis. 2008 Jan 15;197(2):262–5.

491 32. dos Santos B, Valverde J, Rohr P, Monticielo O, Brenol J, Xavier R, et al. TLR7/8/9
492 polymorphisms and their associations in systemic lupus erythematosus patients from
493 Southern Brazil. Lupus. 2012 Mar 1;21(3):302–9.

494 33. Willie B, Hall NB, Stein CM, Jurevic RJ, Weinberg A, Mehlotra RK, et al.
495 Association of Toll-like receptor polymorphisms with HIV status in North
496 Americans. Genes Immun. Nature Publishing Group; 2014 Dec 25;15(8):569–77.

497 34. Martínez-Robles E, Yebra-Bango M, Mellor-Pita S, Tutor-Ureta P, Vargas JA,
498 Citores MJ. Genotypic distribution of common variants of endosomal toll like
499 receptors in healthy Spanish women. A comparative study with other populations.
500 Gene. Elsevier B.V.; 2016 Mar;578(1):32–7.

35. - -, Gibbs RA, Belmont JW, Hardenbol P, Willis TD, Yu F, et al. The International HapMap Project. Nature. 2003 Dec 18;426(6968):789–96.

36. Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature. 2001 Feb 15;409(6822):928–33.

37. Robinson J, Halliwell JA, McWilliam H, Lopez R, Marsh SGE. IPD--the Immuno Polymorphism Database. Nucleic Acids Res. 2013 Jan 1;41(Database issue):D1234-40.

38. Lefranc M-P, Giudicelli V, Duroux P, Jabado-Michaloud J, Folch G, Aouinti S, et al. IMGT®, the international ImMunoGeneTics information system® 25 years on. Nucleic Acids Res. 2015 Jan 28;43(Database issue):D413-22.

39. Flicek P, Amode MR, Barrell D, Beal K, Billis K, Brent S, et al. Ensembl 2014. Nucleic Acids Res. 2014 Jan;42(D1):D749–55.

40. NCI-NHGRI Working Group on Replication in Association Studies, Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, et al. Replicating genotype-phenotype associations. Nature. 2007 Jun 7;447(7145):655–60.

41. Ko DC, Urban TJ. Understanding Human Variation in Infectious Disease Susceptibility through Clinical and Cellular GWAS. Heitman J, editor. PLoS Pathog. 2013 Aug 1;9(8):e1003424.

42. Lewis CM, Knight J. Introduction to genetic association studies. Cold Spring Harb Protoc. 2012 Mar 1;2012(3):297–306.

43. Lee YH. Meta-analysis of genetic association studies. Ann Lab Med. 2015 May;35(3):283–7.

44. Stringer S, Wray NR, Kahn RS, Derks EM. Underestimated effect sizes in GWAS: fundamental limitations of single SNP analysis for dichotomous phenotypes. Timpson NJ, editor. PLoS One. 2011 Nov 28;6(11):e27964.

45. Niel C, Sinoquet C, Dina C, Rocheleau G. A survey about methods dedicated to epistasis detection. Front Genet. 2015 Sep 10;6(September).

46. Lieberman-Blum SS, Fung HB, Bandres JC. Maraviroc: A CCR5-receptor antagonist for the treatment of HIV-1 infection. Clin Ther. 2008 Jul;30(7):1228–50.

47. Endres MJ, Clapham PR, Marsh M, Ahuja M, Turner JD, McKnight A, et al. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. Cell. 1996 Nov 15;87(4):745–56.

48. Azevedo-Pereira JM, Santos-Costa Q, Mansinho K, Moniz-Pereira J. Identification and characterization of HIV-2 strains obtained from asymptomatic patients that do not use CCR5 or CXCR4 coreceptors. Virology. 2003 Aug 15;313(1):136–46.

49. Berry N, Ariyoshi K, Jaffar S, Sabally S, Corrah T, Tedder R, et al. Low peripheral blood viral HIV-2 RNA in individuals with high CD4 percentage differentiates HIV-2 from HIV-1 infection. J Hum Virol. 1(7):457–68.

50. Rauch A, Nolan D, Thurnheer C, Fux CA, Cavassini M, Chave J-P, et al. Refining abacavir hypersensitivity diagnoses using a structured clinical assessment and genetic testing in the Swiss HIV Cohort Study. Antivir Ther. 2008;13(8):1019–28.

51. Baniasadi S, Shokouhi SB, Tabarsi P, Alehashem M, Khalili H, Fahimi F, et al. Prevalence of HLA-B*5701 and Its Relationship with Abacavir Hypersensitivity Reaction in Iranian HIV-Infected Patients. Tanaffos. 2016;15(1):48–52.

52. Zucman D, Truchis P De, Majerholc C, Stegman S, Caillat-Zucman S. Prospective screening for human leukocyte antigen-B*5701 avoids abacavir hypersensitivity reaction in the ethnically mixed French HIV population. J Acquir Immune Defic Syndr. 2007 May 1;45(1):1–3.

53. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna K V, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. Nature Publishing Group; 2009 Sep 17;461(7262):399–401.

54. Shi K-Q, Liu W-Y, Lin X-F, Fan Y-C, Chen Y-P, Zheng M-H. Interleukin-28B polymorphisms on the SVR in the treatment of naïve chronic hepatitis C with pegylated interferon-α plus ribavirin: a meta-analysis. Gene. Elsevier B.V.; 2012 Oct 1;507(1):27–35.

ANEXO D:

HOSPITAL N. S. DA CONCEIÇÃO S.A.
Av. Francisco Trein, 596
CEP 91350-200 - Porto Alegre - RS
Fone: 3357.2000
CNPJ: 92.767.116/0001-20

HOSPITAL DA CRIANÇA CONCEIÇÃO
(Unidade Pediátrica do Hospital Nossa Senhora da Conceição S.A.)

HOSPITAL CRISTO REDENTOR S.A.
Rua Domingos Rubbo, 20
CEP 91040-000 - Porto Alegre - RS
Fone: 3357.4100
CNPJ: 92.767.126/0001-76

HOSPITAL FÊMINA S.A.
Rua Mostardeiro, 17
CEP 91430-001 - Porto Alegre - RS
Fone: 3314.5200
CNPJ: 92.693.134/0001-63

Vinculados ao Ministério da Saúde - Decreto nº 99.244/90

COMITÊ DE ÉTICA EM PESQUISA - CEP/GHC

O Comitê de Ética em Pesquisa do Grupo Hospitalar Conceição (CEP/GHC), que é reconhecido pela Comissão Nacional de Ética em Pesquisa (CONEP)/MS desde 31/10/1997, pelo Office For Human Research Protections (OHRP)/USDHHS, como Institutional Review Board (IRB0001105) e pelo FWA - Federalwide Assurance (FWA 00000378), em 30 de novembro de 2010, reavaliou o seguinte projeto de pesquisa:

Projeto: 10-213 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
JOSÉ ARTUR BOGO CHIES
LUIZ FERNANDO JOBIM
MARIA CRISTINA COTTA MATTE
RÚBIA MARÍLIA MEDEIROS
DENNIS MALETICH JUNQUEIRA
LEONARDO AUGUSTO LUVISON ARAÚJO
CYNARA CARVALHO NUNES
MARINEIDE GONÇALVES DE MELO
BRENO RIEGEL SANTOS
MARIA LÚCIA ROSA ROSSETTI
SABRINA ESTEVES DE MATOS ALMEIDA

Título: Avaliação de polimorfismos em genes envolvidos na resposta imunológica de pacientes infectados com HIV-1.

Documentação: Aprovados
Aspectos Metodológicos: Aprovados
Aspectos Éticos: Aprovados

Parecer final: Este projeto, por estar de acordo com as Diretrizes e Normas Internacionais e Nacionais especialmente as Resoluções 196/96 e complementares do Conselho Nacional de Saúde, obteve o parecer de APROVADO.

Considerações Finais: Toda e qualquer alteração do projeto, deverá ser comunicada imediatamente ao CEP/GHC. Lembramos do compromisso de encaminhar dentro dos prazos estipulados, o(s) relatório(s) parcial(ais) e/ou final ao Comitê de Ética em Pesquisa do Grupo Hospitalar Conceição e ao Centro de Resultado onde a pesquisa for desenvolvida.

Porto Alegre, 30 de novembro de 2010.

Daniel Demétrio Faustino da Silva
Coordenador-geral do CEP/GHC

COMITÊ DE ÉTICA EM PESQUISA DA FEPPS
Av Ipiranga 5400, Prédio Administrativo.
CEP 90.610-000 – PORTO ALEGRE/RS
e-mail: cep_fepps@fepps.rs.gov.br

PARECER DO COMITÊ DE ÉTICA EM PESQUISA

CEP/FEPPS-RS Nº: 13/2010
PROCESSO Nº: 002964-20.69/ 10-5
PROJETO PADCT Nº: 12/2010
Deliberação conforme reunião realizada em: (27/09/2009)

Título do Projeto:

"AVALIAÇÃO DE POLIMORFISMOS EM GENES ENVOLVIDOS NA RESPOSTA IMUNOLÓGICA DE PACIENTES INFECTADOS COM HIV-1".

Nome do pesquisador principal:

Sabrina Esteves de Matos Almeida

	PARECER
x	APROVADO
	APROVADO COM RECOMENDAÇÕES
	NECESSITA DE ADEQUAÇÕES
	NÃO APROVADO

PARECER DO COMITÊ:

O Comitê de Ética em Pesquisa da FEPPS/RS em reunião do dia 27/09/2010, Ata nº 13/2010, que o presente projeto está adequado ética e metodologicamente de acordo com as Diretrizes e Normas Regulamentadoras de Pesquisa envolvendo Seres Humanos (Res.196/96/CNS e suas complementares) e portanto, **aprovado** por este Comitê.

Reiteramos que relatórios semestrais do projeto em andamento, relatório final e cópia do trabalho de conclusão e/ou publicação deverão ser entregues ao Comitê de Ética em Pesquisa da FEPPS.

Porto Alegre, 27 de setembro de 2010.

Maria da Graça Boucinha Marques
Coordenadora CEP-FEPPS/RS

ANEXO E:

COMISSÃO NACIONAL DE
ÉTICA EM PESQUISA

PARECER CONSUBSTANCIADO DA CONEP

DADOS DA EMENDA

Título da Pesquisa: Abordagem imunogenética de receptores de quimiocinas e sua correlação com a diversidade do gene Env do HIV-1: Fatores que influenciam a progressão para AIDS

Pesquisador: José Artur Bogo Chies

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);
A critério do CEP

Versão: 6

CAAE: 30491714.0.0000.5347

Instituição Proponente: UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.592.399

Apresentação do Projeto:

INTRODUÇÃO

A caracterização do fenótipo de células T infectadas pelo HIV-1surgiu com alguns estudos que demonstram que o vírus é muito seletivo em escolher seu alvo celular de infecção. Já foi observado que diferentes subpopulações das células T CD4+ são caracterizadas pela combinação da expressão de receptores de quimiocinas. Por exemplo, a literatura observou que o receptor de quimiocina CCR6 em combinação com os receptores CCR4 e CXCR3 caracterizaram dois subtipos diferentes de células T que produzem IL-17. Nesse estudo, as células T CD4+ de memória específicas para Candida albicans estiveram presentes principalmente no conjunto de células T CD4+ com o fenótipo CCR4+CCR6+ as quais produziram IL-17 e expressaram o fator de transcrição ROR (perfil Th17). Já as células T de memória específicas para Mycobacterium tuberculosis estiveram presentes principalmente no conjunto de células T CD4+ com o fenótipo CXCR3+CCR6+ e produziram IL-17 e IFN- e expressaram os fatores de transcrição ROR e T-bet (perfil Th1Th17). Em relação ao HIV-1, mais recentemente foi observado que as células T CD4+ com o perfil de expressão CCR4+CCR6+ (Th17) e CXCR3+CCR6+ (Th1Th17) foram altamente

Endereço: SEPN 510 NORTE, BLOCO A 3° ANDAR, Edifício Ex-INAN - Unidade II - Ministério da Saúde
Bairro: Asa Norte **CEP:** 70.750-521
UF: DF **Município:** BRASILIA
Telefone: (61)3315-5878 **E-mail:** conep@saude.gov.br

Outros	controles.docx	19:04:36		Aceito
Parecer Anterior	Pareceres_controles 2012.pdf	25/05/2014 18:56:07		Aceito
Parecer Anterior	Pareceres_HIV 2010.pdf	25/05/2014 18:55:33		Aceito
Outros	Carta Dra. Sabrina CDCT-FEPPS 2014 assinada.pdf	25/05/2014 18:54:34		Aceito
Outros	Parecer COMPESQ.docx	17/04/2014 14:19:24		Aceito
Folha de Rosto	Folha de rosto assinada.pdf	17/04/2014 13:39:15		Aceito
Outros	Carta Dr. Breno GHC 2013 assinada.pdf	22/12/2013 19:55:51		Aceito

Situação do Parecer:
Aprovado

BRASILIA, 15 de Junho de 2016

Assinado por:
Gabriela Marodin
(Coordenador)

ANEXO F:

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - PACIENTES

Projeto de Pesquisa: **Abordagem imunogenética de receptores de quimiocinas e sua correlação com a diversidade do gene *Env* do HIV-1: Fatores que influenciam a progressão para AIDS**

Pesquisadores: Sabrina Esteves de Matos Almeida[1], José Artur Bogo Chies[2], Jacqueline Valverde Villegas[1,2], Rúbia Marília de Medeiros[1,2], Dennis Maletich Junqueira[1],Tiago Gräf [1], Karine Andrade[1], Breno Riegel Santos[3], Marineide Gonçalves de Melo[3]

1. Centro de Desenvolvimento Científico e Tecnológico – FEPPS Tel: (51) 3352-0336
2. Laboratório de Imunogenética – UFRGS Tel: (51) 3308-6737
3. Serviço de Infectologia – Hospital Nossa Senhora da Conceição Tel: (51) 3357-2126

Caro(a) Senhor(a),

Você está sendo convidado a participar de uma pesquisa intitulada: **"Abordagem imunogenética de receptores de quimiocinas e sua correlação com a diversidade do gene *Env* do HIV-1: Fatores que influenciam a progressão para AIDS"**, que tem como objetivo principal a caracterização do perfil de expressão de proteínas do sistema imunológico e análises genéticas dessas proteínas junto com a diversidade genética de genes virais na progressão da doença. O tema escolhido se justifica pela importância de entender as diferenças na progressão da doença tanto em nível do sistema imune do hospedeiro quanto do vírus. Para alcançar os objetivos do estudo será realizada uma entrevista individual em 90 participantes, previamente selecionados em um projeto anterior. A entrevista constará de um questionário com tempo estimado de preenchimento de 15 minutos. Os dados serão confidenciais e os nomes reservados. Os dados obtidos serão armazenados pelos pesquisadores durante 5 (cinco) anos e após totalmente destruídos (conforme preconiza a Resolução 466/12).

Como são feitas as análises? As análises das proteínas do sistema imune serão realizadas a partir de coleta de sangue, como uma coleta normal para hemograma. Com o uso de agulhas e seringas descartáveis será coletada de você uma amostra de **10 mL de sangue** (uma coleta por participante). Esta coleta será feita por um indivíduo treinado. Após, o sangue será examinado para caracterizar subpopulações celulares referentes ao sistema imune, assim como a diversidade genética do vírus. As amostras serão identificadas por números. Todos os dados que vinculem sua identidade com os dados obtidos a partir de sua amostra de sangue serão mantidos em um banco de dados sigiloso, ao qual só terão acesso os pesquisadores acima citados.

Quais os riscos em participar? Poderá haver formação de um hematoma no braço em função da coleta de sangue. Seus dados pessoais serão mantidos em sigilo e, portanto, risco de perda de confidencialidade dos dados será minimizado por codificação das amostras e entrevistas por números, sem a presença de seu nome.
A sua participação no presente projeto envolve uma coleta de sangue, onde serão avaliados fatores imunológicos, para que possamos ter uma maior compreensão do perfil imunológico das pessoas HIV+. Independente de sua participação no estudo, o tratamento que você recebe não será alterado e, você poderá retirar sua autorização a qualquer momento, apenas comunicando sua nova decisão a um participante do grupo de pesquisa.

O que o participante ganha com este estudo? Embora este trabalho não possa gerar qualquer beneficio imediato aos participantes, este estudo poderá trazer vários benefícios em longo prazo (por exemplo, o conhecimento das características genéticas presentes na nossa população) podendo assim, auxiliar em novas diretrizes do tratamento e acompanhamento futuro dos pacientes que vivem com HIV/AIDS.

Quais são os seus direitos? Os seus registros médicos serão sempre tratados confidencialmente. Os resultados deste estudo só poderão ser usados para fins científicos, e você não será identificado por nome. Sua participação no estudo é voluntária, caso você decida não participar, isto não afetará o tratamento que

você tem direito. Salientamos novamente que você tem a liberdade de retirar seu consentimento a qualquer momento, caso desejar. Você poderá procurar qualquer pesquisador envolvido para responder a qualquer pergunta ou obter esclarecimento acerca dos assuntos relacionados a esta pesquisa a qualquer momento do estudo. Além disso, no caso de eventuais danos decorrentes da pesquisa você será indenizado de acordo ao tipo de dano.

Eu_____recebi as informações sobre os objetivos e a importância desta pesquisa de forma clara e concordo em participar do estudo.

Declaro que também fui informado (a):

• Da garantia de receber resposta a qualquer pergunta ou esclarecimento acerca dos assuntos relacionados a esta pesquisa.
• De que minha participação é voluntária e terei a liberdade de retirar o meu consentimento, a qualquer momento e deixar de participar do estudo, sem que isto traga prejuízo para a minha vida pessoal e nem para o atendimento prestado a mim.
• Da garantia que não serei identificado quando da divulgação dos resultados e que as informações serão utilizadas somente para fins científicos do presente projeto de pesquisa.
• Da garantia que serei indenizado no caso de eventuais danos decorrentes da pesquisa.
• Sobre o projeto de pesquisa e a forma como será conduzido e que em caso de dúvida ou novas perguntas poderei entrar em contato com a pesquisadora: **Sabrina Esteves de Matos Almeida, telefone 3352-0336, email: sabrinamatos.almeida@gmail.com e endereço: Av. Ipiranga, 5400, 3^{eiro} andar. Bairro Jardim Botânico – Porto Alegre.**

• Também que, se houverem dúvidas quanto a questões éticas e aprovação do presente projeto, poderei entrar em contato com o Comitê de Ética em Pesquisa da UFRGS pelo telefone 3308 3738, endereço Av. Paulo Gama, 110 - Sala 317 - Prédio Anexo 1 da Reitoria - Campus Cento- Porto Alegre/RS - CEP: 90040-060; com Daniel Demétrio Faustino da Silva, Coordenador-geral do Comitê de Ética em Pesquisa do GHC pelo telefone 3357-2407, endereço Av. Francisco Trein 596, 3° andar, Bloco H, sala 11, das 09h às 12h e das 14h:30min às 17h; e com o Comitê de Ética em Pesquisa da FEPPS-CDCT pelo telefone 3288-4000, endereço Av. Ipiranga, 5400 - Jardim Botânico - Porto Alegre/RS - CEP: 90610-000 das 8h às 12h e 13h às 18h.

Declaro que recebi via deste Termo de Consentimento Livre e Esclarecido, ficando outra via com os pesquisadores.

Porto Alegre, ___, de _____ de 20__.

Nome e assinatura do participante incluído no estudo:

_____

Nome e assinatura do pesquisador:

_____