MARÍA TERESA MAIDANA GIRET

INFECÇÃO PELO VÍRUS GB-C (GBV-C) EM RECÉM INFECTADOS PELO VÍRUS DA IMUNODEFICIENCIA HUMANA TIPO 1 (HIV-1).

Tese apresentada à Universidade Federal de São Paulo - Escola Paulista de Medicina, para obtenção do título de Doutor em Ciências

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Orientador: Prof. Dr. Esper Georges Kallás

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SUMARIO

LISTA DE FIGURAS vii

LISTA DE ABREVIATURAS E SÍMBOLOS viii

RESUMO ix

1 INTRODUÇÃO 1

1.1 Perspectiva histórica 1

1.2 Biologia Molecular e Filogenia. 3

1.3 Epidemiologia e Transmissão. 5

1.4 Importância clínica. 10

1.5 Interações do GBV-C e o HIV na resposta imunológica do hospedeiro 14

1.6 A ativação celular na infecção pelo HIV 18

2 OBJETIVOS 21

2.1 Objetivo geral 21

2.2 Objetivos específicos 21

3 MATERIAL E METODOS, E RESULTADOS 22

4 MANUSCRITOS 23

4.1 MANUSCRITO 1 23

4.2 MANUSCRITO 2 28

4.3 MANUSCRITO 3 55

5 DISCUSSÃO 68

6 CONCLUSÕES 72

7 REFERÊNCIAS BLIOGRÁFICAS 73

8 ANEXOS 85

8.1 MANUSCRITO 4 85

LISTA DE FIGURAS

Figura 1. Esquema comparativo da organização do genoma do GBV-C e HCV mostrando as fases de leituras abertas e as regiões 5’e 3’UTR...........................4

Figura 2. Múltiplos mecanismos propostos para explicar a ação do GBV-C nos pacientes coinfectados com HIV..18

LISTA DE ABREVIATURAS DE SIMBOLOS

ALT	Alanina aminotransferase
CCR5	Receptor 5 de quimiocinas (C-C), com cisteinas adjacentes
CXCR4	Receptor 4 de quimiocinas (CXC), com cisteinas pareadas separadas por um outro aminoácido
HBV	Vírus da Hepatite B
HCV	Vírus da Hepatite C
HHV-6	Herpesvírus 6.humano
HHV-7	Herpesvírus 7 humano
HIV-1	Vírus da imunodeficiência tipo 1
GBV-A	GB vírus A
GBV-B	GBv vírus B
GBV-C	GB vírus C
HAART	Terapia de Alta Atividade Anti-Retroviral
IL	Interleucina
INF	Interferon
mRNA	Ácido Ribonucleico mensageiro
MIP-1α	Proteína inflamatória de macrófagos
NCR	Região não codificadora
NS	Região não estrutural
NK	Célula *natural killer*
SIDA	Sindrome da imunodeficiência adquirida
SIV	Vírus da imunodeficiência símia
TH	Células T *helper* ou auxiliadoras

RESUMO

O GB vírus C (GBV-C) está constituído por uma fita única de RNA de polaridade positiva e pertence à família *Flaviviridae.* Possui uma seqüência e organização genómica parecida ao vírus da hepatite C, (HCV). A infecção pelo GBV-C não foi associada a nenhuma patologia, embora, na co infecção com o HIV, tenha sido associada a uma sobrevida maior e retardo no desenvolvimento da imunodeficiência. O efeito benéfico do GBV-C parece ser mediado por alterações na resposta imune celular; contudo, os possíveis mecanismos para explicar esse efeito ainda não foram esclarecidos. Neste trabalho investigamos a freqüência e características genotípicas assim como o impacto da infecção pelo GBV-C nos indivíduos infectados pelo HIV-1. No primeiro manuscrito examinamos os conhecimentos descritos na literatura referentes à coinfecção e propusemos algumas hipóteses para explicar esses efeitos.

Posteriormente, descrevemos a taxa de infecção, a prevalência, incidência e características genotípicas do GBV-C nesta população. Assim, uma considerável freqüência de infecção pelo GBV-C foi observada e a análise filogenética dos isolados de GBV-C mostraram ser do genótipo 1 e 2. Foi observada também uma correlação inversa entre a carga viral do GBV-C e a carga viral do HIV na inclusão e um ano depois, assim como uma correlação positiva, mas não significativa, entre a carga viral do GBV-C e a contagem de linfócitos T CD4+.

Finalmente, avaliamos o efeito da viremia pelo GBV-C na ativação celular em recém infectados pelo HIV-1. Os pacientes foram agrupados em GBV-C viremicos e não virémicos e foram avaliados para a contagem de linfócitos T, marcadores de ativação celular e carga viral do GBV-C e HIV-1. Foram realizadas análises de univariada e multivariada para identificar variáveis associadas com ativação celular. Demonstramos que a viremia pelo GBV-C foi correlacionada com uma diminuição da ativação celular nos indivíduos HIV positivos e este efeito mostrou se independente da carga viral do HIV. Assim, esta associação entre a replicação do GBV-C e menor ativação celular

pode explicar, pelo menos em parte, a proteção conferida pelo GBV-C na progressão da doença nos indivíduos infectados pelo HIV-1.

ABSTRACT

GB virus C (GBV-C) is a single stranded positive sense RNA virus, which is a member of the *Flaviviridae.* It has a close sequence homology and genomic organization to hepatitis C virus (HCV). No disease has been associated with GBV-C infection but co-infection with human immunodeficiency virus (HIV) leads to improved morbidity and mortality for the HIV infected subjects. The mechanism of the beneficial effect of GBV-C appears to be mediated by alterations in the cellular immune response. In this study we investigated the frequency and genotyping characteristics as well as the impact of the GBV-C infection among recently HIV-1 infected individuals. In the first manuscript we examined the current knowledge concerning this co-infection and developed hypotheses to explain its effects.

Subsequently, we described the rate of infection, the prevalence, incidence and genotypic GBV-C characteristics in this population. In that regard, a considerable frequency of GBV-C infection was observed and the phylogenetic analysis of the GBV-C isolates revealed the predominance of genotypes 1 and 2. Also, it was observed an inverse correlation between GBV-C load and HIV-1 load at the enrollment and after one year of follow-up, and a positive, but not statistically significant, correlation between GBV-C load and CD4+ T lymphocyte counts.

Finally, we have investigated the effect of GBV-C viremia on T cell activation in early HIV-1-infection. The volunteers were enrolled into two groups: GBV-C viremic and non viremic, all co-infected with HIV-1. They were evaluated for T cell counts, cellular activation markers, GBV-C RNA detection, and HIV-1 viral load. Non-parametric univariate and multivariate analyses were carried out to identify the variables associated with cellular activation. We demonstrated that the GBV-C viremia is correlated with a lower T cell activation in HIV-1-infected individuals and this effect was independent of HIV-1 viral load. The association between GBV-C replication and lower T-cell activation

may explain, at least in part, the protection conferred by this virus against disease progression to immunodeficiency in HIV-1-infected patients.

1 INTRODUÇÃO

Existe uma longa história de interação entre diferentes vírus, geralmente um aumentando a patogenicidade de outro no hospedeiro. A interação entre o vírus da imunodeficiência humana tipo 1 (HIV-1) e o GB Vírus C (GBV-C) é rara e curiosa, beneficiando o indivíduo co-infectado. É, portanto, de grande interesse que os mecanismos envolvidos nos resultados desta interação sejam bem conhecidos, pois poderia resultar em avanços no conhecimento da patogênese de ambas as viroses e contribuir para o desenvolvimento de novas estratégias de tratamento de indivíduos infectados pelo HIV-1. Até o inicio do estudo, foram realizados poucos inquéritos em população de pacientes infectados pelo HIV para a presença da infecção pelo GBV-C no Brasil. No presente trabalho avaliamos a freqüência da infecção pelo GBV-C, a incidência de casos no primeiro ano de seguimento dos pacientes, caracterizamos os genótipos circulantes nesta população e principalmente, definimos o impacto da infecção pelo GBV-C a través do estudo da ativação celular em pacientes recém infectados pelo HIV-1.

1.1 Perspectiva histórica

O GBV-C, chamado inicialmente de “agente GB”, foi descrito pela primeira vez por Deinhardt e colaboradores em 1967, a partir de um relato de caso de hepatite de etiologia desconhecida num cirurgião americano de 34 anos, G. Barker (GB). O paciente apresentou sintomas de hepatite aguda com atividade enzimática moderada e período de icterícia de três semanas. Na tentativa de identificar a etiologia da infecção, foi inoculado o soro do paciente em primatas, sagüi, tamarin (pequeno macaco sul-americano de cauda cumprida pertencente à família *Callithricinae).* Foram registrados quadros clínicos e laboratoriais de hepatite em todos os animais inoculados até a

quarta seqüência de passagem, sugerindo que a causa fosse um agente viral pós-transfusional não A, não B, que foi nomeado “GBV” (Deinhardt, Holmes et al. 1967).

As Investigações do agente recomeçaram 25 anos depois, quando surgiram novos métodos qualitativos e de reconhecimento viral. O vírus foi, então, descoberto por dois grupos independentes de pesquisadores em estudos de casos de hepatites não A, não B, não E (Simons, Leary et al. 1995; Simons, Pilot-Matias et al. 1995; Linnen, Wages et al. 1996). O *Virus Discovery Group* dos Laboratórios Abbott identificou duas cepas de vírus em amostras de soro dos primatas infectados com derivados do soro GB e o denominaram GBV tipo A (GBV-A) e tipo B (GBV-B) (Muerhoff, Leary et al. 1995; Simons, Pilot-Matias et al. 1995). Caracterização daqueles dois vírus (GBV-A e GBV-B) revelou como sendo muito relacionados com o HCV, contudo, estudos subseqüentes demonstraram que tratavam se de um vírus diferente. O mesmo grupo isolou uma cepa de vírus semelhante ao GBV-A e GBV-B em amostras de sangue de uma população da África ocidental considerada de risco, durante um estudo retrospectivo e o denominou GBV-C (Simons, Leary et al. 1995). Logo depois dois grupos descobriram vírus relacionados ao HCV em humanos. Um desses vírus foi isolado de um paciente com hepatite de origem desconhecida e de outros pacientes com hepatite aguda e crônica não A - E, e o nome “HGV” (Vírus da hepatite G) foi logo sugerido (Linnen, Wages et al. 1996).

Estudos em GBV-C e HGV revelaram 96% de similaridade entre os dois genomas, indicando que eles seriam duas cepas do mesmo vírus (Alter 1996; Leary, Muerhoff et al. 1996) e, não tendo sido associado com hepatite em vários estudos epidemiológicos, muitos pesquisadores da área consideram incorreta a denominação de vírus da hepatite G.

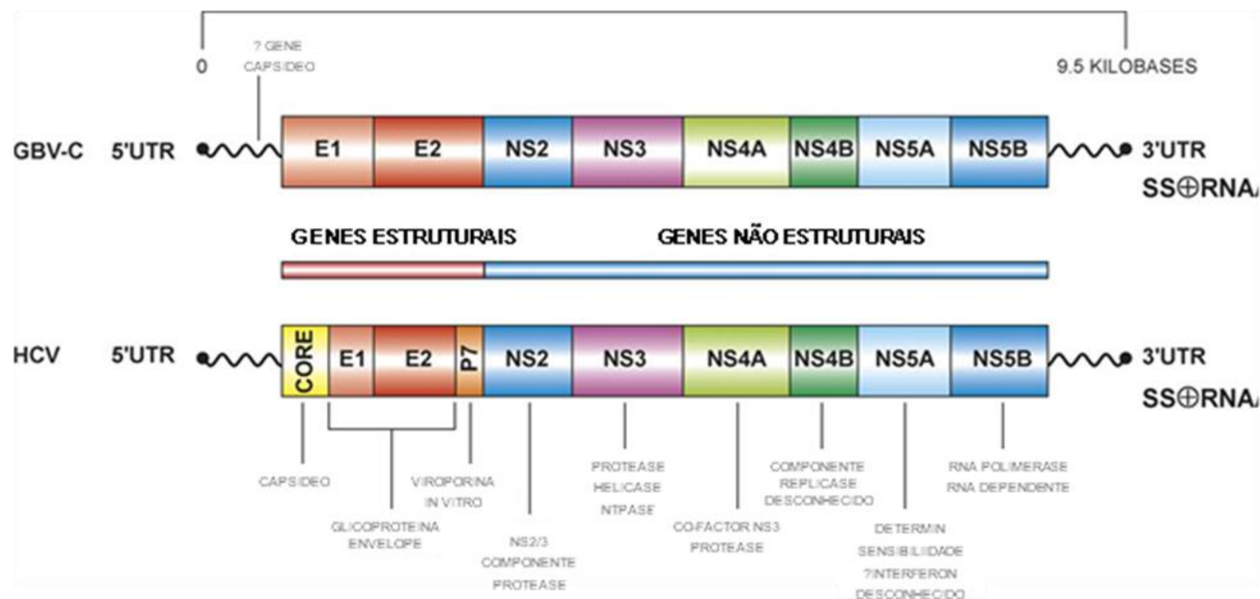
1.2 Biologia Molecular e Filogenia.

O GBV-C é um vírus RNA da família *Flaviviridae*. Apresenta um genoma muito similar ao HCV. GBV-C e o HCV se diferenciam de outros vírus RNA humanos pela capacidade de poder causar infecção persistente sem um DNA intermediário ou uma forma latente conhecida durante o ciclo de replicação (Xiang, Wunschmann et al. 2001). Uma exibição de diagrama de organização do genoma comparativo de GBV-C e HCV, assim como as diferenças potenciais e as várias funções dos genes são mostradas na Figura 1. O genoma do GBV-C é constituído por uma fita única de RNA de polaridade positiva constituído de aproximadamente 9400 nucleotídeos. A fita única de RNA contém proteínas estruturais e não estruturais além da região não codificadora 5' (NCR), o GVB-C apresenta duas glicoproteínas de envelope, chamadas E1 e E2, no entanto a composição exata do nucleocapsídeo ainda não foi definida. Possui uma fase de leitura aberta *(Open Reading Frame, ORF)* que codifica uma poli proteína de aproximadamente 3000 aminoácidos (Leary, Muerhoff et al. 1996; Linnen, Wages et al. 1996). Contém um sítio de entrada ribosomal *(internal ribosomal entry site, IRES)* na região não codificadora 5' (NCR), que serve de sítio de entrada do ribossomo dirigindo a tradução do RNA mensageiro (Leary, Muerhoff et al. 1996); duas glicoproteínas de envelope E1 e E2, uma helicase de RNA com domínios de serina protease do tipo tripsina NS3 e uma região RNA polimerase (NS5B) RNA dependente. A fase de leitura aberta do GBV-C apresenta 29% de homologia na seqüência de aminoácido com o HCV assim como em áreas conservadas, o que implica produtos dos genes de funções semelhantes.

As semelhanças com HCV geralmente são limitadas a seqüência padrões específicas nas regiões NS3 e NS5B enquanto há pequena ou nenhuma semelhança nas regiões que codificam os genes de envelope entre GBV-C e HCV. Entre as seqüências isoladas de GBV-C existe uma variação limitada nos genes que codificam as

glicoproteínas de envelope ao contrário de HCV e HIV onde a variabilidade foi relacionada com a persistência viral (Simmonds 2001)

Ainda não foram determinadas características importantes no genoma do GBV-C. A região codificadora para proteínas do núcleo (*core)* ainda não foi definida. É provável que o gene do *core* do GBV-C utilize uma fase de leitura aberta alternativa ou que este seja codificado pela seqüência de RNA antisense (Xiang, Daniels et al. 1999; Pavesi 2000), porém, esta última seria pouco provável considerando a disponibilidade de programas de análise de seqüências que analisam fases de leituras abertas em ambas às direções, positiva e negativa. Uma possível explicação seria a formação de estruturas secundárias dentro do genoma de RNA que torna a identificação do gene do *core* de GBV-C difícil.



Fonte:Barzsenyi e colaboradores. Journal of Clinical Virology.2005; 33:257-266

Figura 1: Esquema comparativo da organização do genoma do GBV-C e HCV mostrando as fases de leituras abertas e as regiões 5'e 3'UTR

Diferentes isolados ou genótipos de GBV-C apresentaram variabilidade limitada, com seqüência de nucleotídeo diferindo no máximo em 13%. Comparações epidemiológicas de diferentes isolados de GBV-C sugerem que há seis agrupamentos filogenéticos principais ou genótipos que são igualmente divergentes de seqüências de GBV-C isoladas em primatas como determinado de análise do gene E2. Assim, já foram descritos seis genótipos do GBV-C com 12% de divergência entre eles (Okamoto, Nakao et al. 1997; Tucker, Smuts et al. 1999). O genótipo 1 predomina na África Ocidental (Muerhoff, Simons et al. 1996) , o genótipo 2 dividido em 2a e 2b, na Europa e EUA (Muerhoff, Smith et al. 1997), o genótipo 3 na Ásia (Mukaide, Mizokami et al. 1997; Okamoto, Nakao et al. 1997; Katayama, Kageyama et al. 1998), o genótipo 4 no sudeste asiático (Naito, Win et al. 1999) e o genótipo 5 na África do Sul (Tucker, Smuts et al. 1999) e o novo genótipo 6 na Indonésia (Muerhoff, Dawson et al. 2006). Na Venezuela foram encontrados os genótipos 1 e 2 enquanto que o genótipo 3 (asiático) esteve presente somente em população rural e indígena (Loureiro, Alonso et al. 2002). No Brasil predomina o genótipo 2, seguido do genótipo 1 (Oliveira, Martins et al. 2002; Nishiya, Ribeiro-dos-Santos et al. 2003).

A distribuição geográfica mundial das variantes de GBV-C e a sua natureza não patogênica sugerem uma história evolutiva longa que permite fazer uma analogia com a migração humana, insinuando que a evolução deste vírus RNA ao longo do tempo tem sido extremamente lento (Smith, Basaras et al. 2000; Duffy, Shackelton et al. 2008)

1.3 Epidemiologia e Transmissão.

Plasma de pacientes infectados pelo GBV-C foram avaliados por meio de ensaios imunológicos com peptídeos específicos e foi sugerido que a partícula viral possui um nucleocapsídeo e pelo menos parte do *core* expresso *in vivo* Assim, baseado na

densidade muito baixa das partículas de GBV-C, acredita-se que o vírus se associe a lipídeos em soro humano e, como ocorre com o HCV, liga-se aos receptores das lipoproteínas de baixa densidade para entrar na célula (Xiang, Klinzman et al. 1998). Foi demonstrado pela primeira vez e em condições experimentais que se replica em cultura de células mononucleares de sangue periférico (PBMC) (Fogeda, Navas et al. 1999) em linfócitos T CD4+ (Xiang, Wunschmann et al. 2000) no baço e na medula óssea (Tucker, Smuts et al. 2000). A replicação do GBV-C nos hepatócitos não esta completamente elucidada. Alguns grupos têm mostrando replicação no fígado (Seipp, Scheidel et al. 1999; Tucker, Smuts et al. 2000) e outros tem descrito ausência de evidências de replicação hepática após estudos em biopsia de fígado de pacientes co infectados pelos vírus GBV-C/HIV-1/ HCV (Barbosa AJ 2008).

A Infecção pelo GBV-C é comum e a resolução ocorre em aproximadamente 60 a 75% das pessoas sem deficiência imunológica, caracterizada pelo aparecimento dos anticorpos contra a glicoproteína do E2 do envelope (Thomas, Vlahov et al. 1998). A Infecção pelo GBV-C não está associada a nenhuma doença conhecida e não parece representar uma ameaça à saúde pública; é por isso que o *Food and Drug Administration* (FDA) não recomenda sua triagem em bancos de sangue.

De ampla distribuição mundial, o GBV-C RNA é detectado em 1% a 4% dos doadores saudáveis (Moaven, Hyland et al. 1996; Alter, Nakatsuji et al. 1997; Stapleton 2003); no Brasil essa prevalência em doadores é maior, em torno de 9% (Bassit, Kleter et al. 1998; Nishiya, Ribeiro-dos-Santos et al. 2003). A viremia pelo GBV-C é definida pela detecção de RNA viral no soro ou no plasma. Os métodos empregados incluem sistema de amplificação de ácidos nucléicos (Simons, Leary et al. 1995; Linnen, Wages et al. 1996), e a quantificação tem sido realizada com métodos de diluição terminal (Xiang, Wunschmann et al. 2000), reação em cadeia da polimerase em tempo real (*TaqMan*) (Lefrere, Ferec et al. 1999) e ensaio em cadeia de DNA ramificado (*branched –DNA*) (Tillmann, Heiken et al. 2001).

A maioria das pessoas depura o vírus e desenvolve anticorpos, o que fortemente sugere que a resposta imunológica humoral contra as glicoproteína de envelope E2 está associada ao controle da viremia (Dille, Surowy et al. 1997; Tacke, Kiyosawa et al. 1997), enquanto que a presença de GBV-C RNA em soro ou plasma é resultado de infecção ativa.

A prevalência de anticorpos contra as glicoproteínas E2 (anti-E2) no soro foi de dois a seis vezes maiores que a presença de RNA GBV-C (viremia), sugerindo que a depuração espontânea do vírus na infecção pelo GBV-C é mais comum nos doadores de sangue (Tacke, Schmolke et al. 1997; Thomas, Vlahov et al. 1998). Ainda não está claro o mecanismo de depuração do GBV-C. Também não sabemos, como mostrado nos primeiros trabalhos na década dos noventa, porque, ao contrário de pessoas saudáveis (Tacke, Schmolke et al. 1997), a maioria dos indivíduos infectados pelo HIV não desenvolvem anticorpos contra o GBV-C (Bjorkman, Flamholc et al. 2004; Williams, Klinzman et al. 2004; Van der Bij, Kloosterboer et al. 2005).

Considerando que o GBV-C replica em células T CD4+ (Xiang, Wunschmann et al. 2000; George, Varmaz et al. 2006); foi sugerido que a depuração viral poderia refletir ser comprometida pela perda dessas células na infecção pelo HIV (Van der Bij, Kloosterboer et al. 2005). Poderíamos ainda especular que os controladores de elite, um grupo de indivíduos com uma preservada população de células T CD4+, apresentassem uma resposta de anticorpos para GBV-C sustentada e uma prevalência menor de viremia pelo GBV-C. Infelizmente, não existem testes de anticorpos disponíveis para avaliar a resposta contra o GBV-C suficientes para esta avaliação (comunicação pessoal, Georg Hess, Roche Diagnóstics and A. Scott Muerhoff, Abbott Laboratories) (Blankson, Klinzman et al. 2008)

Embora tenha sido demonstrado que muitas pessoas estejam infectadas por este vírus em todo o mundo, não foi demonstrada associação com doença clínica. O GBV-C é transmitido predominantemente por via parenteral, mas também por via sexual. São descritos índices de alta soroprevalência entre os usuários de drogas intravenosas, (Frey, Homan et al. 2002).

Existem extensas evidências de transmissão do GBV-C pela via sexual e percutânea de maneira muito parecida à transmissão do HIV. A transmissão sexual entre homossexuais foi proposta como o modo mais efetivo de disseminação do GBV-C (Berzsenyi, Bowden et al. 2005), mas há evidências de transmissão entre indivíduos heterossexuais (Sawayama, Hayashi et al. 1999), transmissão intra familiar (Pinho, Zanotto et al. 1999) e transmissão vertical e horizontal, evidenciadas por análises de seqüências genéticas (Seifried, Weber et al. 2004). Relacionado com esta ultima via, foi demonstrada diminuição da transmissão do HIV-1 de mãe para filho associada com a aquisição do GBV-C pelo filho, mas não associada com a infecção materna pelo GBV-C (Supapol, Remis et al. 2008).

Em relação à prevalência de infecção pelo GBV-C, um estudo em indivíduos saudáveis, sem fatores de risco para doenças sexualmente transmissíveis e com níveis normais de alanina aminotransferase (ALT) mostrou que a presença de RNA do GBV-C foi de 1,9% enquanto que essa porcentagem foi de 6,8% de pacientes submetidos à hemodiálise, 18,2% em indivíduos infectados pelo HIV-1, com 16% de viremia e 56% de anticorpos anti E2 em homossexuais infectados pelo HIV (Tillmann, Heiken et al. 2001), e 39% e 46%, respectivamente, em outra coorte com as mesmas características (Williams, Klinzman et al. 2004). Estudos em outros grupos incluem 21,1% de viremia em pacientes politransfundidos, 24,4% em indivíduos HCV positivos, 28,8% em usuários de drogas intravenosas e 35,2% em pacientes hemofílicos (Feucht, Zollner et al. 1997). Logo após a descoberta do GBV-C, foi encontrada uma freqüência de 11%

em homossexuais e bissexuais que não eram usuários de drogas intravenosas e outra maior, 35%, em usuários de drogas intravenosas. (Stark, Bienzle et al. 1996).

A prevalência atual de GBV-C obviamente variará de acordo com a população estudada, mas é claro é que a viremia pelo GBV-C é mais comum em grupos de risco para transmissão sexual e percutânea. No que diz respeito a este ultimo grupo, a viremia pelo GBV-C foi notificada em 25,3% de pacientes transplantados de fígado com doença hepática terminal devida a causas virais, porém não foi mostrada nenhuma influência na evolução ou ocorrência de hepatite no enxerto (Vargas, Laskus et al. 1997). Da mesma forma que a aquisição da infecção pelo GBV no momento do transplante, não houve nenhum impacto no resultado da enxertia nos receptores por hepatite não infecciosa (Fried, Khudyakov et al. 1997).

A situação não é diferente na América do Sul. A infecção pelo GBV-C também foi estudada na Venezuela com prevalência de 3% em trabalhadores da saúde, 7% em doadores de sangue, 9% em hemodialisados, 5% em população indígena da região oeste e 25% em ameríndios do sul da Venezuela (Loureiro, Alonso et al. 2002).

Dados brasileiros de infecção pelo GBV-C foram disponibilizados logo após a descoberta do vírus (Pinho, Capacci et al. 1996; Pinho and da Silva 1996), por diferentes grupos em várias regiões, com 9,7% em doadores de sangue de São Paulo (Levi, Contri et al. 2003), 7% na região central (Oliveira, Martins et al. 2002; Ramos Filho, Carneiro et al. 2004) e 8,3% na população geral de São Paulo (Ribeiro-dos-Santos, Nishiya et al. 2002); 15% em hemodialisados e 19% em pacientes com hepatite não A não C (Lampe, Saback et al. 1997), 2.3% em crianças menores de 10 anos e 18% em adultos jovens, além de porcentagens diminuídas na população mais velha de até 80 anos (Lampe, Saback et al. 1998). No mesmo estudo, a prevalência de

anticorpos contra a glicoproteina E2 foi de 6% em adultos jovens (entre 18 e 24 anos) e 35% em adultos de meia idade (entre 43 e 60 anos). Na Região Central do Brasil, a proporção foi de 16% em transplantados de rins, 15% em hemodialisados e 7% em pacientes submetidos a diálise peritoneal (Ramos Filho, Carneiro et al. 2004). No Nordeste, 10% em pacientes com hepatite C crônicos (Pereira, Spinelli et al. 2002) e 16% de viremia e 18% de anticorpos em população rural (Gallian, Rodrigues et al. 1998). O único dado disponível de viremia pelo GBV-C em pacientes infectados pelo HIV-1 é disponível em estudo de resposta terapêutica, que revelou prevalência de 24% (Souza, Zhang et al. 2006). Dado o limitado número de estudos epidemiológicos da coinfecção GBV-C e HIV-1, surgiu o nosso interesse em determinar a prevalência, incidência e distribuição genotípica de GBV-C em uma coorte de indivíduos recém infectados pelo HIV-1.

1.4 Importância clínica.

Apesar das numerosas investigações, o GBV-C não foi associado a nenhuma doença em particular. Ao contrário, vários relatos têm mostrado um papel "protetor" na co infecção com o HIV, mas nenhum papel similar foi atribuído na coinfecção com o vírus da hepatite C ou B.

A infecção pelo HIV esta associada a um amplo cenário clínico que envolve desde a preservação do sistema imunológico, particularmente na presença de tratamento antiretroviral, até infecções assintomáticas e oportunistas que podem levar ao estabelecimento da imunodeficiência (Pantaleo and Fauci 1996). Particularmente, a infecção pelo HIV apresenta taxas elevadas de coinfecção com GBV-C e que variam entre 14% e 45%, com as maiores proporções em homossexuais e usuários de drogas intravenosas (Lau, Miller et al. 1999; Rey, Fraize et al. 1999; Puig-Basagoiti, Cabana et al. 2000; Tillmann, Heiken et al. 2001). A taxa de depuração espontânea de GBV-C

RNA e o aparecimento de anticorpos anti-E2 em pacientes co-infectados HIV/GBV-C acontecem de forma mais lenta que em indivíduos não infectados pelo HIV ou ainda a depuração da viremia de GBV-C sem o desenvolvimento de anticorpos anti-E2 no paciente coinfetado (Stapleton 2003).

Os trabalhos começaram a aparecer no final de 1990, sugerindo que os pacientes co-infectados apresentam uma sobrevida maior e retardo no desenvolvimento da imunodeficiência quando comparados com indivíduos infectados somente pelo HIV (Heringlake, Ockenga et al. 1998; Lefrere, Roudot-Thoraval et al. 1999; Yeo, Matsumoto et al. 2000; Tillmann, Heiken et al. 2001; Xiang, Wunschmann et al. 2001; Williams, Klinzman et al. 2004). Como evidência adicional do efeito protetor da infecção dupla, Williams e cols. (Williams, Klinzman et al. 2004) também demonstraram que o curso da doença pelo HIV-1 estava inversamente influenciado pela ausência replicativa do GBV-C. O mecanismo deste efeito protetor ainda não foi esclarecido e há algumas hipóteses para explicá-lo.

Vários achados importantes foram publicados. Primeiramente, existe uma prevalência elevada de viremia pelo GBV-C entre os pacientes infectados pelo HIV, como já mencionado. Nesses pacientes, a taxa de mortalidade era significativamente menor e independente de tratamento antiretroviral prévio ou profilaxia contra *Pneumocystis jiroveci*, contagem basal de células T CD4+, idade, raça, sexo, ou modo de transmissão do HIV (Xiang, Wunschmann et al. 2001). Em estudos *in vitro,* Xiang e colaboradores observaram que a replicação do HIV foi diminuída na coinfecção com o GBV-C, assim o GBV-C não impede a entrada do HIV na célula nem a queda na contagem de linfócitos T CD4+, mas exerceria um efeito inibitório na replicação do HIV em cultura de células. Foi proposto que esta ação não seria conseqüência da toxicidade celular, considerando que a replicação do GBV-C em linfócitos humanos cultivados não apresenta efeito citopático e é incapaz de inibir a síntese de proteínas celulares (Xiang,

Wunschmann et al. 2001). Esta ultima observação é importante e sugere que o GBV-C atua primordialmente alterando a replicação viral do HIV do que através de outros mecanismos.

Em outro estudo, com relevância no cuidado ao do paciente infectado pelo HIV, 197 pacientes foram avaliados para a presença de viremia pelo GBV-C e acompanhados prospectivamente. Os pacientes positivo para o GBV-C apresentaram sobrevida significativamente maior e progressão lenta para AIDS, além de uma sobrevida maior após a instalação da imunodeficiência. Curiosamente, em uma análise restrita ao período de pós-tratamento, a viremia pelo GBV-C permaneceu como fator preditor de maior sobrevida. A carga viral do HIV também foi menor nos pacientes virêmicos para o GBV-C e uma correlação inversa entre as cargas virais dos dois vírus foi observada e ainda, a carga viral de GBV-C aumentou em todos os pacientes que tinham iniciado tratamento antiretroviral (Tillmann, Heiken et al. 2001)

Mais recentemente, amostras coletadas na era pré-HAART *(Highly Active Antiretroviral Therapy)*, na coorte MACS *(Multicenter AIDS Cohort Study)*, fruto de estudo colaborativo em vários centros no Estados Unidos, analisaram o impacto da viremia pelo GBV-C na progressão para AIDS. O estudo mostrou que a viremia pelo GBV-C estava significativamente associada com maior sobrevida entre os individuos HIV positivos 5 a 6 anos após a soroconversão para o HIV, mas não após dos primeiros 12 a 18 meses da soroconversão, e que a negativação do GBV-C RNA após 5 a 6 anos foi associada com piore prognóstico. Os autores do estudo concluíram que houve uma significativa vantagem em termos de sobrevida associada à persistência da viremia pelo GBV-C no paciente co-infetado com HIV (Williams, Klinzman et al. 2004). Ainda não se sabe se estes dados podem ser aplicados na era pós HAART

Posteriormente, num estudo retrospectivo em uma coorte de pacientes que respondeu questionários sobre a qualidade de vida, foi observado que pacientes infectados por HIV e GBV-C apresentaram melhor qualidade de vida comparados com os GBV-C negativos, o que poderia apoiar o curso favorável da doença nos pacientes coinfectados (Tillmann, Manns et al. 2004).

Nem todos os estudos de co-infecção com o HIV e GBV-C, porém, têm demonstrado um feito benéfico na progressão da doença (Birk, Lindback et al. 2002; Brumme, Chan et al. 2002; Bjorkman, Flamholc et al. 2004; Van der Bij, Kloosterboer et al. 2005). Não foi observada nenhuma influência clinica ou imunológica atribuída ao GBV-C em um estudo sueco com 157 indivíduos coinfetados, mesmo considerando que os pacientes tiveram níveis elevados de células T CD4+ no começo da infecção (Birk, Lindback et al. 2002). Foi então sugerido que a viremia pelo GBV-C na infecção pelo HIV-1 pode representar um fenômeno secundário e não um fator de prognóstico independente e, ainda, que a persistência da viremia do GBV-C dependeria do número de células T CD4+ circulantes. Assim, a diminuição do número de células T CD4+ associadas com a progressão da imunodeficiência seria a causa e não a conseqüência da depuração do GBV-C (Van der Bij, Kloosterboer et al. 2005).

Em subanálise de um estudo prospectivo de coorte *(Multicenter Hemophilia Cohort Study)*, os mesmos efeitos favoráveis na sobrevida foram observados e foram independentes da idade, carga viral de HIV e HCV, contagem de células T CD4+ e CD8+ e genótipo do receptor de quimiocinas CCR5 (Yeo, Matsumoto et al. 2000). Como mencionado em linhas anteriores, não existe ainda consenso sobre a influência exercida, se existe, da viremia de GBV-C na resposta terapêutica. Logo após do trabalho de Tillmann e cols., em 2001, surgiram novas informações, algumas indicando ausência de influência na resposta após inicio de tratamento (Brumme, Chan et al. 2002); outros mostrando melhores respostas independentemente da contagem de

células T CD4+ e carga viral do HIV nos pacientes co infectados (Rodriguez, Woolley et al. 2003) e respostas semelhantes as observadas com inibidores nucleosídeos da transcriptase reversa (INTR) (Souza, Zhang et al. 2006).

Durante os últimos anos, houve controvérsia em relação às interações entre GBV-C e HIV *in vivo*. Vários estudos demonstraram níveis de sobrevivência surpreendentes entre pacientes co-infectados quando comparados com aqueles infectados somente com HIV. Outros estudos, porém, não conseguiram reproduzir tais resultados. O trabalho de Williams e cols. poderia sugerir algumas explicações para essas divergências, já que demonstra claramente que os pacientes infectados somente pelo HIV-1 apresentavam maior probabilidade de morte se comparados aos coinfectados com o HIV-1 e o GBV-C, analisados cinco a seis anos após a infecção pelo HIV-1. Esta vantagem de sobrevivência significativa não foi observada durante os primeiros 12 a 18 meses após a infecção pelo HIV-1. Assim, esta dependência de tempo poderia explicar os resultados contraditórios dos efeitos protetores do GBV-C observados em alguns dos estudos (Williams, Klinzman et al. 2004).

1.5 Interações do GBV-C e o HIV na resposta imunológica do hospedeiro

Alguns estudos sugerem que a replicação do HIV-1 estaria diretamente reduzida pelo GBV-C. A identificação dos mecanismos pelo qual o GBV-C inibiria a replicação do HIV pode levar ao desenvolvimento de novas estratégias de tratamento. O exato mecanismo que explicaria o efeito benéfico do GBV-C não foi identificado, embora pesquisas recentes tenham proposto algumas vias. A Figura 2 resume as vias propostas para explicar esta interação.

O fato de que HIV-1 e o GBV-C infectam e se replicam nas células mononucleares de sangue periférico sugere que os dois vírus, possam interagir direta ou indiretamente no ciclo celular. Vários estágios do ciclo celular do HIV podem ser afetados pelo GBV-C, incluindo a fase de apresentação, a ligação à célula alvo, devido à alta afinidade pelo receptor e vários receptores de quimiocinas, internalização e transcrição reversa, integração no genoma da célula hospedeira para originar um pró-vírus, transcrição, tradução e morfogênese viral; outra possibilidade seria considerar uma série de interações que envolvem o CCR5, fator importante na transmissão de HIV e progressão da doença (Cocchi, DeVico et al. 2000).

A importância da resposta das células T auxiliadoras contra o HIV é sustentada por estudos mostrando que a progressão para imunodeficiência é relacionada com a incapacidade das células mononucleares para produzir interleucina 2 (IL-2), interleucina 12 (IL-12) e Interferon gamma (INFγ), citocinas da resposta Th1 e produção aumentada de interleucina 4 (IL-4) e interleucina 10 (IL10) (resposta Th2) (Spellberg and Edwards 2001). Na coinfecção GBV-C/HIV a resposta tida como TH1 foi preservada com níveis estáveis de citocinas características deste perfil, em comparação aos pacientes infectado semente pelo HIV-1 nos quais houve um aumento progressivo de citocinas do perfil Th2, assim, os níveis sorológicos das interleucinas IL-2, IL-12, IL-4, e IL-10 permanecem relativamente estáveis ao longo do tempo no grupo GBV-C RNA positivo enquanto que indivíduos GBV-C RNA negativos apresentam uma diminuição nos níveis de IL-2 e IL-12 de aproximadamente 85% e 83%, e aumento de IL-4 e IL-10 em 654% e 395%, respectivamente, (Nunnari, Nigro et al. 2003).

Certas cepas do HIV (R5) utilizam o receptor CCR5 para entrar na célula, enquanto outras cepas, usam o co receptor CXCR4 (X4). Em um estudo *in vitro,* a infecção de células mononucleares de sangue periférico com o GB vírus C produziu uma diminuição na replicação de cepas isoladas de HIV que usam CCR5 ou CXCR4 como co-receptores para entrar na célula. O GBV-C induziu a secreção de citocinas que levaram a uma diminuição da expressão destes coreceptores. Essas quimiocinas

implicadas e evidenciadas pelos altos níveis de mRNA, incluem RANTES, *(Regulated on Activation Normal T cell-Expressed and Secreted)*, proteínas inflamatórias dos macrófagos 1α (MIP-1α), proteínas inflamatórias dos macrófagos 1 β (MIP-1 β), ligantes naturais de CCR5 e o fator derivado de estroma (SDF-1) único ligante de CXCR4 conhecido, e, mais interessante ainda, o efeito inibitório de GBV-C sobre o HIV foi neutralizado por de anticorpos dirigidos contra essas quimiocinas.(Xiang, George et al. 2004). Em outros estudos a proteína E2 induziu apenas RANTES e não MIP-1α ou MIP-1β, outros ligantes de CCR5 (Nattermann, Nischalke et al. 2003).

Inicialmente foi observado que as glicoproteínas do envelope, especificamente E2, do GBV-C se ligam à CD81, membro da família das tetraspanina, é expressa na maioria das células nucleadas. Esta interação induziria uma liberação dose-dependente de RANTES, ligante natural de CCR5, provocando a internalização de CCR5 e inibição da expressão na superfície da célula com o concomitante acúmulo intracelular de proteínas de CCR5 nos linfócitos T CD4+ e T CD8+ de pacientes infectados com GBV-C em 53% e 36% respectivamente. Assim, essa internalização dos receptores constituiria um mecanismo de bloqueio efetivo da entrada do HIV nas células (Alkhatib, Locati et al. 1997; Brandt, Mariani et al. 2002). Novas evidências sugerem que a sua ligação à célula não parece depender da expressão do receptor CD81 (Kaufman, McLinden et al. 2007).

A variabilidade genética dos indivíduos é um fator determinante na progressão para a imunodeficiência após a infecção pelo HIV-1 ter-se estabelecido e o papel de alguns alelos específicos continua sendo estudado, como o do CCR5Δ32, um alelo que contem a deleção de 32 pares de base, e que codifica para um co-receptor não funcional. Assim, os indivíduos homozigotos para CCR5Δ32 são fortemente resistentes à infecção pelo HIV-1. Já os indivíduos heterozigotos continuam susceptíveis à infecção pelo HIV-1, mas progridem mais lentamente para SIDA quando comparados

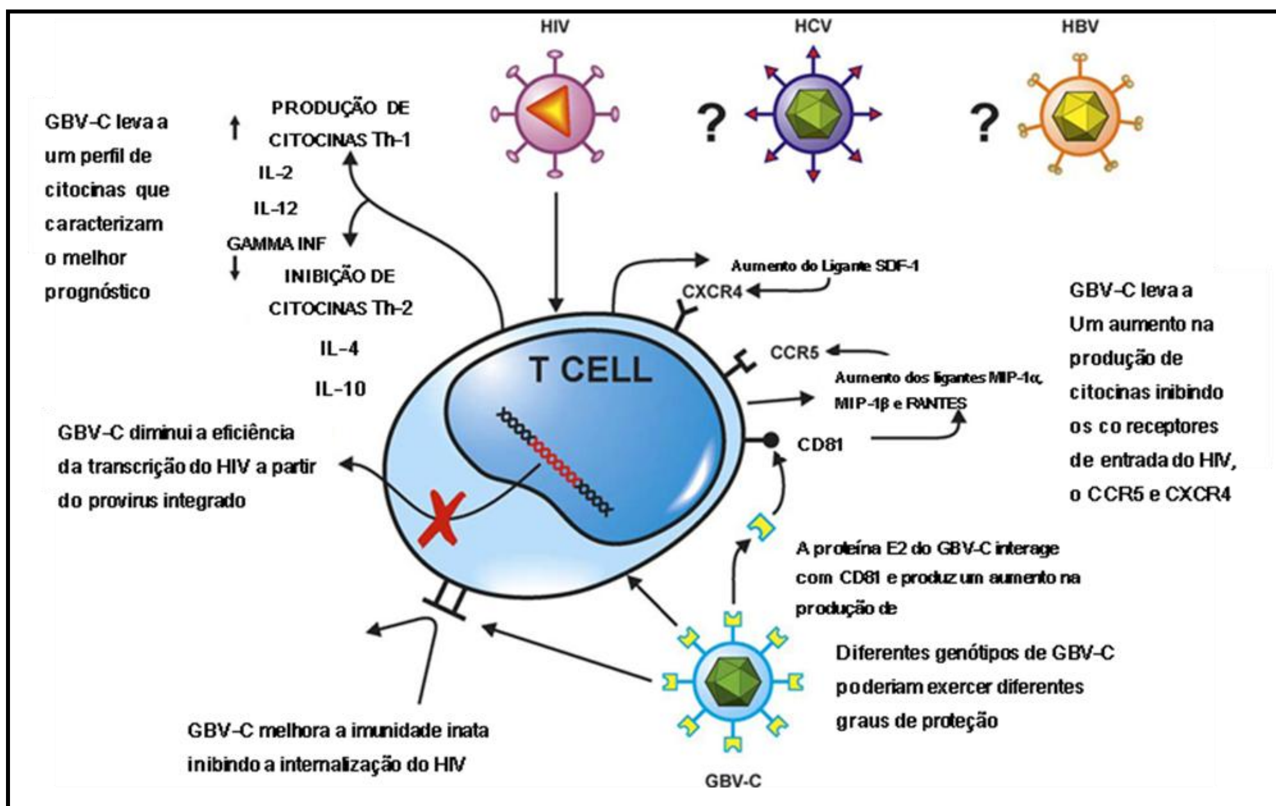
com indivíduos que possuem os alelos do tipo selvagem (Dean, Carrington et al. 1996; O'Brien and Goedert 1998).

Em trabalhos mais recentes e por meio de ensaios *in vitro* foi demonstrado que a proteína não estrutural GBV-C NS5A parece ter diferentes efeitos na célula, incluindo a alteração do ambiente necessário para a replicação do HIV, diminuição da expressão do coreceptor do HIV-1, CXCR4, na superfície, e aumento da liberação do ligante correspondente SDF-1 o que poderia explicar a inibição do HIV-1 (Xiang, McLinden et al. 2006). Foi observado também que após expressão da proteína NS5A em células *Jurkat* os genótipos 1, 2, 3, e 5 inibiram a replicação do HIV (Chang, McLinden et al. 2007) e ainda, que a expressão dos amino ácidos 152-165 na seqüência de GBV-C NS5A seriam suficientes para inibir a replicação do HIV *in vitro* e uma serina na posição 158 seria importante para este efeito, através da fosforilação ou mudanças estruturais no peptídeo (Xiang, McLinden et al. 2008). Mais recentemente, foi demonstrado que a replicação do GBV-C foi significativamente inibida *in vitro* por ativação de culturas primarias de células T CD4+ com interleucina 2 (IL-2) e fitohemoaglutinina (PHA), sugerindo que a viremia pelo GBV-C seria um fator importante na resposta ao tratamento com IL-2.

Os mecanismos moleculares da regulação de CCR5 em células que constituem alvo primário do HIV-1 ainda não foram explicados. Por outro lado, seria interessante saber se a depuração do GBV-C estaria associada à destruição das células hospedeiras mediadas pelo HIV-1, necessárias para a produção de GBV-C, precedendo à perda das células T CD4+.

Em relação aos diferentes genótipos de GBV-C, existe evidência limitada sugerindo que diferentes genótipos poderiam oferecer diferentes graus de “proteção” contra o HIV no hospedeiro coinfectado. Foi descrito que o número de células T CD4+ tende a ser

menor em pacientes infectados pelo genótipo 2a, embora estudos de coortes mais amplos e de diferentes regiões geográficas sejam necessários.



Fonte: Barzsenyi e colaboradores. Journal of Clinical Virology.2005; 33:257-266

Figura 2: Múltiplos mecanismos propostos para explicar a ação do GBV-C nos pacientes coinfectados com HIV.

1.6 A ativação celular na infecção pelo HIV

A ativação crônica do sistema imunológico está associada a progressão da infecção pelo HIV. Constitui um forte preditor da imunodeficiência (Liu, Cumberland et al. 1997; Giorgi, Hultin et al. 1999; Deeks, Kitchen et al. 2004; Hunt, Brenchley et al. 2008) e se relaciona com uma deficiente reconstituição do sistema imunológico nos pacientes tratados (Hunt, Martin et al. 2003).

Manifestações de ativação imunológica crônica incluem aumento da freqüência das células T fenotipicamente ativadas (Hazenberg, Stuart et al. 2000), aumento da capacidade repositória das células T *(turnover)* (Hellerstein, Hanley et al. 1999), ativação policlonal de células B (Lane, Masur et al. 1983) e aumento dos níveis séricos de citocinas pró-inflamatórias (Valdez and Lederman 1997).

Apesar da ativação imunológica poder ter algumas conseqüências benéficas, como a proliferação de células T e conseqüente reposição parcial de células T CD4+ de memória (Okoye, Meier-Schellersheim et al. 2007), há consenso geral de que tais efeitos acontecem em detrimento do paciente. A reposição acelerada das células T CD4+ e T CD8+ altera os mecanismos homeostáticos (Kovacs, Lempicki et al. 2001), resultando em uma diminuição da vida meia destas células (Hellerstein and McCune 1997), exaustão dos clones de células T e perda total das células T de memória (Grossman, Meier-Schellersheim et al. 2002; Brenchley, Karandikar et al. 2003).

Além disso, e talvez o ponto mais importante, a ativação celular resulta na geração de células T ativadas, alvo da replicação viral (Grossman, Feinberg et al. 1998; Douek, Picker et al. 2003). Assim, o HIV é um vírus que gera seu próprio substrato para replicação pela indução de ativação celular. Criticamente, a ativação, infecção e posterior depleção das células T de memória central seriam marcadores representativos da progressão para a imunodeficiência (Okoye, Meier-Schellersheim et al. 2007).

Embora o HIV ative também as células dendríticas e células *natural killer* (NK) do sistema imunológico inato, não se pode afirmar que a ativação é totalmente dependente da replicação viral, da mesma forma que os controladores de elite podem

ter ativação imune aumentada e correlacionada com perda de células T CD4+ (Hunt, Brenchley et al. 2008). Pacientes em tratamento antiretroviral, contudo, conseguem eliminar o vírus, apresentam uma ativação celular elevada e também uma deficiente reposição de células T CD4+. Por outro lado, na infecção natural não patogênica pelo SIV em *sooty mangabeys* e macacos verdes africanos também ocorre um aumento da ativação celular na fase aguda que rapidamente é atenuada na fase crônica, mesmo na presença de carga viral elevada (Estes, Gordon et al. 2008).

Finalmente, é de extrema importância conhecer as bases imunológicas da ativação do sistema imunológico para compreender a patogênese da progressão da infecção pelo HIV. No intuito de avaliar os mecanismos modulatorios da viremia pelo GBV-C fomos estudar o estado de ativação celular em uma coorte de pacientes recém infectados pelo HIV-1

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o impacto da infecção pelo GB vírus C na dinâmica de marcadores imunológicos e virológicos em indivíduos recém infectados pelo HIV-1.

2.2 Objetivos específicos

1. Determinar a prevalência da viremia pelo GBV-C e de anticorpos direcionados contra as glicoproteínas E2 do envelope do GB vírus C na inclusão no estudo e um ano depois.

2. Caracterizar a distribuição genotípica do GBV-C na população de pacientes coinfectados pelo GBV-C e HIV-1.

3. Determinar a taxa de infecção e a taxa de depuração da viremia pelo GBV-C no período de um ano no mesmo grupo de pacientes.

4. Avaliar o impacto da infecção pelo GBV-C na ativação celular nesses pacientes através de marcadores de ativação, correlacionando com marcadores laboratoriais de progressão.

5. Estudar a correlação entre a carga viral do GB vírus C e a carga viral do HIV-1

3 MATERIAL E METODOS, E RESULTADOS

Na seguinte seção apresentamos a descrição da metodologia e os resultados através de três manuscritos. Assim, para cumprir os nossos objetivos iniciais utilizamos diferentes metodologias e geramos resultados que deram origem ao manuscritos, um deles já publicado, outro aceito para publicação e o terceiro em fase final de preparação.

No manuscrito número 1 apresentamos o panorama da co-infecção do GBV-C e o HIV-1 no momento do inicio do estudo e descrevemos os mecanismos propostos para explicar essa interação.

No manuscrito número 2 pretendemos cumprir os nossos três primeiros objetivos, determinar a prevalência da viremia pelo GBV-C e de anticorpos do GB vírus C na inclusão no estudo e um ano depois, assim como caracterizar a distribuição genotípica do GBV-C na população e determinar a taxa de infecção e depuração da viremia pelo GBV-C. O manuscrito está em fase final de correção.

No manuscrito número 3 pretendemos respondemos o dos últimos objetivos e nele descrevemos pela primeira vez o efeito modulador da viremia pelo GBV-C na ativação celular nos pacientes coinfectados. O manuscrito foi aceito para publicação e está em fase final de impressão.

4 MANUSCRITOS

4.1 MANUSCRITO 1

Título e autores

Co-infecção GBV-C/HGV e HIV-1

Maria Teresa Maidana, Ester Cerdeira Sabino e Esper Georges Kallas

Resumo

Um padrão de interação interessante foi descrito entre HIV-1 e GBV-C/HGV, resultando em uma proteção contra progressão para a imunodeficiência. Os mecanismos envolvidos nesta interação ainda não foram esclarecidos. Nós examinamos os conhecimentos atuais relativos a esta coinfeção e desenvolvemos hipóteses para explicar os efeitos. Uma melhor compreensão da interação poderia resultar em novos conceitos; os que podem conduzir a estratégias novas para controlar a replicação do HIV-1 e progressão da imunodeficiência.

GBV-C/HGV and HIV-1 Coinfection

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An interesting interaction pattern has been found between HIV-1 and GBV-C/HGV, resulting in protection against progression to AIDS. The mechanisms involved in this interaction remain to be clarified. We examined the current knowledge concerning this coinfection and developed hypotheses to explain its effects. A better understanding of this interaction could result in new concepts, which may lead to new strategies to control HIV-1 replication and progression to AIDS.
Key Words: HIV, HGV, GBV-C, pathogenesis, review

Patients infected with the human immunodeficiency virus (HIV) are often co-infected with other pathogens, especially hepatitis viruses [1]. Coinfection with hepatitis B virus (HBV) or hepatitis C virus (HCV) appears to increase the mortality rate among HIV-infected patients [2], in the same way as the course of HCV infection is accelerated in patients who are coinfected with HIV, when compared with immunocompetent individuals [3,4]. A new virus related to hepatitis C virus was identified in 1995; it was initially thought to be another hepatitis agent [5,6]. It has been described as either GB virus C (GBV-C) [5] or hepatitis G virus (HGV) [6]. GBV-C and HGV are closely related, with more than 95 percent sequence homology [7]. The name GBV-C has been more widely used, since it appears that this virus is not a causative agent of hepatitis [7,8].

GB virus C (GBV-C), an RNA virus in the Flaviviridae family, has a genome very similar to that of hepatitis C virus (HCV), coding for structural and nonstructural proteins. There is a nontranslated region (NTR) at 5' that can serve as an internal ribosomal entry site to direct translation of the uncapped message (genome) RNA [9]. GVB-C appears to encode two structural glycoproteins, which constitute the virus envelope proteins E1 and E2. However, the precise composition of the nucleocapsid has yet to be defined. To date, five genotypes of HGV have been described, with 12 percent divergence between them [10,11]. Genotype 1 is predominant in West Africa, genotype 2 in Europe and the USA, genotype 3 in Asia, genotype 4 in Southeast Asia and genotype 5 in South Africa [12]. In Brazil, two reports have described the genotype distribution. Oliveira et al. evaluated 17 GVB-C-infected blood donors using RFLP, of which 10 (59%) were infected by genotype 2b, four infected by genotype 2a (24%), and three by genotype 1 (18%) [13]. Nishiya et al. screened over 1,000 healthy subjects, and analyzed 24 GVB-C-infected patients, using RT-PCR followed by genome sequencing; they found 12 subjects infected by genotype 2a (50%), 10 by genotype 2b (42%), and two by genotype 1 (8.3%) [14].

Although many people are infected with this virus throughout the world, no clear association with a known disease state has been demonstrated. GBV-C is transmitted predominantly through parenteral routes, with a high seroprevalence among intravenous illicit drug users, although sexual transmission has also been reported [15].

Based on the very low density of the virus particles, it appears that GBV-C associates with lipids in human serum, and like HCV, it may use the low density

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lipoprotein receptor for virus binding and entry into target cells [16]. Virus derived from an infectious molecular clone can replicate in CD_4^+ cells in peripheral blood mononuclear cell (PBMC) cultures [17].

GBV-C clearance is common in immunocompetent subjects, occurring in approximately 60 to 75% of GBV-C-infected persons, along with the development of antibodies against the envelope glycoprotein E2 [18]. GBV-C infection has not been associated with any known disease and does not appear to represent a substantial threat to public health. For this reason, the Food and Drug Administration (FDA) has not recommended screening blood donors for GBV-C RNA, although this persistent infection is common, present in approximately 1.8% of American donors and more than 35% of the HIV-infected subjects [19]. In Brazil, the prevalence of HGV in blood donors is higher, being around 9% [20]. In a study conducted in a representative sample of the city of São Paulo, it was found that the prevalence varies according to the age group, peaking in individuals between 30 and 40 years old. The prevalence in children between 5 and 9 years old was found to be high (2.9%), suggesting that other transmission routes, besides parenteral and sexual, are important in this age group [21].

Whereas the presence of antibodies against the envelope protein E2 (anti-E2) indicates viral clearance, the presence of GBV-C RNA in serum or plasma indicates ongoing GBV-C infection [22]. GBV-C RNA can be detected by nucleic acid amplification systems [23-25], and quantification has been accomplished using branched chain DNA assays [26] and real-time (Taq Man) polymerase chain reaction (PCR).

GBV-C viremia is associated with a lower mortality rate among HIV-1 infected patients, slower progression to AIDS, and longer survival once AIDS has developed [26-29]. In another study, these effects were found to be independent of age, HIV-1 load, HCV load, CD_4^+ and CD_8^+ T cell counts, and CC chemokine receptor 5 (CCR5) genotype [30]. It was also observed that serum levels of interleukin-2 (IL-2), IL-12, IL-4, and IL-10 remained relatively stable over time in the GBV-C RNA-positive group, while the GBV-C RNA negative group had a decrease in IL-2 and IL-12 of approximately 85% and 83%, respectively, and IL-4 and IL-10 increased by 654 and 395%, respectively, preserving a TH1 cytokine response [29].

GBV-C virus did not prevent the entry of HIV in the cell or the depletion of T CD_4^+ cells, but it did have an inhibitory effect in replication and HIV growth in cell culture [31]. This effect did not appear to be a result of cellular toxicity, considering that GBV-C replication in peripheral-blood mononuclear cells appeared to be noncytopathic and did not inhibit the synthesis of cellular proteins [31].

Over the past several years, there has been substantial controversy over the interactions between GBV-C and HIV *in vivo*. A number of studies have demonstrated a surprising survival benefit among patients who are coinfected, when compared with those who are only infected with HIV-1. Other studies, however, have not shown such effects. The article by Williams et al. [32] may settle a few aspects of this controversy, since it clearly demonstrates that five to six years after HIV-1 seroconversion patients were more likely to be dead if they were infected only with HIV-1 than if they were coinfected with both HIV-1 and GBV-C. This significant survival advantage was not observed during the first 12 to 18 months after HIV-1 seroconversion. Likewise, the time dependence could explain the contradictory results of some studies regarding the observed protective effects of GBV-C [32].

As further evidence of the protective effect of dual infections, Williams et al. also found that the course of HIV-1 disease was adversely affected by the clearance of GBV-C viremia [32]. The mechanism of this protective effect remains to be determined and there are a number of possibilities to explain it.

In addition, some studies suggest that HIV-1 replication is directly reduced by GBV-C. The fact that both HIV-1 and GBV-C can infect and replicate within peripheral-blood mononuclear cells suggests that these two viruses interact either directly or indirectly throughout the cell cycle. Various stages of the HIV life cycle may be affected by GBV-C, including retroviral binding and fusion to target cells through the high-affinity receptor and several chemokine

coreceptors, internalization and reverse transcription, integration into the host-cell genome to create the HIV provirus, viral transcription, translation and viral morphogenesis; another possibility is a series of interactions that involve CCR5.

The level of CCR5 expression is an important factor for HIV transmission and disease progression [33]. It was observed that the envelope glycoproteins of GBV-C, specifically E2, bind to CD_{81} on T lymphocytes. This interaction induces a dose-dependent secretion of RANTES, a natural ligand of CCR5, which induces internalization of CCR5 and down-regulation of CCR5 surface expression, with concomitant intracellular accumulation of CCR5 proteins on CD_4^+ and CD_8^+ T lymphocytes of HGV-infected subjects, at a rate of 53 and 36%, respectively. Hence, the internalization of chemokine receptors is an effective mechanism to block cellular entry of HIV into the cells [34,35]. However, it remains unclear why HGV E2 specifically induces RANTES, but not MIP-1αor MIP-1β, which are other ligands of CCR5 [36]. On the other hand, it would be of interest to know whether GBV-C clearance is associated with HIV-mediated destruction of host cells necessary for the production of GBV-C, preceding the loss of CD_4^+ T cells.

There is a long history of interactions between different viruses; in most cases one virus increases the pathogenicity of the other. The interaction between HIV-1 and GBV-C is unusual and curious, and it is beneficial to patients who are dually infected. It is, therefore, of great interest to understand the mechanisms involved in this interaction, because it could result in progress in our understanding of viral pathogenesis and in a contribution towards the development of novel HIV-1 treatment strategies.

References

1. McNair A.N., Main J., Thomas H.C. Interactions of the human immunodeficiency virus and the hepatotropic viruses. Semin Liver Dis **1992**;12:188-96.
2. Ockenga J., Tillmann H.L., Trautwein C., et al. Hepatitis B and C in HIV-infected patients: prevalence and prognostic value. J Hepatol **1997**;27:18-24.
3. Fischer H.P., Willsch E., Bierhoff E., Pfeifer U. Histopathologic findings in chronic hepatitis C. J Hepatol **1996**;24:Suppl:35-42.
4. Pol S., Fontaine H., Carnot F., et al. Predictive factors for development of cirrhosis in parenterally acquired chronic hepatitis C: a comparison between immunocompetent and immunocompromised patients. J Hepatol **1998**;29:12-9.
5. Simons J.N., Leary T.P., Dawson G.J., et al. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med **1995**;1:564-9.
6. Linnen J., Wages J. Jr, Zhang-Keck Z.Y., et al. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science **1996**;271:505-8.
7. Alter H.J. The cloning and clinical implications of HGV and HGBVC. N Engl J Med **1996**;334:1536-7.
8. Tillmann H.L., Heringlake S., Trautwein C., et al. Antibodies against the GB virus C envelope 2 protein before liver transplantation protect against GB virus C de novo infection. Hepatology **1998**;28:379-84.
9. Simons J.N., Desai S.M., Schultz D.E., et al. Translation initiation in GB viruses A and C: evidence for internal ribosome entry and implications for genomic organization. J Virol **1996**;70:6126-35.
10. Okamoto H., Nakao H., Inoue T., et al. The entire nucleotide sequences of two GB virus C/hepatitis G virus isolates of distinct genotypes from Japan. J Gen Virol **1997**;78(4):737-45.
11. Tucker T.J., Smuts H., Eickhaus P., et al. Molecular characterization of the 5' non-coding region of South African GBV-C/HGV isolates: major deletion and evidence for a fourth genotype. J Med Virol **1999**;59(1):52-9.
12. Tucker T.J., Smuts H.E. GBV-C/HGV genotypes: proposed nomenclature for genotypes 1-5. J Med Virol **2000**;62(1):82-3.
13. Oliveira L.A., Martins R.M., Carneiro M.A., et al. Prevalence and genotypes of GB virus C/hepatitis G virus among blood donors in Central Brazil. Mem Inst Oswaldo Cruz. **2002** ;97(7):953-7.
14. Nishiya A.S., Ribeiro-dos-Santos G., Bassit L., et al. Genotype distribution of the GB virus C in citizens of Sao Paulo City, Brazil. Rev Inst Med Trop Sao Paulo **2003**;45(4):213-6.
15. Frey S.E., Homan S.M., Sokol-Anderson M., et al. Evidence for probable sexual transmission of the hepatitis G virus. Clin Infect Dis **2002**;34(8):1033-8.
16. Xiang J., Klinzman D., McLinden J., et al. Characterization of hepatitis G virus (GB-C virus) particles: evidence for a nucleocapsid and expression of sequences upstream of the E1 protein. J Virol **1998**;72:2738-44.

17. Xiang J., Wunschmann S., Schmidt W.N., et al. Fulllength GB virus C (hepatitis G virus) RNA transcripts are infectious in primary CD4-positive T cells. J Virol **2000**;74:9125-33.
18. Thomas D.L., Vlahov D., Alter H.J., et al. Association of antibody to GB virus C (hepatitis G virus) with viral clearance and protection from reinfection. J Infect Dis **1998**;177:539-42.
19. Dawson G.J., Schlauder G.G., Pilot-Matias T.J., et al. Prevalence studies of GB virus-C infection using reverse transcriptase-polymerase chain reaction. J Med Virol **1996**;50:97-103.
20. Bassit L., Kleter B., Ribeiro dos Santos G., et al. Hepatitis G virus: prevalence and sequence in blood donors of São Paulo, Brazil. Vox Sanguinis **1998**;74:83-7.
21. Ribeiro-dos-Santos G., Nishiya A.S., Nascimento C.M., et al. Prevalence of GB virus C (hepatitis G virus) and risk factors for infection in Sao Paulo, Brazil. Eur J Clin Microbiol Infect Dis **2002**;21(6):438-43.
22. Dille B.J., Surowy T.K., Gutierrez R.A., et al. An ELISA for detection of antibodies to the E2 protein of GB virus C. J Infect Dis **1997**;175:458-61.
23. Linnen J., Wages J., Zhang-Keck Z.-Y., et al. Molecular cloning and disease association of hepatitis G virus: A transfusion-transmissible agent. Science **1996**;271:505-8.
24. Simons J.N., Leary T.P., Dawson G.J., et al. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med **1995**;1:564-9.
25. Dawson G.J., Schlauder G.G., Pilot-Matias T.J., et al. Prevalence studies of GB virus-C using reverse-transcriptase polymerase chain reaction. J Med Virol **1996**;50:97-103.
26. Tillmann H.L., Heiken H., Knapik-Botor A., et al. Infection with GB virus C and reduced mortality among HIV-infected patients. N Engl J Med **2001**;345:715-24.
27. Heringlake S., Ockenga J., Tillmann H.L. GB virus C/ hepatitis G virus infection: a favorable prognostic factor in human immunodeficiency virus-infected patients? J Infect Dis **1998**;177:1723-6.
28. Rodriguez B., Valdez H., Lederman M.M. Effect of hepatitis G co-infection on response to antiretroviral treatment in HIV-infected patients. Presented at the 39th Infectious Diseases Society of America Meeting. **2001**; San Francisco, CA. Abstract 698.
29. Nunnari G., Nigro L., Palermo F., et al. Slower progression of HIV-1-infection in HGV/GB virus C co-infected individuals correlates with an intact T helper 1 cytokine profile. Presented at the Retrovirus Conference. **2002**; Seattle, WA .
30. Yeo A.E.T., Matsumoto A., Hisada M., et al. Effect of hepatitis G virus infection on progression of HIV infection in patients with hemophilia: Multicenter Hemophilia Cohort Study. Ann Intern Med **2000**;132:959-63.
31. Xiang J.I., Wünschmann S.A., Diekema D.J., et al. Effect of coinfection with GB virus on survival among patients with HIV infection. N Engl J Med **2001**;345(10):707-14 .
32. Williams C.F., Klinzman D.B.A., Yamashita T.E., et al. Persistent GB Virus C Infection and Survival in HIV-Infected Men. N Engl J Med **2004**;350(10):981-90.
33. Cocchi F., DeVico A.L., Yarchoan R., et al. Higher macrophage inflammatory protein (MIP)-1alpha and MIP-1beta levels from CD_8^+ T cells are associated with asymptomatic HIV-1 infection. Proc Natl Acad Sci USA **2000**,97:13812-7.
34. Brandt S.M., Mariani R., Holland A.U., et al. Association of chemokine-mediated block to HIV entry with coreceptor internalization. J Biol Chem **2002**;277:17291-9.
35. Alkhatib G., Locati M., Kennedy P.E., et al. HIV-1 coreceptor activity of CCR5 and its inhibition by chemokines: independence from G protein signaling and importance of coreceptor downmodulation. Virology **1997**;234:340-8.
36. Nattermann J., Nischalke H.D., Kupfera B.,et al. Regulation of CC chemokine receptor 5 in Hepatitis G virus infection. AIDS **2003**;17(10):1457-62.

4.2 MANUSCRITO 2

Título e autores

Infecção pelo GBV-C em indivíduos recém infectados pelo HIV-1 em Brasil: Prevalência, Genótipos Distribuição, e Impacto na progressão da doença.

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Resumo

A infecção pelo GB vírus C(GBV-C) é freqüente em pacientes infetados com o Vírus da Imunodeficiência humana tipo 1 (HIV-1) devido aos dois vírus apresentarem vias de transmissão semelhantes. O objetivo deste estudo foi determinar a taxa de infecção e características genotípica de GBV-C nesta população. A presença de RNA do GBV-C foi determinada em plasma de 233 pacientes recém infetados pelo HIV pela reação em cadeia da polimerase e quantificada por PCR em tempo real. Os genótipos de GBV-C foram identificados por seqüenciamento. Foi analisada também a carga viral do HIV, o número de linfócitos TCD4+ e o número de linfócitos TCD8+. Nestes pacientes a prevalência da infecção pelo GBV-C foi de 23%. Não houve diferencia significativa entre os pacientes GBV-C positivos ou negativos ou presença de anticorpos com relação à idade, sexo, carga viral do HIV, contagem de linfócitos T CD4+, linfócitos T CD8+ e tratamento antiretroviral. Foi observada uma correlação inversa entre a carga viral do GBV-C e a carga viral do HIV-1 na inclusão dos pacientes e após um ano. Da mesma forma, uma correlação positiva, mas não significativa foi observada entre a carga viral do GBV-C e o número de linfócitos T CD4+. Análise filogenética das seqüencias de GBV-C revelaram como sendo do genótipo 1 e do genótipo 2, classificado como subtipo 2a e 2b. A infecção pelo GBV-C é relativamente comum em pacientes infectados pelo HIV. Este é o primeiro reporte naquele grupo de pacientes e

a prevalência do genótipo 2b de GBV-C neste grupo de estudo coincide com dados de outros grupos e partes do País.

GBV-C Infection in HIV-recently infected subjects in Brazil: Prevalence, Genotype Distribution, and Impact on HIV Disease Progression.

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Summary (236 words)

Background:

GB virus C (GBV-C) infection is frequent in patients infected with the human immunodeficiency virus type 1 (HIV-1) due to similar transmission routes of both viruses. The aim of this study was to determine the rate of infection and genotypic characteristics of GBV-C in this population. The presence of GBV-C RNA was determined in plasma samples of 233 patients infected recently with HIV by reverse transcriptase-nested polymerase chain reaction and quantified by real-time PCR. GBV-C genotypes were determined by direct sequencing. HIV viral load, CD4+ T lymphocyte and CD8+ T lymphocyte count were also tested in all patients. The overall prevalence of GBV-C infection was 23% in HIV patients. There was no significant difference between patients with and without GBV-C infection and Glycoprotein E2 antibody presence regarding age, sex, HIV-1 viral load, CD4+ and CD8+T cell count and treatment with antiretroviral drugs. An inverse correlation was observed between GBV-C load and HIV-1 load at the enrollment and after one year. Also, a positive but no significant correlation was observed between GBV-C load and CD4+ T lymphocyte. Phylogenetic analysis of the GBV-C isolates revealed being genotype 1 and genotype 2, classified as subtype 2a and 2b. GBV-C infection is relatively common in patients infected with HIV. This is the first report in that group of patients and the prevailing GBV-C genotype 2b in this study group concurred with reports from other groups and parts of the Country.

INTRODUCTION

GB virus type C (GBV-C), an enveloped positive stranded RNA virus belonging to the *Flaviviridae* family and closely related to hepatitis C virus (HCV), was identified in serum from individuals with idiopathic hepatitis in 1995 [1-4]. Although infection with GB virus type C is common, it has not been associated with chronic disease nor affects the clinical course in patients with hepatitis A, B, or C [5]. Over the past several years a number of studies have found GBV-C to have a favorable impact on the course of HIV infection [6-9] or HCV [10] while other studies have failed to demonstrate this effect [11-14].

GBV-C is highly prevalent among subjects at high risk for HIV-1 acquisition, as it is transmitted via blood, blood products, intravenous drug use, from mother to child through pregnancy and/or delivery, [15, 16] and is likely to be sexually transmitted. Phylogenetic analyses of GBV-C/HGV isolates have demonstrated the presence of multiple genotypes with consistent geographical clustering. The first three genotypes described were genotype 1 that predominates in West Africa[17]; genotype 2, most present in US and Europe, divided into 2a and 2b [18]; and genotype 3, described in parts of Asia [19-21]. Subsequently, two groups published data on sufficiently distinct isolates from Myanmar and Vietnam [22], classified as genotype 4, and from South African, classified as genotype 5 [23]. One study identified a novel group of GBV-C sequences among individuals living in Indonesia [24]. These isolates, detected in blood donors and hepatitis patients, were designated by the authors as genotype 5. Further analysis of these Indonesian sequences by Muerhoff *et al.* based on phylogenetic

analysis of a small segment of the GBV-C 50-UTR demonstrated that they belong to a novel distinct group from the other five genotypes, and were designated as genotype 6 [25].

The frequency of GBV-C infection in patients with HIV ranges from as low as 13.5% [26] in an hemophiliacs cohort, to 37% in a study including mainly men who have sex with men [27], or 24% [28, 29] and 45% in a subgroup of 56 intravenous drug users (IDU) [30]. In another study the GBV-C frequency observed was 10.97% with no statistically significant difference in two groups: 13.5% among IDUs *vs*. 6.7% among heterosexuals [31], supporting the theory that although the parenteral route is the most effective way of viral spread, other routes such as sexual and intra-familial contact [32] may also play a role in GBV-C transmission. GBV-C infection was also studied in others groups with reported prevalence of 9% [33] to 13.6% in hemodialysis patients [34], 5% to 25% in West and South Amerindian from Venezuela [33] and in 9.7% [35], 8.3% [36] or 1% [37] in blood donors.

Brazilian data started to be available soon after the GBV-C description [38-40], reporting the prevalence of GBV-C genotypes in HIV-1-uninfected groups [35, 41-46]. Because limited data on the epidemiology of GBV-C infection among Brazilian HIV-1-infected patients has been available, the aim of this study was to determine the prevalence, incidence and genotypic distribution of GBV-C in a cohort of recently HIV-1-infected subjects.

PATIENTS AND METHODS

In this prospective study, 245 recently HIV-1-infected, antiretroviral naïve subjects were included in the study. All patients were enrolled as a part of the prospective cohort of recently HIV-1-infected subjects, after providing Institutional Review Board-approved written informed consent. All recently HIV-1-infected patients were identified by STARHS, utilizing the Serologic Testing Algorithm for Recent HIV Seroconversion [47].

The participants were questioned during the medical evaluation for the presence of symptoms suggestive of acute HIV-1-infection syndrome within the four months before the first serological test. All of the necessary laboratory exams were performed in the period 3-4 months after the initial clinical appointment. The initiation of treatment followed the Ministry of Health Guidelines for HIV-1 therapy in Brazil [48], which recommends treatment for patients with CD4+ T cell counts less than 200 cells/μL as well as the consideration of treatment for patients with CD4+ T cell counts between 200 and 350 cells/μL, or in the presence of clinical AIDS. In clinical practice we initiated treatment after confirmation of CD4+ T cell counts less than 300 cells/μL.

The patients were tested for clinical and virological status at enrollment and after one year of follow up. Plasma was separated from blood obtained from the patients and peripheral blood mononuclear cells (PBMC) were obtained from leukapheresis with Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation. Age, gender, race, transmission route, and laboratory data were obtained as previously described [47].

CD4+ and CD8+ T cell counts were performed using a lymphocyte marking technique with CD3, CD4 and CD8 conjugated monoclonal antibodies (TriTest, BD Biosciences, San Diego, California, USA). The plasma RNA measurements were performed using the Amplicor HIV-1 Monitor test, version 1.5 (Roche Diagnostics, Indianapolis, IN, USA) until January 2007, and was then subsequently substituted for the bDNA (branched DNA) (Versant® - bDNA HIV-1 RNA 3.0 ASSAY, Bayer Health Care LLC Tarrytown, NY). Both assays were performed according to manufacturer protocols and carried out at the Immunology and Retrovirology Laboratories in the Department of Infectious Diseases at the Federal University of São Paulo.

All baseline plasma samples were also tested for the presence of E2 antibodies against GBV-C envelope glycoprotein E2 and the presence of GBV-C RNA in plasma was determined by a GBV-C-specific nested reverse-transcriptase-polymerase-chain-reaction assay and GBV-C viral load was determined by real-time PCR. The evaluation was performed after HIV seroconversion in order to establish the prevalence of GBV-C infection near the time of HIV seroconversion and after one year of enrollment.

Detection of E2 antibody

As markers of GBV-C RNA clearance and prior exposure [49] plasma E2 antibodies were detected using an immunoassay using recombinant E2 (mPlate Anti-Hgenv test; Roche Diagnostics, kindly provided by Dietmar Zdunek), in accordance with the manufacturer's instructions. Plates were incubated with diluted (1:20) serum, and E2 antibodies were detected using anti-human IgG peroxidase conjugate and ABTS substrate. In accordance

with the manufacturer's cutoff, an OD < 0.10 was considered to be negative, and an OD ≥ 0.10 was considered to be positive.

Detection and quantification of GBV-C RNA

Viral RNA was extracted from 140 µL plasma samples using QIAamp Viral RNA Mini Kit (QIAGEN Inc, CA), according to the manufacturer's instructions. The quantity of 5 µL of the RNA extracted was diluted in a mix containing 150 ng of random primer (Random Primer –Pharmacia Biotech, Sweden) and 10 mM deoxyribonucleosides trifosfate (dNTPs by Invitrogen Inc.); the solution was kept at 65°C for 5 minutes. cDNA synthesis was carried out by the addition of 200U of Super Script III Reverse transcriptase (Invitrogen Inc, CA) in a buffer solution with 10 U of ribonuclease inhibitor (Invitrogen Inc, CA) at 25°C for 5 minutes, 50°C for 60 minutes and 70°C for 15 minutes at a final volume of 20µL.

A fragment of 344 bp of the 5' non coding region (5 ' NCR) was amplified by nested RT PCR using the followings primers located at positions 108 (5'-AGGTGGTGGATGGGTGAT-3'; sense, outer), 134 (5'-TGGTAGGTCGTAAATCCCGGT-3'; sense, inner), 476 (5'-GGAGCTGGGTGGCCCCATGCAT-3'; antisense, inner) and 531 (5'-TGCCACCCGCCCTCACCCGAA-3'; antisense, outer) [23, 50]. Amplification was over 40 cycles for both first and second rounds of PCR, with the following times and temperatures: 94°C 30 s, 50°C 30 s, and 72°C 30 s for the first round and 94°C 30 s, 60°C 30 s, and 72°C 30 s for the second round. After amplification, 5 µl of the PCR product was used for electrophoresis analysis on a 2% agarose gel.

The GBV-C load was quantified in all GBV-C RNA-positive samples in triplicate by Real-Time PCR using a TaqManTM PCR detection kit (Perkin-Elmer Applied Biosystems). The following oligonucleotides were used in the Real-Time PCR located at positions 111-130 (5'-GTGGTGGATGGGTGATGACA-3'; sense), 192-171(5'-GACCCACCTATAGTGGCTACCA-3', antisense). The HGV specific probe tagged with fluorescence FAM CCGGGATTTACGACCTACC NFQ (MGB = Minor Groove Binder invest de NFQ = non-fluorescent quencher) antisense 154-136 numbered according to Accession NC_001710.1 [51] synthesized by Applied Biosystems. A strongly positive GBV-C RNA plasma bag from an HIV negative blood donor was obtained and serial dilutions of it were used to estimate the assay end-point sensitivity. This corresponded to a 10,000x dilution of the original plasma. Based on that, this standard plasma bag was estimated to contain 10,000 detectable units of HGV-RNA and was therefore used on real-time assays to quantify viral load in HIV patients. Results are provided in relation to this “standard”. The lower limit of detection was 1 arbitrary unit (au)/mL.

Sequencing and genotyping

The 344 bp PCR product was purified with the QIAquick kit (QIAGEN, Germany), and directly sequenced in the MegaBACE – DNA Sequencing with the DYEnamic ET Dye Terminator Cycle Sequencing kit (Amersham Biosciences), following their protocol. The sequences were edited using the SEQUENCHER program (Genecodes) and aligned with the sequences of the 5 main genotypes. The phylogenetic analysis was carried out in 5’NCR fragments with the PHYLIP 3.5c program. Bootstrap values were determined on 100 replicates of the sequence data with the SEQBOOT program. Phylogenetic reconstructions

were generated by the Neighbor-Joining program, and the distances were calculated by the maximum likelihood with the DNADIST program. The consensus tree was found with the CONSENSE program. The GBV-C sequences used in this study were deposited in GenBank under accession numbers…..

Statistical analysis.

Comparisons between groups were carried out using two-sided Student's *t* test, Mann-Whitney or Kruskal-Wallis non-parametric tests. Correlation between outcomes and independent variables was analyzed in linear regression analysis, Spearman non-parametric test, and ANOVA univariate models. To perform the Mann-Whitney tests and *logrank* tests for the comparison of Kaplan-Meier survival curves we used GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA). Values of $p<0.05$ were considered statistically significant.

RESULTS

A total of 233 patients in the Brazilian cohort of recently HIV-1 infected individuals with mean age 30.6 (24.6–36.3) years were enrolled in the study [47] . Twenty one (9.01%) were female and 212 (90.9%) of were male, 191 (91.9%) of the latter reported to have had sex with men (MSM). Among all participants, 138 (59%) were white, 46 (19.7%) were mulatto, and 18 (7.7%) were black. The demographics as well as the baseline characteristics of the cohort, classified according GBV-C and E2 antibody status are described in Table 1.

At the baseline, the median CD4+ T lymphocyte count was 522 cells/μL (interquartile range 25-75% [IQR], 400-698), median CD8+ T lymphocyte count was 902.5 cells/μL (IQR 621.8-1201.5), and the median viral load of 18,417 copies/mL (IQR 3,908-65,750), which in log scale (log_{10}) corresponds to 4.3 (IQR 3.60-4.82), as shown in Table 2. In the same Table, we further describe such laboratory characteristics of the HIV-1 infected patients according to their RNA GBV-C and Glycoprotein E2 antibody status.

Active GBV-C infection (defined by the presence of GBV-C RNA without E2 antibody detection) was present in 54 of the 233 individuals (23%), and past GBV-C infection (defined by the presence of E2 antibody and undetectable GBV-C RNA) was present in 31 of the 143 individuals (22%) (Table 1). Thus, a total of 85 individuals (45%) had evidence of current or previous infection with GBV-C. After one year of fallow up the GBV-C RNA was present in 32 of the 126 individuals (25%), and E2 antibody and undetectable GBV-C RNA in 25 of the 126 individuals (20%).

Laboratory characteristics, did not differ significantly between patients with GBV-C viremia and those without GBV-C viremia or anti-glycoprotein E2 antibodies, at the enrollment [CD4+ T lymphocyte (P=0.7731), CD8+ T lymphocyte (P=0.4827) and HIV-1 viral load (0.9394)], or after one year of enrollment [CD4+ T lymphocyte (P=0.9087), CD8+ T lymphocyte (P=0.1366) and HIV-1 viral load (0.9996)]. Moreover, there was no significant difference between those groups regarding age, sex and ethnicity.

During the first year of follow-up, four men cleared the GBV-C virus and three new cases turned positive for GBV-C RNA, reflecting an annual viral clearance rate of 7.4% and an annual incidence rate of 1.6%. Interestingly, no patient developed E2 antibodies after clearance of GBV-C RNA. From those who cleared de virus, just one was under treatment and from the incident cases, two out of three needed to start on antiretroviral therapy after 48 and 204 days after enrollment, respectively. Between the three groups, no statistically significant difference was observed in the outcomes of the antiretroviral treatment initiation (p=0.3385).

At the baseline, the median viral load of GBV-C was 1,157 au/mL (interquartile range 25-75% [IQR], 276-27,679), which in log scale (log_{10}) corresponds to 3.06 (IQR 2.44-4.44); and twelve month after enrollment the median viral load of GBV-C was 15,563 au/mL (interquartile range 25-75% [IQR], 1,289-58,802), which in log scale (log_{10}) corresponds to 4.19 (IQR 3.08-4.76) as shown in Table 2. We then proceeded with correlation analyses between GBV-C viral load and HIV-1 viral load or CD4+ T cell counts. At the earlier visit, an inverse but not significant correlation (r=-0.24, P=0.1071) was found (Figure 1A), together with no apparent correlation between the GBV-C load and the CD4+ cell count (r= 0.09,

P=0.5069) (data not shown). After one year, when we performed the same analysis excluding those on antiretroviral therapy, a persistent trend of negative correlation between the GBV-C load and the HIV-1 load continued (r=-0.30, P=0.1608) (Figure 1B). At the same time, a trend of positive correlation between the GBV-C load and the CD4+ cell count (r=0.43, P=0.07) was observed in the group of untreated patients (data not shown).

From 54 GBV-C RNA-positive samples, we were able to amplify 45 GBV-C isolates for phylogenetic analysis. The phylogenetic three revealed that 5 (11%) GBV-C strains were grouped as genotype 1, 13 (29%) as genotype 2a and 27 (60%) as genotype 2b (Figure 2). Although, no difference was observed in terms of CD4+ T lymphocytes (P=0.3767), CD8+ T lymphocytes (P=0.4843), and HIV-1 viral load (0.4722), a significant difference was observed in GBV-C load between the subjects infected by distinct genotypes, with the median of 3.917 $\log_{10}$ RNA copies for genotype 2a (interquartile range 25-75% [IQR], 2.887-4.912), 2.705 $\log_{10}$ RNA copies in genotype 2b (interquartile range 25-75% [IQR], 1.872-3.091), and 5.037 $\log_{10}$ RNA copies in genotype 1 (interquartile range 25-75% [IQR], 2.289-6.010) (P=0.0072).

To examine the effect of GBV-C status on survival, we classified the 81 antiretroviral naïve patients from 126 individuals with data for both visits into three mutually exclusive categories: 22 of 32 (68.7%) individuals with persistent GBV-C viremia; 15 of 25 (60%) individuals with a prior GBV-C infection, as indicated by the absence of GBV-C viremia at both visits and the presence of E2 antibody at one or both visit and 44 of 69 (63.7%) individuals who did not have GBV-C viremia or E2 antibody at either visit. One GBV-C RNA-positive individual was also positive for E2 antibody and was excluded from the

analysis. Survival rates after the early visit did not differ significantly between the patient with GBV-C viremia, E2 antibody positive, and those without GBV-C viremia when we compared the outcomes of the CD4+ T cell count drop under 300 cells/μL (Figure 3A). Similarly, when the analysis was restricted to the GBV-C viremic subject classified according the GBV-C genotype, survival curves did not differ significantly (Figure 3B).

DISCUSSION

In this study, the prevalence, rate of infection, and genotypic characteristics of GBV-C virus infection in a cohort of recently HIV-1-infected subjects were described. This cohort is primarily constituted by ethnically diverse young men who have sex with men, with no intravenous drug users predominantly infected by clade B virus in the city of São Paulo [52], which is at the epicenter of the Brazilian epidemic. Evidence of GBV-C infection was documented in 45% of participants (23% with ongoing viral replication and 22% with evidence of previous infection). The prevalence of GBV-C found in our cohort is similar to described among other HIV-positive groups from North America [7] and Europe [6, 28] or higher than other similar group from Argentina [26]. Also, the observed prevalence is higher as compared to previous studies performed in other non HIV-infected Brazilians [35, 41, 42, 44, 53-55]. Actual prevalence of GBV-C should vary according to the particular studied population but it has been flagrant that GBV-C infection is more common in groups with risk factors for percutaneous and sexual transmission of infecting agents.

After one year, clearance of GBV-C was found in 7% of patients, similar to 9% observed in a larger study cohort [7], whereas GBV-C RNA infection was documented in only one subject (1,6%), at a lower rate than observed in a group of patients co-infected with GBV-C, Hepatitis C, and HIV-1 viruses on antiretroviral therapy [56]. The factors that influence the clearance of GBV-C are not known and may depend on the presence of sufficient numbers of CD4+ T cells [6, 57]. Our result do not support the hypothesis of antiretroviral therapy interfering with GBV-C clearance, considering that only one out of the four subjects who cleared GBV-C RNA was on treatment. Likewise, the role of E2 antibody development in

GBV-C RNA clearance remains unclear. In our study, no patients who cleared de GBV-C virus developed E2 antibodies during the first year after enrollment. Whether more time is necessary or the spontaneous resolution of GBV-C viremia is not necessarily followed by the appearance of E2 antibodies [57], we still do not know. We also cannot rule out fluctuating GBV-C RNA levels in the four subjects with undetectable results after baseline positive viremia, which may bounce back up again. A longer follow-up would necessary to assess this possibility.

At enrollment, dual HIV-1 and GBV-C viremic subject exhibit a trend toward an inverse correlation between GBV-C and HIV-1 viral loads (Figure 1A), which was maintained after one year of follow-up if they remained off antiretroviral therapy (Figure 1B). We hypothesized that if GBV-C exert some beneficial effect on HIV infection, then the GBV-C load should correlate with either higher CD4+ cell counts or lower HIV load, because of an inhibition of HIV replication. We found a trend of negative correlation between the GBV-C load and the HIV load but no correlation between the GBV-C load and the number of CD4+ cells. In the same way, the more severe immunodeficiency created over time and the limited ability of the immune system to control viral replication therefore increasing the GBV-C load cannot be an argument to explain this tendency.

The Philogenetic analysis of our GBV-C isolates among HIV-1-infected patients identified genotypes 1, 2a, and 2b consistent to reports from other regions of the country [35, 41, 42]. The isolates were not clustered, since they exhibit closer identity to isolates from USA, Africa, and to other isolates from Brazil .The presence of both genotypes 1 and 2 in Sao Paulo is likely to reflect European and African population origins. Lower GBV-C viral load

also characterized the genotype 2b comparing to the genotypes 2a and 1. Few investigators have examined sequence diversity of GBV-C in their studies of GBV-C and HIV co-infection. A recent study has shown that CD4 cell counts tended to be lower in patients infected with genotype 2a compared with those with genotype 2b [58]. It is difficult to conclude whether the GBV-C genotype influences the clinical outcome due to the limited number of cases in this cohort after such stratification. Larger series may be able to demonstrate whether the genotype has a stronger impact in the clinical course of HIV-1 infection or on surrogate markers of progression. The survival analyses performed in our study suggested that the different genotypes had quite similar outcomes of CD4+ T cell loss overtime, pointing towards similar behavior in that matter.

Exploring the interactions between HIV-1 and GBV-C sheds light in the possible interventions to ameliorate the course of HIV-1 infection. In this work, we performed detailed evaluations in a cohort of recently infected subjects and were able to describe several aspects of GBV-C infection in a population of recently HIV-1-infected subjects in Southeast Brazil. Potential diverse distribution of GBV-C virus may also account for different interaction between both viruses in different world regions. Further studies in different countries and regions are important to address these issues.

REFERENCES

1. Simons JN, Leary TP, Dawson GJ*, et al.* **Isolation of novel virus-like sequences associated with human hepatitis**. *Nat Med* 1995,1:564-569.
2. Simons JN, Pilot-Matias TJ, Leary TP*, et al.* **Identification of two flavivirus-like genomes in the GB hepatitis agent**. *Proc Natl Acad Sci U S A* 1995,92:3401-3405.
3. Muerhoff AS, Leary TP, Simons JN*, et al.* **Genomic organization of GB viruses A and B: two new members of the Flaviviridae associated with GB agent hepatitis**. *J Virol* 1995,69:5621-5630.
4. Schlauder GG, Pilot-Matias TJ, Gabriel GS*, et al.* **Origin of GB-hepatitis viruses**. *Lancet* 1995,346:447-448.
5. Alter MJ, Gallagher M, Morris TT*, et al.* **Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. Sentinel Counties Viral Hepatitis Study Team**. *N Engl J Med* 1997,336:741-746.
6. Tillmann HL, Heiken H, Knapik-Botor A*, et al.* **Infection with GB virus C and reduced mortality among HIV-infected patients**. *N Engl J Med* 2001,345:715-724.
7. Williams CF, Klinzman D, Yamashita TE*, et al.* **Persistent GB virus C infection and survival in HIV-infected men**. *N Engl J Med* 2004,350:981-990.
8. Xiang J, Wunschmann S, Diekema DJ*, et al.* **Effect of coinfection with GB virus C on survival among patients with HIV infection**. *N Engl J Med* 2001,345:707-714.
9. Heringlake S, Ockenga J, Tillmann HL*, et al.* **GB virus C/hepatitis G virus infection: a favorable prognostic factor in human immunodeficiency virus-infected patients?** *J Infect Dis* 1998,177:1723-1726.
10. Berzsenyi MD, Bowden DS, Kelly HA*, et al.* **Reduction in hepatitis C-related liver disease associated with GB virus C in human immunodeficiency virus coinfection**. *Gastroenterology* 2007,133:1821-1830.
11. Birk M, Lindback S, Lidman C. **No influence of GB virus C replication on the prognosis in a cohort of HIV-1-infected patients**. *Aids* 2002,16:2482-2485.
12. Bjorkman P, Flamholc L, Naucler A, Molnegren V, Wallmark E, Widell A. **GB virus C during the natural course of HIV-1 infection: viremia at diagnosis does not predict mortality**. *Aids* 2004,18:877-886.
13. Brumme ZL, Chan KJ, Dong WW*, et al.* **No association between GB virus-C viremia and virological or immunological failure after starting initial antiretroviral therapy**. *Aids* 2002,16:1929-1933.
14. Quiros-Roldan E, Maroto MC, Torti C*, et al.* **No evidence of benefical effect of GB virus type C infection on the course of HIV infection**. *Aids* 2002,16:1430-1431.
15. Supapol WB, Remis RS, Raboud J*, et al.* **Reduced mother-to-child transmission of HIV associated with infant but not maternal GB virus C infection**. *J Infect Dis* 2008,197:1369-1377.
16. Sathar MA, York DF, Gouws E, Coutsoudis A, Coovadia HM. **GB virus type C coinfection in HIV-infected African mothers and their infants, KwaZulu Natal, South Africa**. *Clin Infect Dis* 2004,38:405-409.
17. Muerhoff AS, Simons JN, Leary TP*, et al.* **Sequence heterogeneity within the 5'-terminal region of the hepatitis GB virus C genome and evidence for genotypes**. *J Hepatol* 1996,25:379-384.
18. Muerhoff AS, Smith DB, Leary TP, Erker JC, Desai SM, Mushahwar IK. **Identification of GB virus C variants by phylogenetic analysis of 5'-untranslated and coding region sequences**. *J Virol* 1997,71:6501-6508.

19. Okamoto H, Nakao H, Inoue T*, et al.* **The entire nucleotide sequences of two GB virus C/hepatitis G virus isolates of distinct genotypes from Japan**. *J Gen Virol* 1997,78 (Pt 4):737-745.
20. Mukaide M, Mizokami M, Orito E*, et al.* **Three different GB virus C/hepatitis G virus genotypes. Phylogenetic analysis and a genotyping assay based on restriction fragment length polymorphism**. *FEBS Lett* 1997,407:51-58.
21. Katayama K, Kageyama T, Fukushi S*, et al.* **Full-length GBV-C/HGV genomes from nine Japanese isolates: characterization by comparative analyses**. *Arch Virol* 1998,143:1063-1075.
22. Naito H, Win KM, Abe K. **Identification of a novel genotype of hepatitis G virus in Southeast Asia**. *J Clin Microbiol* 1999,37:1217-1220.
23. Tucker TJ, Smuts H, Eickhaus P, Robson SC, Kirsch RE. **Molecular characterization of the 5' non-coding region of South African GBV-C/HGV isolates: major deletion and evidence for a fourth genotype**. *J Med Virol* 1999,59:52-59.
24. Handajani R, Soetjipto, Lusida MI*, et al.* **Prevalence of GB virus C/Hepatitis G virus infection among various populations in Surabaya, Indonesia, and identification of novel groups of sequence variants**. *J Clin Microbiol* 2000,38:662-668.
25. Muerhoff AS, Dawson GJ, Desai SM. **A previously unrecognized sixth genotype of GB virus C revealed by analysis of 5'-untranslated region sequences**. *J Med Virol* 2006,78:105-111.
26. Massud I, Corti M, de Tezanos Pinto M, Perez Bianco R, Picchio G, Bare P. **[Prevalence of hepatitis G virus infection in a cohort of hemophilic HIV positive patients]**. *Medicina (B Aires)* 2002,62:173-175.
27. Lau DT, Miller KD, Detmer J*, et al.* **Hepatitis G virus and human immunodeficiency virus coinfection: response to interferon-alpha therapy**. *J Infect Dis* 1999,180:1334-1337.
28. Ryt-Hansen R, Katzenstein TL, Gerstoft J, Eugen-Olsen J. **No influence of GB virus C on disease progression in a Danish cohort of HIV-infected men**. *AIDS Res Hum Retroviruses* 2006,22:496-498.
29. Souza IE, Zhang W, Diaz RS, Chaloner K, Klinzman D, Stapleton JT. **Effect of GB virus C on response to antiretroviral therapy in HIV-infected Brazilians**. *HIV Med* 2006,7:25-31.
30. Rey D, Vidinic-Moularde J, Meyer P*, et al.* **High prevalence of GB virus C/hepatitis G virus RNA and antibodies in patients infected with human immunodeficiency virus type 1**. *Eur J Clin Microbiol Infect Dis* 2000,19:721-724.
31. Ramezani A, Mohraz M, Vahabpour R*, et al.* **Frequency of hepatitis G virus infection among HIV positive subjects with parenteral and sexual exposure**. *J Gastrointestin Liver Dis* 2008,17:269-272.
32. Pinho JR, Zanotto PM, Ferreira JL*, et al.* **High prevalence of GB virus C in Brazil and molecular evidence for intrafamilial transmission**. *J Clin Microbiol* 1999,37:1634-1637.
33. Loureiro CL, Alonso R, Pacheco BA*, et al.* **High prevalence of GB virus C/hepatitis G virus genotype 3 among autochthonous Venezuelan populations**. *J Med Virol* 2002,68:357-362.
34. Hosseini-Moghaddam SM, Keyvani H, Samadi M*, et al.* **GB virus type C infection in hemodialysis patients considering co-infection with hepatitis C virus**. *J Med Virol* 2008,80:1260-1263.
35. Levi JE, Contri DG, Lima LP*, et al.* **High prevalence of GB virus C/hepatitis G virus RNA among Brazilian blood donors**. *Rev Inst Med Trop Sao Paulo* 2003,45:75-78.
36. Ribeiro-dos-Santos G, Nishiya AS, Nascimento CM*, et al.* **Prevalence of GB virus C (hepatitis G virus) and risk factors for infection in Sao Paulo, Brazil**. *Eur J Clin Microbiol Infect Dis* 2002,21:438-443.
37. Ramezani A, Gachkar L, Eslamifar A*, et al.* **Detection of hepatitis G virus envelope protein E2 antibody in blood donors**. *Int J Infect Dis* 2008,12:57-61.

38. Lampe E, Saback FL, Yoshida CF, Niel C. **Infection with GB virus C/hepatitis G virus in Brazilian hemodialysis and hepatitis patients and asymptomatic individuals**. *J Med Virol* 1997,52:61-67.
39. Pinho JR, Capacci ML, da Silva LC*, et al.* **Hepatitis G virus/GB virus C in Brazil. Preliminary report**. *Rev Inst Med Trop Sao Paulo* 1996,38:243-246.
40. Pinho JR, da Silva LC. **GB virus C/hepatitis G virus and other putative hepatitis non A-E viruses**. *Rev Inst Med Trop Sao Paulo* 1996,38:441-450.
41. Ramos Filho R, Carneiro MA, Teles SA*, et al.* **GB virus C/hepatitis G virus infection in dialysis patients and kidney transplant recipients in Central Brazil**. *Mem Inst Oswaldo Cruz* 2004,99:639-643.
42. Nishiya AS, Ribeiro-dos-Santos G, Bassit L, Focaccia R, Chamone DF, Sabino EC. **Genotype distribution of the GB virus C in citizens of Sao Paulo City, Brazil**. *Rev Inst Med Trop Sao Paulo* 2003,45:213-216.
43. Pereira LM, Spinelli V, Ximenes RA*, et al.* **Chronic hepatitis C infection: influence of the viral load, genotypes, and GBV-C/HGV coinfection on the severity of the disease in a Brazilian population**. *J Med Virol* 2002,67:27-32.
44. Oliveira LA, Martins RM, Carneiro MA*, et al.* **Prevalence and genotypes of GB virus C/hepatitis G virus among blood donors in Central Brazil**. *Mem Inst Oswaldo Cruz* 2002,97:953-957.
45. Gallian P, Rodrigues V, Cantaloube JF*, et al.* **High prevalence of GB-C/hepatitis G virus in a Brazilian population with helminth infection**. *J Med Virol* 1998,56:310-315.
46. Lampe E, Saback FL, Viazov S, Roggendorf M, Niel C. **Age-specific prevalence and genetic diversity of GBV-C/hepatitis G virus in Brazil**. *J Med Virol* 1998,56:39-43.
47. Kallas EG, Bassichetto KC, Oliveira SM*, et al.* **Establishment of the serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion (STARHS) strategy in the city of Sao Paulo, Brazil**. *Braz J Infect Dis* 2004,8:399-406.
48. **Ministério da Saúde Recomendações para terapia anti-retroviral em adultos e adolescentes infectados peloHIV: 2005/2006**. In: P. Secretaria de Vigilância em Saúde and N.d.D.e. AIDS., Editors; 2007.
49. Thomas DL, Vlahov D, Alter HJ*, et al.* **Association of antibody to GB virus C (hepatitis G virus) with viral clearance and protection from reinfection**. *J Infect Dis* 1998,177:539-542.
50. Jarvis LM, Davidson F, Hanley JP, Yap PL, Ludlam CA, Simmonds P. **Infection with hepatitis G virus among recipients of plasma products**. *Lancet* 1996,348:1352-1355.
51. Linnen J, Wages J, Jr., Zhang-Keck ZY*, et al.* **Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent**. *Science* 1996,271:505-508.
52. Sa-Filho D, Kallas EG, Sanabani S*, et al.* **Characterization of the full-length human immunodeficiency virus-1 genome from recently infected subjects in Brazil**. *AIDS Res Hum Retroviruses* 2007,23:1087-1094.
53. Leao-Filho GC, Lopes EP, Ferraz AA*, et al.* **Hepatitis G virus infection in patients with hepatocellular carcinoma in Recife, Brazil**. *Jpn J Clin Oncol* 2007,37:632-636.
54. Lyra AC, Pinho JR, Silva LK*, et al.* **HEV, TTV and GBV-C/HGV markers in patients with acute viral hepatitis**. *Braz J Med Biol Res* 2005,38:767-775.
55. Watanabe MA, Milanezi CM, Silva WA, Jr.*, et al.* **Molecular investigation of GB virus C RNA in hemodialysis and thalassemics patients from Brazil**. *Ren Fail* 2003,25:67-75.
56. Schwarze-Zander C, Blackard JT, Zheng H*, et al.* **GB virus C (GBV-C) infection in hepatitis C virus (HCV)/HIV-coinfected patients receiving HCV treatment: importance of the GBV-C genotype**. *J Infect Dis* 2006,194:410-419.
57. Van der Bij AK, Kloosterboer N, Prins M*, et al.* **GB virus C coinfection and HIV-1 disease progression: The Amsterdam Cohort Study**. *J Infect Dis* 2005,191:678-685.

58. Muerhoff AS, Tillmann HL, Manns MP, Dawson GJ, Desai SM. **GB virus C genotype determination in GB virus-C/HIV co-infected individuals**. *J Med Virol* 2003,70:141-149.

Table 1: Baseline Characteristics of the HIV-1 infected patients , According to their GB Virus C (GBV-C) and Glycoprotein E2 Antibody status

Characteristic	GBV-C RNA Negative E2 Antibody Negative	GBV-C RNA Negative E2 Antibody Positive N = 31/143 (22%)	GBV-C RNA Positive E2 Antibody Negative N = 54/233 (23%)
Age			
Mean	31.6	31.5	27.7
(IC 25%-75%)	(26.3-38.14)	(26.0-35.6)	(23.9-34.7)
Ethnicity			
White-no.(%)	87 (58.7)	21 (67.7)	30 (55.55)
Mulatto-no.(%)	32 (21.6)	3 (9.7)	11 (20.37)
Black-no.(%)	14 (9.4%)	0 (0.0)	4 (7.4)
Other*-no.(%)	15 (10.1)	7 (22.6)	9 (16.66)
Sex			
Male-no.(%)	136 (91.9)	26 (83.9)	50 (92.6)
Female-no.(%)	12 (8.1)	5 (16.1)	4 (7.4)
Exposure			
HSH**-no.(%)	126 (85.1)	24 (77.4)	45 (83.3)
Heterosexual-no.(%)	22 (14.8)	7 (22.6)	9 (16.7)
Symptoms			
Absent-no.(%)	97 (65.6)	21 (67.7)	36 (66.7)
Present-no.(%)	51 (34.4)	10 (32.2)	18 (33.3)

* indigenous or Asian descendant

** men who have sex with men

Figure Legends

Figure 1: Correlation between GBV-C loads and HIV-1 load. Analysis of the relation between the HIV-1 load and the GBV-C load revealed an inverse correlation at the enrollment (A) (r=-0.24, P=0.1071) and a trend to an inverse correlation after one year in individuals GBV-C positive without treatment, represented as a solid symbol (B) (r=-0.10, P=0.6867). The P values were determined by the Spearman rank correlation test.

Figure 2: Kaplan-Meier estimates of survival among HIV-1 positive patients according to the GBV-C and E2 antibody status (A) and GBV-C genotype distribution (B) comparison the outcomes at the CD4+ T cell count drop under 300 cells/µL. The three men who were negative for GBV-C at the early visit and positive for RNA at the late visit were excluded from this analysis.

Figure 3: Phylogenetic tree constructed with the DNA sequences of 344 bp from the GBV-C 5’NCR fragments from HIV-1/GBV-C positive Brazilian isolates. Phylogenetic reconstructions were generated by the Neighbor-Joining program, and the distances were calculated by the maximum likelihood with the DNADIST program.

Figure 1

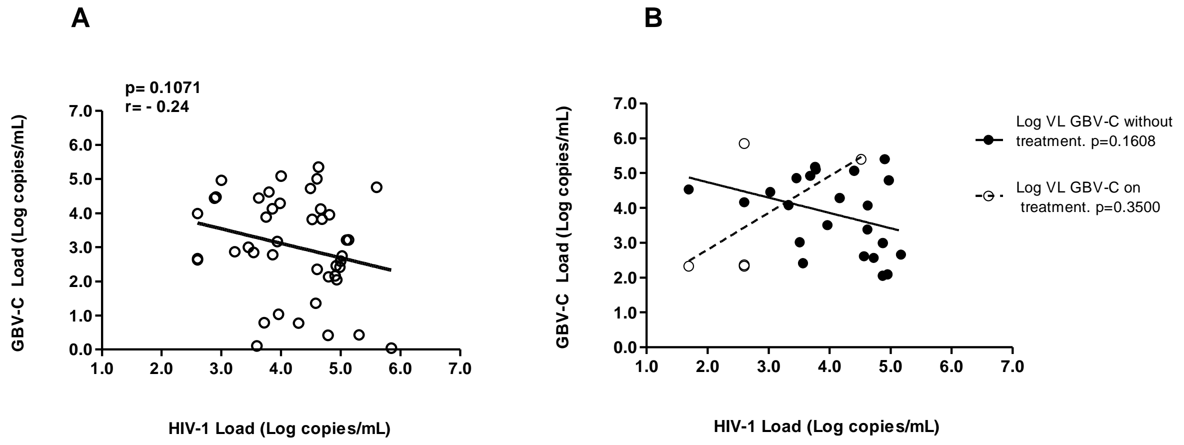


Figure 2

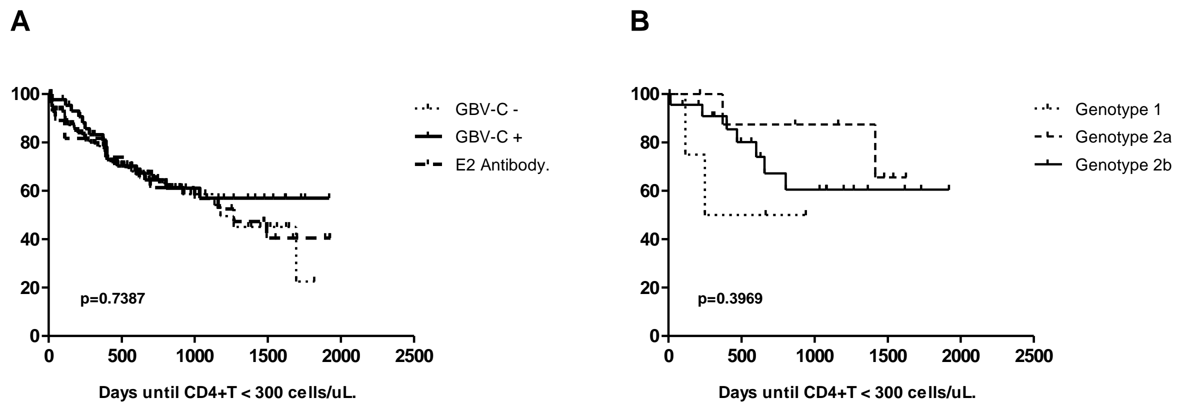
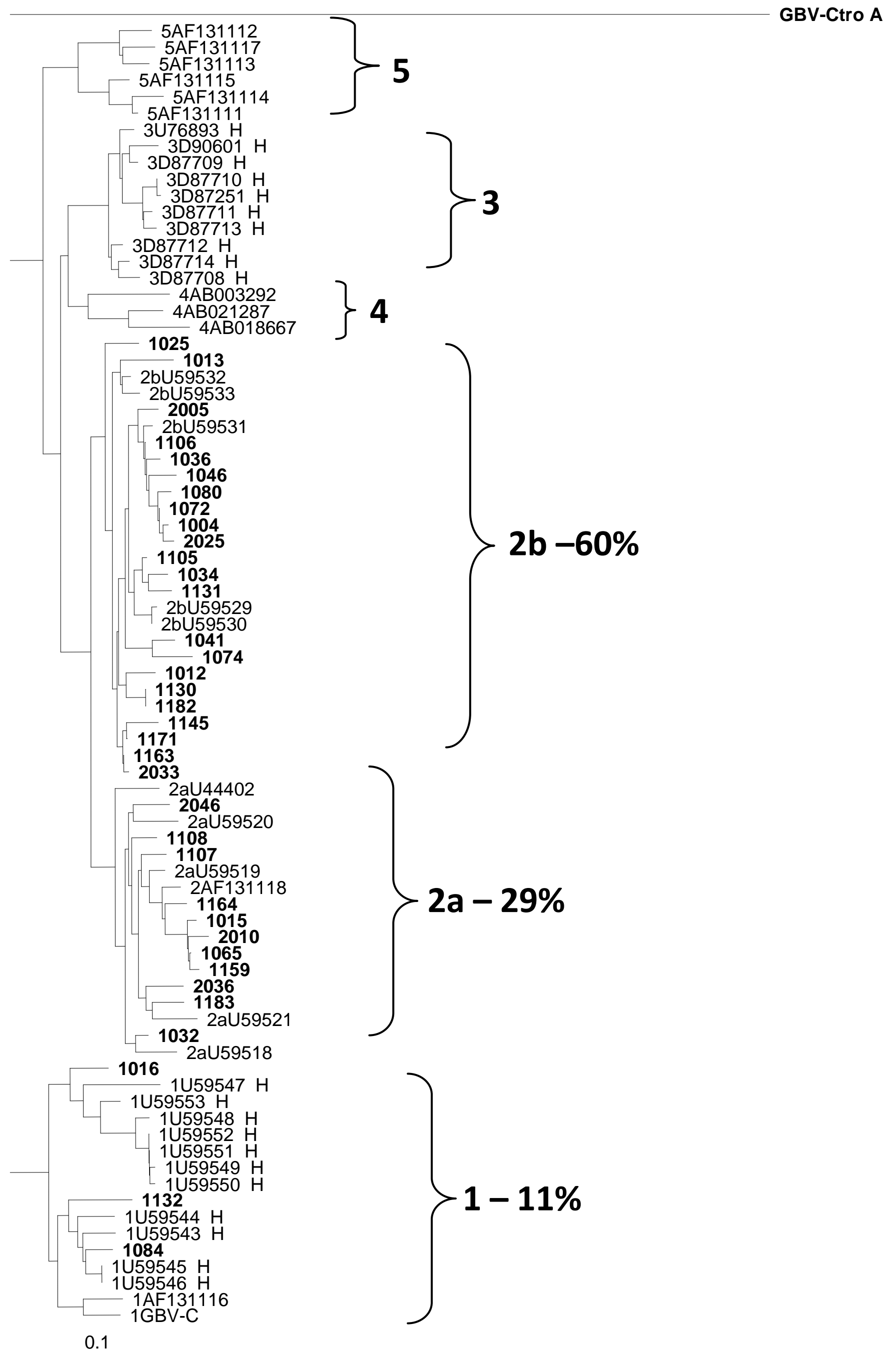


Figure 3



4.3 MANUSCRITO 3

Título e autores

A infecção pelo GBV-C modula a ativação das células T independentemente da carga viral do HIV-1.

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Resumo

Introdução: Muitos estudos clínicos têm sugerido o efeito benéfico do GB vírus C (GBV-C) no curso da infecção pelo HIV-1, mas os mecanismos envolvidos nessa evolução favorável não são claros. Investigações recentes têm relacionado à ativação celular com a patogênese do HIV. Decidimos, por tanto, investigar o efeito da viremia pelo GBV-C na ativação celular das células T na infecção recente pelo HIV.

Métodos: Quarenta e oito pacientes recém infectados pelo HIV-1,(23 virémicos pelo GBV-C) foram avaliados na contagem dos linfócitos T, imunofenotipagem, detecção de RNA do GBV-C e carga viral do HIV-1. Foram realizadas analise não paramétrica univariada e multivariada para identificar variáveis associadas com a ativação celular, que incluíram o estado de viremia pelo GBV-C, carga viral do HIV-1, contagem de linfócitos T CD4+ e CD8+, assim como expressão dos marcadores de superfície CD38 e o receptor de quimiocinas CCR5.

Resultados: Não somente confirmamos a correlação positiva entre a carga viral do HIV-1 e a percentagem de células T CD8+CD38+, se não também observamos que os pacientes virémicos para GBV-C tinham uma menor percentagem de células de células T CD4+CD38+, CD8+CD38+, CD4+CCR5+, e CD8+ CCR5+ comparados com os pacientes HIV-1 positivos não infectados pelo GBV-C. Em modelos de regressão matemática, observamos que a viremia pelo GBV-C estava associada à uma

diminuição da expressão de CD38 nas células T CD4+ e T CD8+ e CCR5 nas células T CD8+, independente da carga viral do HIV-1, contagem de células T CD4+ e células T CD8+. Estes resultados foram sustentados pela menor expressão dos marcadores CD69 e CD25 nos pacientes GBV-C positivos

Conclusão: Os indivíduos coinfectados pelo HIV e GBV-c possuem uma menor ativação celular na fase inicial da infecção. A associação entre a replicação do GBV-C e a menor ativação celular poderiam constituir uns dos mecanismos chaves envolvidos na proteção conferida por este vírus na progressão para a imunodeficiência nos pacientes infectados pelo HIV.

GB virus type C infection modulates T-cell activation independently of HIV-1 viral load

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Background: Many clinical studies have suggested a beneficial effect of GB virus type C (GBV-C) on the course of HIV-1 infection, but the mechanisms involved in such amelioration are not clear. As recent evidence has implicated cellular activation in the HIV-1 pathogenesis, we investigated the effect of GBV-C viremia on T-cell activation in early HIV-1 infection.

Methods: Forty-eight recently infected HIV-1 patients (23 GBV-C viremic) were evaluated for T-cell counts, expanded immunophenotyping GBV-C RNA detection, and HIV-1 viral load. Nonparametric univariate and multivariate analyses were carried out to identify variables associated with cellular activation, including GBV-C status, HIV-1 viral load, T lymphocyte counts, and CD38 and chemokine (C–C motif) receptor 5 (CCR5) surface expression.

Finding: We not only confirmed the positive correlation between HIV-1 viral load and the percentage of T cells positive for $CD38^+CD8^+$ but also observed that GBV-C viremic patients had a lower percentage of T cells positive for $CD38^+CD4^+$, $CD38^+CD8^+$, $CCR5^+CD4^+$, and $CCR5^+CD8^+$ compared with HIV-1-infected patients who were not GBV-C viremic. In regression models, GBV-C RNA^+ status was associated with a reduction in the CCR5 on $CD4^+$ or $CD8^+$ T cells and $CD38^+$ on $CD8^+$ T cells, independent of the HIV-1 viral load or $CD4^+$ and $CD8^+$ T-cell counts. There results were also supported by the lower expression of CD69 and CD25 in GBV-C viremic patients.

Interpretation: The association between GBV-C replication and lower T-cell activation may be a key mechanism involved in the protection conferred by this virus against HIV-1 disease progression to immunodeficiency in HIV-1-infected patients.

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Introduction

GB virus type C [GBV-C, also previously called hepatitis G virus (HGV)] is a common human flavivirus closely related to hepatitis C virus (HCV) [1–3]. GBV-C is not associated with any disease; however, persistent infection is associated with a lower mortality rate among HIV-1-infected patients, slower progression to AIDS, and a

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longer survival period once AIDS has developed, and better response to antiretrovirals [4–8]. Further evidence of this coinfection protective effect is that the course of HIV-1 disease is adversely affected by the clearance of GBV-C viremia [9].

GBV-C is lymphotropic and replicates in T lymphocytes (both CD4$^+$ and CD8$^+$) and B lymphocytes [10–12]. GBV-C infection is very common in HIV-1-infected patients, and up to 43% of HIV-1-infected patients are viremic with GBV-C in cross-sectional studies [13,14]. In addition, concurrent transmission of HIV and GBV-C appears to be relatively common in HIV-1 seroconvertors [15].

The mechanisms of such a protective effect remain elusive. The fact that both HIV-1 and GBV-C can infect and replicate within peripheral blood mononuclear cells (PBMCs) suggests that these two viruses interact either directly or indirectly throughout the cell cycle. One potential mechanism of GBV-C protective effect may involve the downregulation of expression of chemokine (C–C motif) receptor 5 (CCR5) on infected cells, a coreceptor for HIV-1, which influences HIV-1 transmission and progression to immunodeficiency [16]. Because CCR5 is downregulated in GBV-C-infected cells [17,18], it has been hypothesized that GBV-C might also use this HIV-1 coreceptor as a receptor. Jurkat cells, however, do not have detectable CCR5 on their surface and they are permissive to the envelope glycoprotein E2 cell binding. Alternatively, an earlier study suggested that CD81, belong to a family of tetraspanins that presumably acts as a receptors for HCV as it binds to HCV E2 envelope glycoprotein, would be a cellular receptor for GBV-C E2, but a recent study [19] demonstrated that E2 bound to cells independent of CD81 expression, suggesting that the virus could use more than one receptor. Taken together, it is still unclear whether the effect of GBV-C on HIV-1 progression could be a result of an HIV-1 cellular receptor interference.

As in many viral infections, chronic immune activation is a hallmark of progressive HIV disease. Indeed, polyclonal B-cell activation was one of the first immunological abnormalities described in HIV-1-infected patients [20]. Subsequently, increased T-cell turnover [21], increased frequencies of T cells with an activated phenotype [22], and increased serum levels of proinflammatory cytokines and chemokines [23] were also described. Notably, the degree of immune activation can be a better predictor of disease progression than plasma viral load [24]. Therefore, the basis for cellular activation upregulation might be a result of the dynamic interactions occurring *in vivo* between HIV-1 and the immune response [25]. Among the different surrogate markers for cellular activation, CD38 is not only an important prognostic marker but also an active player in HIV-1 pathogenesis. Previous studies [26,27] have shown a positive correlation between the level of viral replication and the activation status of HIV-1-specific CD8$^+$ T cells. In contrast to viremic controls, CD38 expression by HIV-1-specific CD8$^+$ T cells from HIV-1 controllers was very low [28].

As cellular immune response play a critical role in the control of viral replication, dissecting the effect of coinfection with GBV-C on T-cell responses directed against HIV-1 will be important to understand disease progression. Therefore, we decided to explore whether GBV-C could impact cellular activation in the context of HIV-1 infection.

Patients and methods

Study design and patients

A total of 48 recently HIV-1-infected (23 GBV-C viremic), antiretroviral-naive patients were included in the study. All patients were enrolled as a part of the prospective cohort of recently HIV-1-infected patients, after providing Institutional Review Board-approved written informed consent. All recently HIV-1-infected patients were identified by the serologic testing algorithm for recent HIV seroconversion (STARHS) [29].

To investigate the putative protective effect of GBV-C viral infection among HIV-seropositive patients and the effect on T-cell activation, plasma was separated from blood obtained from the patients and PBMCs were obtained from leukapheresis with Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation. Age, sex, race, transmission mode, and laboratory data were obtained as previously described [29]. T lymphocyte counts, and expression of CD38 and CCR5 as well as expanded immunophenotyping were determined by flow cytometry, and HIV plasma viral load was determined using an Amplicor Monitor (Roche Diagnostics Systems, Branchburg, New Jersey, USA), with a lower detection limit of 400 copies/ml.

All baseline plasma samples were also tested for the presence of E2 antibodies against GBV-C envelope glycoprotein E2 and the presence of GBV-C RNA in plasma was determined by reverse-transcription PCR (RT-PCR) after HIV seroconversion in order to establish the prevalence of GBV-C infection near the time of HIV seroconversion. Positive samples were confirmed by a GBV-C-specific nested RT-PCR assay, and GBV-C viral load was determined by real-time PCR.

Detection and quantification of GB virus type C RNA

Viral RNA was extracted from 140 μl plasma samples using QIAamp Viral RNA Mini Kit (QIAGEN Inc., California, USA), according to the manufacturer's instructions. The quantity of 5 μl of the RNA extracted

was diluted in a mix containing 150 ng of random primer (Random Primer, Pharmacia Biotech, Sweden) and 10 mmol/l deoxyribonucleosides triphosphate (dNTP by Invitrogen Inc., Carlsbad, California, USA); the solution was kept at 65°C for 5 min. Complementary DNA (cDNA) synthesis was carried out by the addition of 200 U of Super Script III Reverse transcriptase (Invitrogen Inc.) in a buffer solution with 10 U of ribonuclease inhibitor (Invitrogen Inc.) at 25°C for 5 min, 50°C for 60 min, and 70°C for 15 min at a final volume of 20 μl.

A fragment of the nonstructural 5a region (NS5a) was amplified in a reaction mixture containing 5 μl of the cDNA solution, 1 μl of the primer-mix solution with 10 pmol/μl of NS5a 1–5′-CTCTTTGTGGTAGTAGCCGAGAGAT-3′ and NS5a 2–5′-CGAATGAGTCAGAGGACGGGGTAT-3′ [30], 0.3 μl of Taq-DNA Polymerase 5 U/μl (Invitrogen Inc.), and 1.5 mmol/l $MgCl_2$ (Invitrogen Inc.) to a final volume of 50 μl. The reaction was performed as follows: an initial step at 95°C for 5 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension period of 10 min at 72°C for the final 156 bp amplified fragment. After amplification, 5 μl of the PCR product was used for electrophoresis analysis on a 2% agarose gel. The positive and negative samples were corroborated with nested RT-PCR that amplified a fragment of 344 bp of the 5′ noncoding region (5′ NCR) using the followings primers located at positions 108 (5′-AGGTGGTGGATGGGTGAT-3′; sense, outer), 134 (5′-TGGTAGGTCGTAAATCCCGGT-3′; sense, inner), 476 (5′-GGAGCTGGGTGGCCCCATGCAT-3′; antisense, inner), and 531 (5′-TGCCACCCGCCCTCACCCGAA-3′; antisense, outer) [31,32]. Amplification was over 40 cycles for both first and second rounds of PCR, with the following times and temperatures: 94°C 30 s, 50°C 30 s, and 72°C 30 s for the first round and 94°C 30 s, 60°C 30 s, and 72°C 30 s for the second round. After amplification, 5 μl of the PCR product was used for electrophoresis analysis on a 2% agarose gel.

The GBV-C load was quantified in all GBV-C RNA-positive samples in triplicate by real-time PCR using a TaqMan PCR detection kit (Perkin-Elmer Applied Biosystems, Foster City, California, USA). The following oligonucleotides were used in the real-time PCR located at positions 111–130 (5′-GTGGTGGATGGGTGATGACA-3′; sense), 192–171(5′-GACCCACCTATAGTGGCTACCA-3′, antisense). The HGV-specific probe tagged with fluorescence FAM CCGGGATTTACGACCTACC NFQ (MGB, Minor Groove Binder ao invest de; NFQ, nonfluorescent quencher) antisense 154-136 numbered according to Accession NC_001710.1 [1] synthesized by Applied Biosystems. A strongly positive GBV-C RNA plasma bag from an HIV negative blood donor was obtained and serial dilutions of it were used to estimate the assay endpoint sensitivity. This corresponded to a 10 000× dilution of the original plasma. On the basis of that, this standard plasma bag was estimated to contain 10 000 detectable units of HGV-RNA and was therefore used on real-time assays to quantify viral load in HIV patients. Results are provided in relation to this 'standard'. The lower limit of detection was one arbitrary unit (au)/ml.

Detection of E2 antibody

As markers of GBV-C RNA clearance and prior exposure [33], plasma E2 antibodies were detected using an immunoassay using recombinant E2 (mPlate Anti-Hgenv test; Roche Diagnostics, kindly provided by Dietmar Zdunek) in accordance with the manufacturer's instructions. Plates were incubated with diluted (1 : 20) serum, and E2 antibodies were detected using anti-human immunoglobulin G peroxidase conjugate and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate. In accordance with the manufacturer's cutoff, an optical density (OD) of less than 0.10 was considered to be negative, and an OD of at least 0.10 was considered to be positive.

Monoclonal antibodies and sample preparation

The following monoclonal antibodies (mAbs) were used in cell surface staining assays: tube 1 – CD38 fluorescein isothiocyanate (FITC) clone HI T2, CCR5 phycoerythrin clone 2D7, CD3 peridin chlorophyll protein (PerCP) clone SK7, CD4 R-phycoerythrin cyanine dye Cy7 (phycoerythrin Cy7) clone SK3, CD8-allophycocyanin (APC) clone SK1. Tube 2 – CD25-FITC clone 2A3, Human Leukocyte Antigen DR-1 (HLA-DR)-PerCP clone L243, CD69-phycoerythrin clone L78, CD3-PECy7 clone SK7, CD4-APC clone SK3, CD8 APC carbocyanin 7 (APC-Cy7) clone SK1. mAbs were obtained from Becton Dickinson Immunocytometry Systems (BDIS, San Jose, California, USA), and CCR5-phycoerythrin (clone 2D7) was obtained from PharMingen (San Diego, California, USA).

In all experiments, thawed PBMCs were transferred into 96 V bottom well plates (Nunc, Denmark) with 100 μl of MACS buffer (Miltenyi Biotec, Bergisch Gladbach, Germany) [phosphate buffered saline (PBS) with 500 mmol/l EDTA and 0.5% bovine serum albumin] according to the manufacturer's instructions. Cells were washed and centrifuged at 1500 rpm for 5 min, staining with the surface mAbs panel and incubated at 4°C in darkness for 30 min. After, incubation cells were washed in MACS buffer, centrifuged, and resuspended in 200 μl of fixation buffer 1% paraformaldehyde (Polysciences, Warrington, Pennsylvania, USA in PBS, pH 7.4–7.6) for flow cytometry analysis. Samples were acquired on a FACSCanto, using FACSDiva software (BD Biosciences) and analyzed with FlowJo software (Tree Star, San Carlo, California, USA). Fluorescence voltages were determined using matched unstained cells. Compensation was carried out with CompBeads (BD Biosciences) single stained with CD3-PerCP, CD4-FITC, CD8-APC-Cy7,

CD4-phycoerythrin-Cy7, CD3-phycoerythrin, and CD3-APC. Samples were acquired until at least 100 000 events in a live lymphocyte gate were obtained.

Statistical analysis

Percentage of $CD38^+$ and $CCR5^+$ in $CD4^+$ and $CD8^+$ T cells were used as outcomes in statistical analysis. Comparisons between groups were carried out using two-sided Student's *t* tests or Mann–Whitney nonparametric tests. Correlation between outcomes and independent variables was analyzed in linear regression analysis, Spearman nonparametric test, and ANOVA univariate and multivariate models. All the analyses were performed in SPSS 15.0 (SPSS, Inc., Chicago, Illinois, USA). Values of P less than 0.05 were considered statistically significant.

Results

Study population

A total of 175 patients who acquired HIV infection in the previous 170 days (by STARHS) were evaluated for GBV-C infection. Active GBV-C infection (defined by the presence of GBV-C RNA without E2 antibody) was detected in 42 out of 175 patients (24%), and past GBV-C infection (defined by the presence of E2 antibody without GBV-C RNA) was detected in 31 out of 123 patients (25%). Only one of the RNA-positive patients was also positive for E2 antibody. To study the impact of GBV-C viremia on cellular activation, we randomly selected 48 patients: 23 patients with persistent GBV-C viremia (the persistent GBV-C viremia group), 10 patients with anti-E2-positive (the GBV-C clearance group), and 15 patients without GBV-C viremia of anti-E2 antibody (the HIV-1 monoinfected group) were compared. The baseline demographics and clinical characteristics of the patients are summarized in Table 1. Overall, 46 of the 48 patients (96%) were young men and the median age was 32 years (P_{25-75}, 24.6–35.6). The median $CD4^+$ T-cell count was 519 cells/μl (P_{25-75}, 433–626) and the median viral load was 24 100 HIV-RNA copies/ml (P_{25-75}, 6350–53 200), reflecting their recent HIV-1 infection status.

Correlation between the GB virus type C viral load, HIV viral load, and $CD4^+$ T-cell counts

There were no significant differences regarding age, sex, baseline CD4 cell count, and HIV-1 viral load. However, in a bivariate analysis, we found a weak positive correlation between the GBV-C load and the $CD4^+$ cell count ($r = 0.23$, $P = 0.2953$) (Fig. 1a), and also an inverse, but not statistically significant, correlation was observed between GBV-C viral load and HIV viral load ($r = -0.17$, $P = 0.4533$) (Fig. 1b). The HIV-1-infected patients that were also infected with GBV-C had lower mean HIV viral loads: $4.57 \pm 0.3\ \log_{10}$ copies/ml as compared with $4.99 \pm 0.5\ \log_{10}$ copies/ml for the GBV-C negative/anti-E2-positive patients and $5.02 \pm 0.5\ \log_{10}$ copies/ml for patients solely infected by HIV-1.

HIV-1-infected patients with active GB virus type C infection possess an unusual activation profile

We examined the activation status of the $CD4^+$ and $CD8^+$ T cells by evaluating cell surface expression of CD38 and CCR5 in HIV-1/GBV-C coinfected (active infection) and HIV-1 monoinfected patients. We observed that patients with HIV-1/GBV-C coinfection had lower expression of CD38 on their $CD4^+$ T cells (median 32.9%; P_{25-75}, 13.3–45.3 versus median 48.5%; P_{25-75}, 39.4–58.4) ($P = 0.031$) and lower expression of CD38 on their $CD8^+$ T cells (median 40.1%; P_{25-75}, 17.5–48.5 versus median 66%; P_{25-75}, 48.1–73.8) ($P = 0.007$) than those HIV-1 monoinfected patients (Fig. 2a and b). When we examined the expression of CCR5 on the $CD4^+$ T cells, no statistical difference was observed (median 18.9%; P_{25-75}, 16.1–24.4 versus median 23.6%; P_{25-75}, 18.3–30.7) ($P = 0.3461$). However, the expression of this marker on the $CD8^+$ T cells was also significantly lower in the group of coinfected patients (median 41.5%; P_{25-75}, 29.3–48.4 versus median 59.18%; P_{25-75}, 51.1–65.3) ($P < 0.000$) (Fig. 2c and d), reflecting also the lower activation status in the GBV-C-infected patients. In the multiple comparisons Tukey's honestly significant difference test (HSD) analysis, we examined the same markers, categorizing them now into three groups, according to their GBV-C status: GBV-C nonviremic-anti-E2-negative, GBV-C viremic, and GBV-C nonviremic-anti-E2-positive individuals. When we analyzed expression of CD38 on $CD8^+$ T cells, we found a statistically significant difference between the group of GBV-C nonviremic anti-E2-negative versus GBV-C viremic ($P = 0.042$); and GBV-C viremic versus GBV-C nonviremic-anti-E2-positive patients ($P = 0.012$) (Fig. 2e). The expression of CD38 on $CD4^+$ T cells showed statistically significant difference between the groups of GBV viremic versus GBV-C nonviremic-anti-E2-positive patients ($P = 0.038$) (Fig. 2f). Multivariate regression analyses revealed that the downregulation of these markers was likely caused by the replication of the GBV-C virus rather than the loss of GBV-C.

Relationship between CD38 expression on $CD8^+$ T cells and HIV-1 plasma viremia

We sought to investigate the correlation between the proportion of $CD8^+$ T cells expressing CD38 and the level of HIV-1 plasma viremia in 48 HIV-1-infected patients (including a number of HIV-1/GBV-C coinfected patients). We first confirmed the positive correlation between HIV-1 viral load and CD38 expression on $CD8^+$ T cells ($r = 0.35$, $P < 0.05$) (data not shown). By contrast, GBV-C viral load showed negative correlation with CD38 expression on $CD8^+$ T cells ($r = -0.1609$, $P > 0.05$) (Fig. 2g). To assess the

Table 1. Profiles of HIV-1 and HIV-1/GB virus type C coinfected patients.

Patient	Sex	Age	$CD4^+$ T-cell count/μl	$CD8^+$ T cell count/μl	HIV-1 plasma viremia[a] (copies/ml)	GBV-C plasma viremia[a] [(au)/ml]	E2 antibody status
1	M	57	734	886	16 400	0	P
2	M	43	481	1197	11 200	0	N
3	F	29	519	1558	32 600	0	N
4	M	39	856	1073	3190	0	P
5	M	29	525	302	<400	0	P
6	M	68	469	1484	419 000	0	N
7	M	33	381	757	45 800	0	P
8	M	28	496	1447	39 600	0	N
9	M	34	475	1672	17 400	0	P
10	M	22	1137	1246	<400	0	N
11	F	25	429	1098	6200	0	N
12	M	24	529	482	<400	0	N
13	M	35	591	1158	54 700	0	N
14	M	35	687	633	48 700	0	P
15	M	31	128	375	654 000	0	N
16	M	34	437	4170	657 000	0	P
17	M	37	709	116	6800	0	N
18	M	69	215	329	248 000	0	N
19	M	34	228	1541	154 000	0	P
20	M	39	361	1011	76 300	0	N
21	M	34	447	1120	23 800	0	N
22	M	23	332	716	24 400	0	P
23	M	28	415	843	15 000	0	P
24	F	44	330	569	14 000	0	N
25	M	40	550	657	<400	0	N
26	M	35	581	896	5230	6	N
27	M	36	602	1382	48 700	6540	N
28	M	31			7230	602	N
29	M	22	792	1002	19 800	5	N
30	M	38	450	1280	<400	9742	N
31	M	42	485	1263	61 900	2	N
32	M	30	590	382	1010	92 038	N
33	M	28	726	894	3500	710	N
34	M	24	603	1002	4270	27 800	N
35	M	36	351	410	3960	1	N
36	M	25	891	701	778	27 315	P
37	M	24	534	283	<400	458	N
38	M	29	478	954	9150	10	N
39	M	32	478	988	399 000	56 907	N
40	M	20	585	1772	2870	1003	N
41	M	28	375	4358	62 900	136	N
42	M	22	546	752	42 800	223 000	N
43	M	21	326	925	85 800	111	N
44	M	34	694	1192	38 300	22	N
45	M	23	735	649	817	29 500	N
46	M	35	491	408	31 100	52 500	N
47	M	19	650	840	31 300	76 800	N
48	M	37	788	1147	1000	5610	N

F, female; M, male; N, negative; P, positive.
[a]Measured by Amplicor Monitor, with a detection limit of 399 copies/ml of plasma.

influence of GBV-C status on CD38 expression on $CD8^+$ T cells and HIV-1 viral load, we further investigated the correlation between HIV-1 viral load and CD38 expression on $CD8^+$ T cells. The significant correlation between CD38 on $CD8^+$ T cells and HIV-1 viral load in the group of patients solely infected by HIV-1 ($r = 0.5780$, $P = 0.0025$) was lost if we considered just HIV-1/GBV-C coinfected patients ($r = 0.2047$, $P = 0.3733$) (Fig. 2h). Additionally, in a regression model, we observed that the association between the GBV-C productive infection and the downregulation of CD38 and CCR5 on $CD8^+$ T cells and CD38 on $CD4^+$ T cells was independent of HIV-1 viral load, CCR5Δ32 polymorphism, $CD4^+$ T cell counts, and $CD8^+$ T cell counts, which have been recognized as predictors of HIV-1 diseases progression (Table 2). In addition, the downregulation of the activations markers observed during GBV-C infection may play a role in containment of viral replication in HIV-1-infected patients.

Other activation markers

To confirm the previous results, we performed additional immunophenotyping on a subset of patients to identify activated CD4 and CD8 lymphocytes looking at the

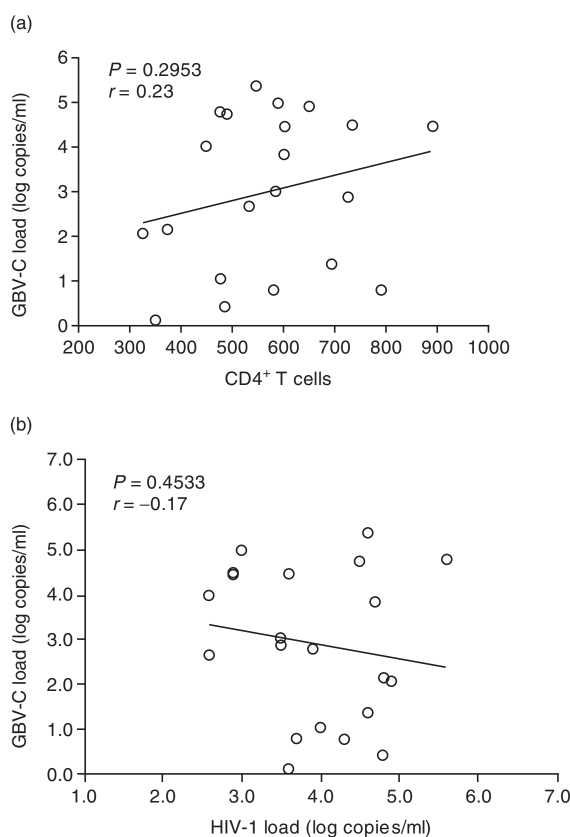

Fig. 1. Correlation between GB virus type C viral loads and $CD4^+$T-cell counts or HIV viral loads. The correlation between the GBV-C viral load and the $CD4^+$ T-cell count revealed a trend to a positive correlation between the two variables (a), whereas between the HIV load and the GBV-C load revealed a trend to an inverse correlation between the two variables (b). The *P* values were determined by the Spearman rank correlation test. GBV-C, GB virus type C.

other activation markers such as HLA-DR, CD25 (a component of the receptor for interleukin 2), and CD69 in the early phase of the immune response. When the same analyses were restricted to the groups of viremic and nonviremic GBV-C patients, we demonstrate that CD69 is also downregulated in HIV-1/GBV-C-positive patients as compared with those HIV-1 singly infected patients on $CD4^+$ T cells (median 2.1%; P_{25-75}, 1.4–2.9 versus median 4.6%; P_{25-75}, 3.4–9.8) ($P = 0.004$) (Fig. 3a) and on $CD8^+$ T cells (median 4.2%; P_{25-75}, 3.4–7.1 versus median 7.6%; P_{25-75}, 6.6–12.6) ($P = 0.022$) (Fig. 3b). When we examined the expression of CD25, we also demonstrated the downregulation of this marker on $CD4^+$ T cells (median 2.7%; P_{25-75}, 2.1–3.3 versus median 10.8%; P_{25-75}, 8.7–12.5) ($P < 0.001$) (Fig. 3c); nevertheless, no statistical difference was observed in CD25-expressing $CD8^+$ T cells (median 0.6%; P_{25-75}, 0.3–1.1 versus median 1.0%; P_{25-75}, 0.8–1.5) ($P = 0.192$) (Fig. 3d). Frequencies of activated $CD4^+$ and $CD8^+$ lymphocytes were comparable between the two study groups when we analyzed the expression of HLA-DR on $CD4^+$ T cells (median 6.7%; P_{25-75}, 4.9–12.2 versus median 8.7%; P_{25-75}, 5.4–10.3) ($P > 0.546$) and HLA-DR on $CD8^+$ T cells (median 16.0%; P_{25-75}, 12.9–32.8 versus median 21.0%; P_{25-75}, 16.6–28.2) ($P = 0.886$) (data not shown). That failure to show the same downregulation by HLA-DR staining may be due to the fact that we worked with frozen samples, which may result in significant changes of its expression on $CD4^+$ and $CD8^+$ T lymphocytes [34].

Discussion

Although immune activation in HIV infection is a subject of increasing interest, no data on activation status are available in HIV-1/GBV-C coinfected patients. Using CD38 expression on T cells as a measure of T-cell activation, together with other activation markers, we assessed the influence of GBV-C viremia during HIV-1 infection. First, we confirmed the positive correlation between HIV-1 viral load and CD38 expression on $CD8^+$ T cells, as previously demonstrated [35,36]. Second, GBV-C viral load was negatively correlated with CD38 expression on $CD8^+$ T cells. Third, the significant correlation between CD38 expression on $CD8^+$ T cells and HIV-1 viral load seen in the patients solely infected by HIV-1 was lost in the HIV-1/GBV-C coinfected patients. Fourth, GBV-C active infection was associated with less $CD4^+$ and $CD8^+$ T-cell activation, measured by different cellular surface markers in HIV-1-infected patients. Fifth, the association between GBV-C active infection and the down-regulation of CD38 and CCR5 on $CD8^+$ T cells and CD38 on $CD4^+$ T cells was independent of HIV-1 viral load, CCR5Δ32 polymorphism, $CD4^+$ T-cell counts, and $CD8^+$ T-cell count. Taken together, these finding suggest that the GBV-C viremia might, in fact, reduce the levels of T-cell activation in HIV-1-infected patients.

Persistent immune activation has been recognized as one of the main factors driving disease progression in HIV-1 infection and is predictive of disease development and treatment outcome [22,27,35,37,38]. The causes of immune activation observed during the acute phase of infection, in contrast to the chronic phase, may include factors in addition to translocation of microbial products [39]. Our results suggest that GBV-C may also interfere in cell activation. We observed that the positive correlation between CD38 expression on $CD8^+$ T cells and HIV-1 viral load was present in patients infected solely by HIV-1, but not in those coinfected with GBV-C. Moreover, a trend towards a negative correlation was observed in the association between HIV-1 viral load and GBV-C viral load. To explore a link between GBV-C infection and cellular activation, we measured the activation profile, through CD38 and CCR5 expression, in three groups categorized according to their GBV-C status. In the

multivariate analysis, surprisingly, the low expression of CD38 and CCR5 on $CD8^+$ T cells and CD38 on $CD4^+$ T cells was observed in the group of GBV-C viremic patients, but not in controls or GBV-C nonviremic anti-E2-seropositive individuals. These results suggest that active GBV-C replication is necessary to down modulate cellular activation, independently of previous exposure to GBV-C. In regression models, we confirmed that this effect was independent of $CD4^+$T-cell counts level, $CD8^+$ T-cell counts, HIV-1 viral load, and CCR5Δ32 polymorphism. One could also argue that the observed effect might be restricted to CD38 and CCR5 expression. However, when we analyzed the other activation markers, downregulation was also seen in CD69 and CD25 expression. In all cases, the GBV-C/HIV-1 coinfected patients exhibited lower levels of these markers with statistically difference in $CD69^+CD4^+$, $CD69^+CD8^+$, and $CD25^+CD4^+$ T cells expression. These observations confirm the role of GBV-C virus in reducing immune activation.

The effect of GBV-C infection was counteract of the high levels of activation and the rapid destructive events at the gut-associated lymphoid tissue seen during the acute phase of HIV-1 infection [40]. Considering that relatively slow progression in the chronic phase of HIV-1 infection is dependent on the degree of immune activation, our findings suggest that this downregulation can also be influenced by the GBV-C viremia.

It has been demonstrated that a substantial proportion of $CD8^+$ T cells expressing high levels of CD38 is highly susceptible to spontaneous and Fas-mediated apoptosis, resulting in a population of cells with poor antiviral effector function [41]. The T-cell activation levels, measured by the CD38 expression on CD8 T cells, appear to be established early in HIV-1 infection. Activation levels decline after successful antiretroviral therapy, but remain elevated when compared with HIV-1-uninfected individuals [35]. This study focused on HIV-1-infected patients before therapy intervention,

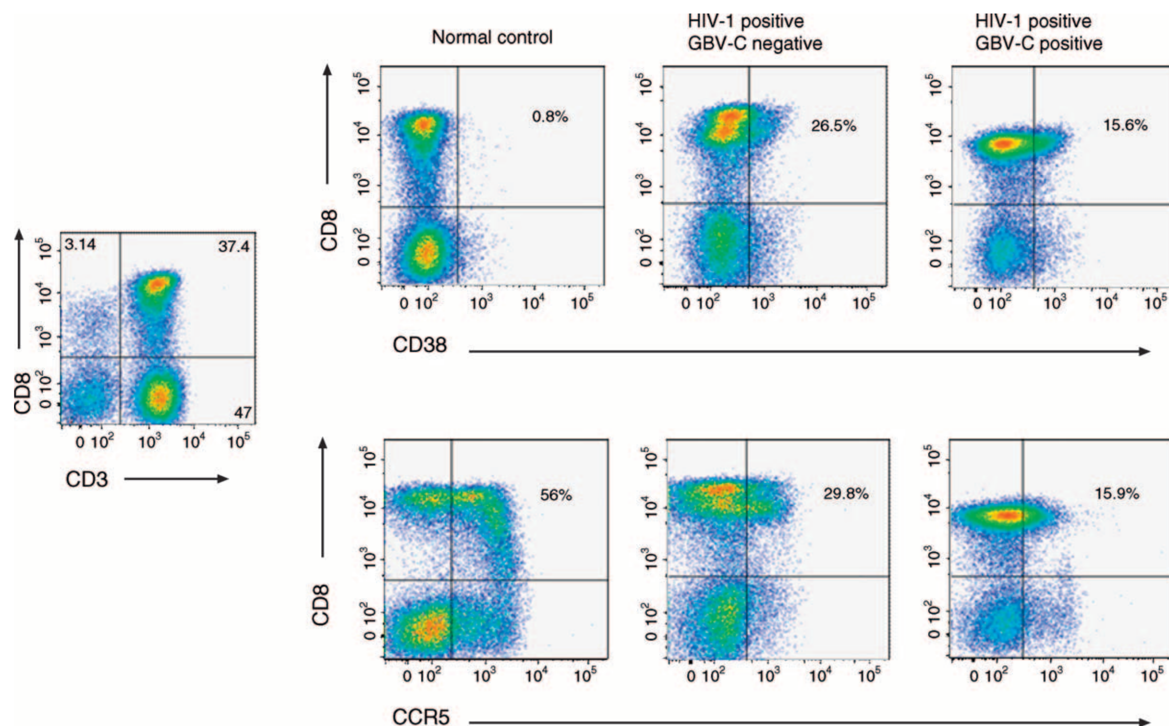

Fig. 2. CD38 expression in GB virus type C nonviremic and viremic patients. On $CD4^+$T cells (a) and $CD8^+$T cells (b) and the CCR5 expression on $CD4^+$T cells (c) and $CD8^+$T cells (d). The level of CD38 and CCR5 expression was determined by flow cytometry by measuring the expression of CD38 and CCR5 on $CD3^+CD8^+$ and $CD3^+CD4^+$ lymphocytes. The *P* values were determined by one-way ANOVA. CD38 expression in GBV-C nonviremic, GBV-C viremic, and GBV-C nonviremic-anti-E2-positive patients on $CD8^+$ T cells (e) and $CD4^+$ T cells (f). The level of CD38 expression was determined by flow cytometry on $CD3^+CD8^+$ T cells and $CD3^+CD4^+$ T cells. The *P* values were determined by one-way ANOVA and the Tukey's HSD multiple comparisons test. CD38 expression on $CD8^+$ T cells and GBV-C viral load (g). The *P* value was determined by the Spearman rank correlation. Correlation between CD38 expression on $CD8^+$ T cells and HIV-1 viral load (h). The open symbols represent the HIV-1/GBV-C-nonviremic patients ($r = 0.5780$, $P = 0.0025$), whereas the solid symbols represent the HIV/GBV-C-viremic patients ($r = 0.2047$, $P = 0.3733$). The *P* values were determined by the Spearman rank correlation. CCR5, chemokine (C–C motif) receptor 5; GBV-C, GB virus type C; HSD, honestly significant difference test; NS, not significant.

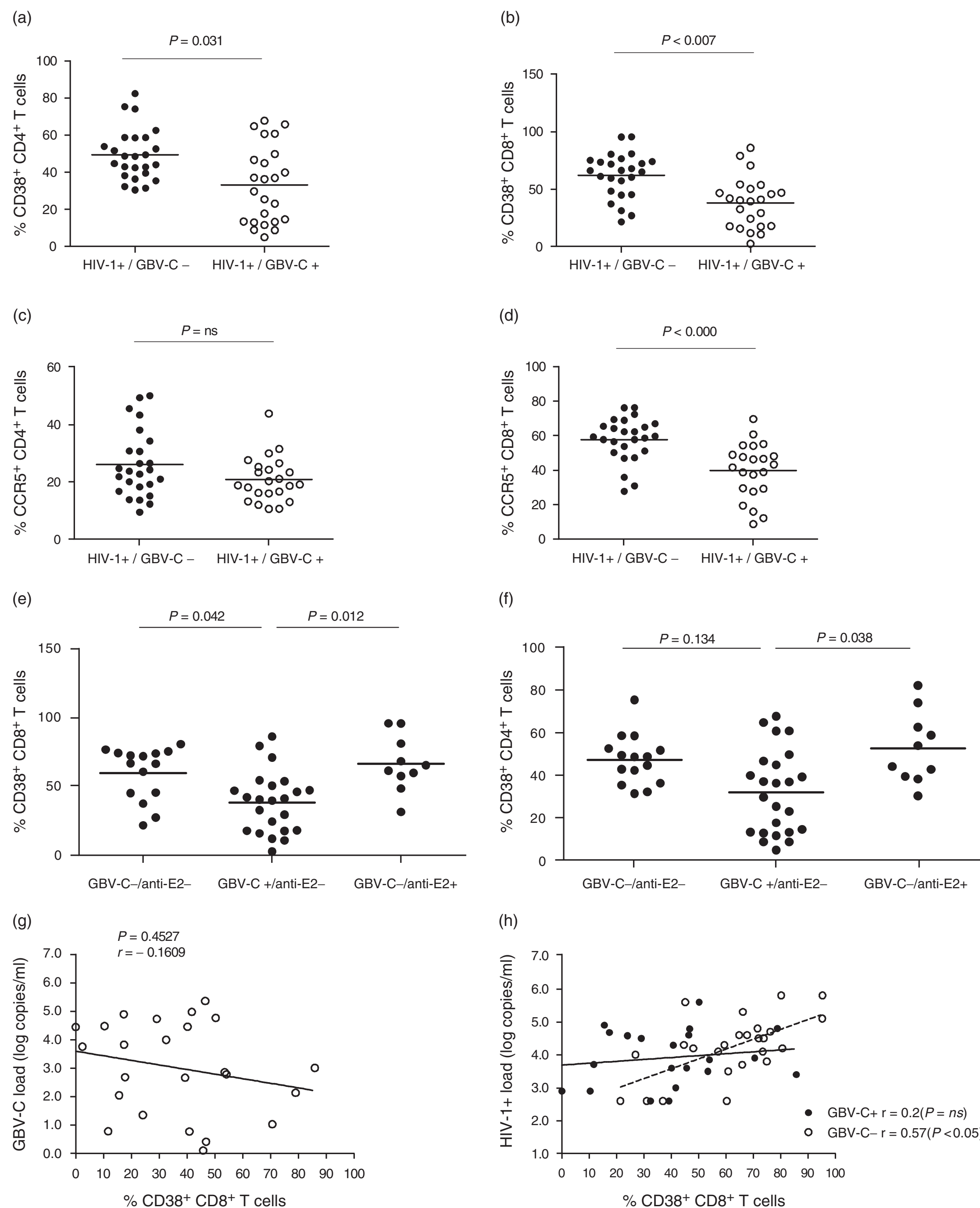

Fig. 2. (*Continued*).

excluding the influence that might be observed after HAART.

We failed to demonstrate a lower expression of CCR5 on CD4 T cells. A fluctuation in the frequency of CCR5 expressing $CD4^+$ T cells during primary infection could be explained in part by the developing hypothesis that $CD4^+$ T cells expressing CCR5 may be short-lived. These cells are constantly undergoing rapid turnover *in vivo* compared with a static population that persists throughout life [42]. Therefore, CCR5 expression can be highly labile and these cells may redistribute throughout the lymphatic system, limiting interpretations of the loss of these cells, as measured in the peripheral blood [43].

The previously described survival advantage conferred by the GBV-C virus among HIV-infected people was dependent on the persistence of GBV-C viremia. Our results are consistent with these observations, suggesting

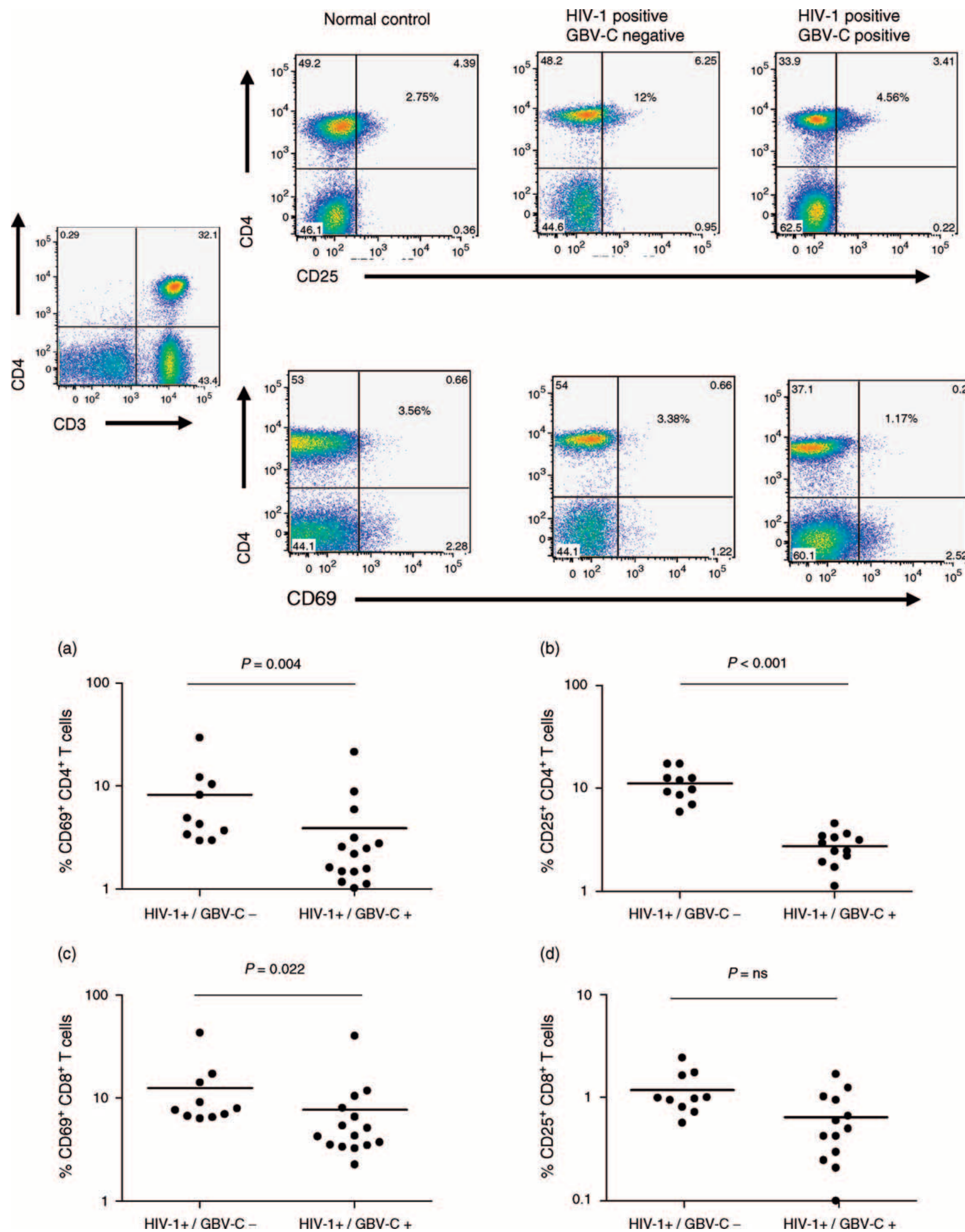

Fig. 3. CD69 expression in GB virus type C nonviremic and viremic patients. On $CD4^+$ T cells (a) and $CD8^+$ T cells (b) and CD25 expression on $CD4^+$ T cells (c) and $CD8^+$ T cells (d). The level of CD69 and CD25 expression was determined by flow cytometry, as depicted in the upper panels. The *P* values were determined by Mann–Whitney test. GBV-C, GB virus type C.

that the increase on activation levels after GBV-C clearance may result in the loss of protective effect of GBV-C and support the notion of GBV-C clearance may also be a contributor for HIV-1 disease progression. Longitudinal studies will be necessary to support the conclusion that GBV-C infection is modulating activation of $CD8^+$ T cells as an important factor for the control of HIV-1 disease progression, reducing the loss of $CD4^+$ cells and development of AIDS.

In summary, we demonstrated that GBV-C infection is associated to a lower T-cell activation independently of

Table 2. Regression model for CD4+ T cells, CD8+ T cells, HIV-1 viral load, chemokine (C–C motif) receptor 5Δ 32 heterozygosis, and GB virus type C infection with chemokine (C–C motif) receptor 5 and CD38 on CD4+ and CD8+ T cells as dependent variables.

	Dependent variables			
	$CD8^+$ T cells		$CD4^+$ T cells	
Parameters of HIV-1 disease progression	$\%CCR5^+$	$\%CD38^+$	$\%CCR5^+$	$\%CD38^+$
$CD4^+$ T cells	NS	NS	NS	NS
$CD8^+$ T cells	NS	NS	2.56 ($P=0.015$)	NS
HIV viral load	2.94 ($P=0.006$)	2.92 ($P=0.006$)	NS	NS
CCR5Δ32 heterozygosis	−2.82 ($P=0.007$)	NS	NS	NS
GBV-C infection	−4.50 ($P<0.001$)	−3.36 ($P=0.007$)	NS	−2.64 ($P=0.011$)

Coefficient and *P* values. CCR5, chemokine (C–C motif) receptor 5; GBV-C, GB virus type C; NS, not significant.

HIV-1 viral load. We recognize that it is not yet possible to discriminate whether the GBV-C replication is a cause or a consequence of the lower T-cell activation, but our findings may reflect the impact of the natural history of GBV-C on HIV-1 disease. Nevertheless, the results suggest that GBV-C infection may be one important mechanism involved in the protection against HIV-1 disease progression and could represent an important topic for future studies, including the development of new therapeutic approaches for HIV infection.

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M.T.M.G. developed the project, performed the experiments, analyzed the results, and wrote the manuscript; T.M.S. performed the experiments and also analyzed the flow cytometry results; M.M.S. followed all the volunteers in the cohort and obtained the clinical data; H.T. organized the sample repository, supported the conduction of experiments, and was in charge of the main database; J.E.L. obtained funds for the study, coordinated the conduction of GBV-C assays, and wrote the manuscript; K.C.B. worked in the set up of the cohort and participated in the volunteers' enrollment; A.N. performed the GBV-C sequence experiments and reviewed the manuscript; R.S.D. obtained funds for the study, coordinated the HIV assays, and reviewed the manuscript; E.C.S. obtained funds for the study, coordinated the GBV-C virus sequencing, and reviewed the manuscript; R.P. did the statistical analyses and reviewed the manuscript; E.G.K. developed the project, obtained funds for the study, reviewed the results, reviewed the statistical analyses, wrote, and reviewed the manuscript.

There are no conflicts of interest.

References

1. Linnen J, Wages J Jr, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, *et al*. **Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent.** *Science* 1996; **271**:505–508.
2. Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, *et al*. **Isolation of novel virus-like sequences associated with human hepatitis.** *Nat Med* 1995; **1**:564–569.
3. Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, *et al*. **Sequence and genomic organization of GBV-C: a novel member of the flaviviridae associated with human non-A-E hepatitis.** *J Med Virol* 1996; **48**:60–67.
4. Rodriguez B, Woolley I, Lederman MM, Zdunek D, Hess G, Valdez H. **Effect of GB virus C coinfection on response to antiretroviral treatment in human immunodeficiency virus-infected patients.** *J Infect Dis* 2003; **187**:504–507.
5. Tillmann HL, Heiken H, Knapik-Botor A, Heringlake S, Ockenga J, Wilber JC, *et al*. **Infection with GB virus C and reduced mortality among HIV-infected patients.** *N Engl J Med* 2001; **345**:715–724.
6. Heringlake S, Ockenga J, Tillmann HL, Trautwein C, Meissner D, Stoll M, *et al*. **GB virus C/hepatitis G virus infection: a favorable prognostic factor in human immunodeficiency virus-infected patients?** *J Infect Dis* 1998; **177**:1723–1726.
7. Nunnari G, Nigro L, Palermo F, Attanasio M, Berger A, Doerr HW, *et al*. **Slower progression of HIV-1 infection in persons with GB virus C co-infection correlates with an intact T-helper 1 cytokine profile.** *Ann Intern Med* 2003; **139**:26–30.
8. Souza IE, Zhang W, Diaz RS, Chaloner K, Klinzman D, Stapleton JT. **Effect of GB virus C on response to antiretroviral therapy in HIV-infected Brazilians.** *HIV Med* 2006; **7**:25–31.
9. Williams CF, Klinzman D, Yamashita TE, Xiang J, Polgreen PM, Rinaldo C, *et al*. **Persistent GB virus C infection and survival in HIV-infected men.** *N Engl J Med* 2004; **350**:981–990.
10. Fogeda M, Navas S, Martin J, Casqueiro M, Rodriguez E, Arocena C, *et al*. **In vitro infection of human peripheral blood mononuclear cells by GB virus C/Hepatitis G virus.** *J Virol* 1999; **73**:4052–4061.

11. George SL, Varmaz D, Stapleton JT. **GB virus C replicates in primary T and B lymphocytes.** *J Infect Dis* 2006; **193**:451–454.
12. Xiang J, Wunschmann S, Schmidt W, Shao J, Stapleton JT. **Full-length GB virus C (Hepatitis G virus) RNA transcripts are infectious in primary CD4-positive T cells.** *J Virol* 2000; **74**:9125–9133.
13. Lefrere JJ, Ferec C, Roudot-Thoraval F, Loiseau P, Cantaloube JF, Biagini P, *et al*. **GBV-C/hepatitis G virus (HGV) RNA load in immunodeficient individuals and in immunocompetent individuals.** *J Med Virol* 1999; **59**:32–37.
14. Rey D, Vidinic-Moularde J, Meyer P, Schmitt C, Fritsch S, Lang JM, *et al*. **High prevalence of GB virus C/hepatitis G virus RNA and antibodies in patients infected with human immunodeficiency virus type 1.** *Eur J Clin Microbiol Infect Dis* 2000; **19**: 721–724.
15. Bisson GP, Strom BL, Gross R, Weissman D, Klinzman D, Hwang WT, *et al*. **Effect of GB virus C viremia on HIV acquisition and HIV set-point.** *Aids* 2005; **19**:1910–1912.
16. Cocchi F, DeVico AL, Yarchoan R, Redfield R, Cleghorn F, Blattner WA, *et al*. **Higher macrophage inflammatory protein (MIP)-1alpha and MIP-1beta levels from $CD8^+$ T cells are associated with asymptomatic HIV-1 infection.** *Proc Natl Acad Sci U S A* 2000; **97**:13812–13817.
17. Xiang J, George SL, Wunschmann S, Chang Q, Klinzman D, Stapleton JT. **Inhibition of HIV-1 replication by GB virus C infection through increases in RANTES, MIP-1alpha, MIP-1beta, and SDF-1.** *Lancet* 2004; **363**:2040–2046.
18. Nattermann J, Nischalke HD, Kupfer B, Rockstroh J, Hess L, Sauerbruch T, *et al*. **Regulation of CC chemokine receptor 5 in hepatitis G virus infection.** *AIDS* 2003; **17**:1457–1462.
19. Kaufman TM, McLinden JH, Xiang J, Engel AM, Stapleton JT. **The GBV-C envelope glycoprotein E2 does not interact specifically with CD81.** *AIDS* 2007; **21**:1045–1048.
20. Lane HC, Masur H, Edgar LC, Whalen G, Rook AH, Fauci AS. **Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome.** *N Engl J Med* 1983; **309**:453–458.
21. Hellerstein M, Hanley MB, Cesar D, Siler S, Papageorgopoulos C, Wieder E, *et al*. **Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans.** *Nat Med* 1999; **5**:83–89.
22. Hazenberg MD, Stuart JW, Otto SA, Borleffs JC, Boucher CA, de Boer RJ, *et al*. **T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART).** *Blood* 2000; **95**:249–255.
23. Valdez H, Lederman MM. **Cytokines and cytokine therapies in HIV infection**. *AIDS Clin Rev* 1997–1998:187–228.
24. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, Jacobson LP, *et al*. **Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage.** *J Infect Dis* 1999; **179**:859–870.
25. Savarino A, Bottarel F, Malavasi F, Dianzani U. **Role of CD38 in HIV-1 infection: an epiphenomenon of T-cell activation or an active player in virus/host interactions?** *AIDS* 2000; **14**:1079–1089.
26. Doisne JM, Urrutia A, Lacabaratz-Porret C, Goujard C, Meyer L, Chaix ML, *et al*. **$CD8^+$ T cells specific for EBV, cytomegalovirus, and influenza virus are activated during primary HIV infection.** *J Immunol* 2004; **173**:2410–2418.
27. Papagno L, Spina CA, Marchant A, Salio M, Rufer N, Little S, *et al*. **Immune activation and $CD8^+$ T-cell differentiation towards senescence in HIV-1 infection.** *PLoS Biol* 2004; **2**:E20.
28. Saez-Cirion A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, *et al*. **HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype.** *Proc Natl Acad Sci U S A* 2007; **104**:6776–6781.
29. Kallas EG, Bassichetto KC, Oliveira SM, Goldenberg I, Bortoloto R, Moreno DM, *et al*. **Establishment of the serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion (STARHS) strategy in the city of Sao Paulo Brazil.** *Braz J Infect Dis* 2004; **8**:399–406.
30. Schlueter V, Schmolke S, Stark K, Hess G, Ofenloch-Haehnle B, Engel AM. **Reverse transcription-PCR detection of hepatitis G virus.** *J Clin Microbiol* 1996; **34**:2660–2664.
31. Jarvis LM, Davidson F, Hanley JP, Yap PL, Ludlam CA, Simmonds P. **Infection with hepatitis G virus among recipients of plasma products.** *Lancet* 1996; **348**:1352–1355.
32. Tucker TJ, Smuts H, Eickhaus P, Robson SC, Kirsch RE. **Molecular characterization of the 5′ noncoding region of South African GBV-C/HGV isolates: major deletion and evidence for a fourth genotype.** *J Med Virol* 1999; **59**:52–59.
33. Thomas DL, Vlahov D, Alter HJ, Hunt JC, Marshall R, Astemborski J, *et al*. **Association of antibody to GB virus C (hepatitis G virus) with viral clearance and protection from reinfection.** *J Infect Dis* 1998; **177**:539–542.
34. Reimann KA, Chernoff M, Wilkening CL, Nickerson CE, Landay AL, The ACTG Immunology Advanced Technology Laboratories. **Preservation of lymphocyte immunophenotype and proliferative responses in cryopreserved peripheral blood mononuclear cells from human immunodeficiency virus type 1-infected donors: implications for multicenter clinical trials.** *Clin Diagn Lab Immunol* 2000; **7**:352–359.
35. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narvaez AB, *et al*. **Immune activation set point during early HIV infection predicts subsequent $CD4^+$ T-cell changes independent of viral load.** *Blood* 2004; **104**:942–947.
36. Giorgi JV, Lyles RH, Matud JL, Yamashita TE, Mellors JW, Hultin LE, *et al*. **Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection.** *J Acquir Immune Defic Syndr* 2002; **29**:346–355.
37. Eggena MP, Barugahare B, Jones N, Okello M, Mutalya S, Kityo C, *et al*. **Depletion of regulatory T cells in HIV infection is associated with immune activation.** *J Immunol* 2005; **174**:4407–4414.
38. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV. **Elevated CD38 antigen expression on $CD8^+$ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than $CD4^+$ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **16**:83–92.
39. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, *et al*. **Microbial translocation is a cause of systemic immune activation in chronic HIV infection.** *Nat Med* 2006; **12**:1365–1371.
40. Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ. **Pathogenesis of HIV infection: what the virus spares is as important as what it destroys.** *Nat Med* 2006; **12**:289–295.
41. Chun TW, Justement JS, Sanford C, Hallahan CW, Planta MA, Loutfy M, *et al*. **Relationship between the frequency of HIV-specific $CD8^+$ T cells and the level of $CD38^+CD8^+$ T cells in untreated HIV-infected individuals.** *Proc Natl Acad Sci U S A* 2004; **101**:2464–2469.
42. Silvestri G, Feinberg MB. **Turnover of lymphocytes and conceptual paradigms in HIV infection.** *J Clin Invest* 2003; **112**: 821–824.
43. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. **Massive infection and loss of memory $CD4^+$ T cells in multiple tissues during acute SIV infection.** *Nature* 2005; **434**:1093–1097.

5 DISCUSSÃO

Os dados do presente estudo sugerem que a infecção pelo GBV-C em pacientes infectados pelo HIV-1 é freqüente e que a ativação celular diminuída nos pacientes poderia explicar, pelo menos em parte, a influência benéfica atribuída ao GBV-C na história natural do HIV-1 por vários outros pesquisadores.

Em 1998 dois grupos reportaram, de forma independentemente, que pacientes infectados pelo HIV e também infectados pelo GBV-C tinham uma sobrevida maior (Heringlake, Ockenga et al. 1998; Toyoda, Fukuda et al. 1998), mas não sabiam a razão dos achados. Foi quando o GBV-C ganhou o interesse de pesquisadores americanos, que afirmaram: "Os pacientes HIV positivos, os coinfectados com GBV-C teriam chance de sobrevida três vezes maior que os pacientes não co infectados'' (Xiang, Wunschmann et al. 2001). Pesquisas subseqüentes de um grupo alemão complementaram os achados mostrando que peptídeos de GBV-C interferem na replicação do HIV, aumentando a produção de substancia química imune que bloqueia a entrada do HIV na célula hospedeira (Tillmann, Heiken et al. 2001).

A infecção pelo HIV é freqüentemente acompanhada de infecção por outros agentes que afetam o curso clinico da infecção. Considerar os aspectos evolutivos desses agentes assim como a escolha dos melhores modelos para estudar a interação podem garantir resultados interessantes. Contudo, a interação entre vírus e outros agentes microbianos no tecido vivo pode ser mais complexa do que o esperado e os agentes infecciosos podem interagir entre eles e com o hospedeiro de maneiras mais imprevisíveis.

Taxas de substituição nucleotídica, ou seja, o numero de alterações mutacionais fixas por sítios nucleotídicos por unidade de tempo, podem ser observados fácilmente em tempo real e permitem elucidar as mudanças que vão surgindo nos vírus RNAs, por exemplo, proporcionando evidências da rápida evolução dos mesmos. Assim, a maioria dos vírus RNA possuem taxas em torno de 10^{-2} a 10^{-3} substituições nucleotídicas por sítio por ano. Um grupo pequeno de vírus RNA, o GBV-C entre eles, apresenta taxas de substituições em torno de 10^{-7} substituições nucleotídicas por sítio por ano, o que poderia ser explicado por longos períodos de latência no hospedeiro e baixas taxas de replicação. Baseado nas taxas de replicação, se presume que o GBV-C é um vírus de evolução lenta e associado com uma infecção crônica mais que aguda (Suzuki, Katayama et al. 1999; Duffy, Shackelton et al. 2008). Assim, os vírus influenciam o sistema imune de forma significativa e eventualmente benéfica, fatos que, baseados nos conceitos de evolução viral não são surpreendentes, se consideramos que têm interagido com o sistema imune por milhões de anos. O GBV-C, assim como outros vírus, estimularia a produção de fatores que influenciam a função imunológica prevenindo ou minimizando futuras infecções mais do que interagindo com outros semelhantes. Outro exemplo desta interação envolve os retrovírus endógenos. E se esses eventos estivessem acontecendo em nível celular, poderia ser mais difícil para o HIV interferir nesses efeitos, o que poderia constituir modelos para o desenvolvimento de drogas terapêuticas

Outros vírus que atuam influenciando a replicação de outros foram descritos, como por exemplo, o HHV-6. Quando infecta a célula aumentando a produção de RANTES, bloqueando a entrada do HIV, pode induzir resistência a essa sustância e à coinfecção, além de exercer uma pressão seletiva gerando vírus altamente resistentes ou mudança de tropismo, mudanças associadas a aumento de virulência. O HHV-7 também inibe a replicação por mecanismos diferentes do HHV-6 (Lisco, Grivel et al. 2007), produzindo uma inibição do receptor CD4.

Por outro lado, descobertas recentes permitiram unificar algumas hipóteses. Ficou claro que a ativação celular é necessária à persistência de níveis elevados de replicação viral no processo de patogêneses da imunodeficiência. Levando em consideração essas afirmações, imaginamos que seria interessante e quase mandatório estudar os mecanismos de ativação celular na nossa coorte durante um período mais extenso e em intervalos talvez menores, acompanhando com outros marcadores imunológicos de resposta imune inata, como já planejamos e começamos a executar,. Além disso, seria importante a avaliação da secreção da ampla gama de citocinas que já está sendo estudada por vários outros grupos. Esperamos ansiosos os desfechos de tais estudos.

Alguns pesquisadores afirmam que se pretendemos estudar determinados agentes patogênicos em modelos de cultura de células, onde não existe sistema imune, não existirão também agentes microbianos que se encontram no estado normal no indivíduo infectado. E como Stapleton menciona, “Estaríamos perdendo fatores muito importantes que influenciam a patogênese”(Wenner 2008).

Assim, foram montados verdadeiros sistemas experimentais para estudar a patogênese do HIV e, com o intuito de reproduzir o que estaria acontecendo no tecido linfóide, utilizaram tecido amigdaliano extraído cirurgicamente. Seguindo esses modelos, seria interessante coinfectar estes “sistemas” com GBV-C e HIV, por exemplo, e pressupondo que o GBV-C é, normalmente, suprimido pero sistema imune, estudar a sua capacidade replicativa no tecido imunocomprometido pela presença do HIV.

Permanecem ainda muitas perguntas que precisam ser respondidas antes de uma completa compreensão do impacto de coinfecção de GBV-C e HIV:

- O que estaria acontecendo com o GBV-C durante o curso de infecção em termos de mudanças nas seqüências do vírus e as alterações que possam ter na resposta imune contra o HIV. Além disso, uma compreensão maior sobre a influência dos diferentes genótipos na progressão, se em realidade existe.

- Evidências claras de replicação nos hepatócitos, como proposto em trabalhos isolados e que ainda não foram totalmente desconsideradas.

- Poucos trabalhos existem ainda estudando a co infecção GBV-C e HBV assim como HCV e HIV.

Realizar mais estudos em coinfecções vem se tornando cada vez mais importantes, inclusive para compreender a patogênese e a história natural da infecção pelo HIV nas diferentes regiões endêmicas. Seremos capazes de elucidar melhor tais aspectos com o avanço do conhecimento na interação de diferentes patógenos com o HIV.

6 CONCLUSÕES

1. A infecção pelo GBV-C é comum em indivíduos recém-infectados pelo HIV-1 na cidade de São Paulo.

2. A prevalência de infecção pelo GBV-C na primeira fase da infecção pelo HIV-1 é semelhante à prevalência após um ano.

3. O genótipo mais freqüente de GBV-C entre os indivíduos estudados é o 2b.

4. No primeiro ano de seguimento, a taxa de incidência de infecção pelo GBV-C foi de 1,6% e a taxa de depuração foi de 7,4%.

5. Há uma tendência a uma correlação negativa entre a carga viral do HIV-1 e a carga viral do GBV-C.

6. Não foi detectada correlação entre a presença da viremia pelo GBV-C ou anticorpos com melhor sobrevida nos pacientes coinfectados.

7. A infecção pelo GBV-C está associada à uma menor ativação celular no indivíduos coinfectados.

8. A diminuição da ativação celular associada ao GBV-C nos pacientes coinfectados foi independente da carga viral do HIV-1, da contagem de linfócitos T CD4+ e CD8+, e da presença do polimorfismo CCR5Δ32

7 REFERÊNCIAS BLIOGRÁFICAS

Alkhatib, G., M. Locati, et al. (1997). "HIV-1 coreceptor activity of CCR5 and its inhibition by chemokines: independence from G protein signaling and importance of coreceptor downmodulation." Virology **234**(2): 340-8.

Alter, H. J. (1996). "The cloning and clinical implications of HGV and HGBV-C." N Engl J Med **334**(23): 1536-7.

Alter, H. J., Y. Nakatsuji, et al. (1997). "The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease." N Engl J Med **336**(11): 747-54.

Barbosa AJ, B. G., Dobo C, Lanzoni VP, Lanzara G, Granato CF. (2008). Análise laboratorial e histologia hepática na infecção pelo GBV-C em pacientes HIV soropositivos coinfectados pelo vírus da Hepatite C. 42 Congresso Brasileiro de Patologia Clínica Medicina Laboratorial. Salvador, BA.

Bassit, L., B. Kleter, et al. (1998). "Hepatitis G virus: prevalence and sequence analysis in blood donors of Sao Paulo, Brazil." Vox Sang **74**(2): 83-7.

Berzsenyi, M. D., D. S. Bowden, et al. (2005). "Male to male sex is associated with a high prevalence of exposure to GB virus C." J Clin Virol **33**(3): 243-6.

Birk, M., S. Lindback, et al. (2002). "No influence of GB virus C replication on the prognosis in a cohort of HIV-1-infected patients." Aids **16**(18): 2482-5.

Bjorkman, P., L. Flamholc, et al. (2004). "GB virus C during the natural course of HIV-1 infection: viremia at diagnosis does not predict mortality." Aids **18**(6): 877-86.

Blankson, J. N., D. Klinzman, et al. (2008). "Low frequency of GB virus C viremia in a cohort of HIV-1-infected elite suppressors." Aids **22**(17): 2398-400.

Brandt, S. M., R. Mariani, et al. (2002). "Association of chemokine-mediated block to HIV entry with coreceptor internalization." J Biol Chem **277**(19): 17291-9.

Brenchley, J. M., N. J. Karandikar, et al. (2003). "Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells." Blood **101**(7): 2711-20.

Brumme, Z. L., K. J. Chan, et al. (2002). "No association between GB virus-C viremia and virological or immunological failure after starting initial antiretroviral therapy." Aids **16**(14): 1929-33.

Chang, Q., J. H. McLinden, et al. (2007). "Expression of GB virus C NS5A protein from genotypes 1, 2, 3 and 5 and a 30 aa NS5A fragment inhibit human immunodeficiency virus type 1 replication in a CD4+ T-lymphocyte cell line." J Gen Virol **88**(Pt 12): 3341-6.

Cocchi, F., A. L. DeVico, et al. (2000). "Higher macrophage inflammatory protein (MIP)-1alpha and MIP-1beta levels from CD8+ T cells are associated with asymptomatic HIV-1 infection." Proc Natl Acad Sci U S A **97**(25): 13812-7.

Dean, M., M. Carrington, 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 Study." Science **273**(5283): 1856-62.

Deeks, S. G., C. M. Kitchen, et al. (2004). "Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load." Blood **104**(4): 942-7.

Deinhardt, F., A. W. Holmes, et al. (1967). "Studies on the transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passages, and description of liver lesions." J Exp Med **125**(4): 673-88.

Dille, B. J., T. K. Surowy, et al. (1997). "An ELISA for detection of antibodies to the E2 protein of GB virus C." J Infect Dis **175**(2): 458-61.

Douek, D. C., L. J. Picker, et al. (2003). "T cell dynamics in HIV-1 infection." Annu Rev Immunol **21**: 265-304.

Duffy, S., L. A. Shackelton, et al. (2008). "Rates of evolutionary change in viruses: patterns and determinants." Nat Rev Genet **9**(4): 267-76.

Estes, J. D., S. N. Gordon, et al. (2008). "Early resolution of acute immune activation and induction of PD-1 in SIV-infected sooty mangabeys distinguishes nonpathogenic from pathogenic infection in rhesus macaques." J Immunol **180**(10): 6798-807.

Feucht, H. H., B. Zollner, et al. (1997). "Prevalence of hepatitis G viremia among healthy subjects, individuals with liver disease, and persons at risk for parenteral transmission." J Clin Microbiol **35**(3): 767-8.

Fogeda, M., S. Navas, et al. (1999). "In vitro infection of human peripheral blood mononuclear cells by GB virus C/Hepatitis G virus." J Virol **73**(5): 4052-61.

Frey, S. E., S. M. Homan, et al. (2002). "Evidence for probable sexual transmission of the hepatitis g virus." Clin Infect Dis **34**(8): 1033-8.

Fried, M. W., Y. E. Khudyakov, et al. (1997). "Hepatitis G virus co-infection in liver transplantation recipients with chronic hepatitis C and nonviral chronic liver disease." Hepatology **25**(5): 1271-5.

Gallian, P., V. Rodrigues, et al. (1998). "High prevalence of GB-C/hepatitis G virus in a Brazilian population with helminth infection." J Med Virol **56**(4): 310-5.

George, S. L., D. Varmaz, et al. (2006). "GB virus C replicates in primary T and B lymphocytes." J Infect Dis **193**(3): 451-4.

Giorgi, J. V., L. E. Hultin, et al. (1999). "Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage." J Infect Dis **179**(4): 859-70.

Grossman, Z., M. B. Feinberg, et al. (1998). "Multiple modes of cellular activation and virus transmission in HIV infection: a role for chronically and latently infected cells in sustaining viral replication." Proc Natl Acad Sci U S A **95**(11): 6314-9.

Grossman, Z., M. Meier-Schellersheim, et al. (2002). "CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause?" Nat Med **8**(4): 319-23.

Hazenberg, M. D., J. W. Stuart, et al. (2000). "T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART)." Blood **95**(1): 249-55.

Hellerstein, M., M. B. Hanley, et al. (1999). "Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans." Nat Med **5**(1): 83-9.

Hellerstein, M. K. and J. M. McCune (1997). "T cell turnover in HIV-1 disease." Immunity **7**(5): 583-9.

Heringlake, S., J. Ockenga, et al. (1998). "GB virus C/hepatitis G virus infection: a favorable prognostic factor in human immunodeficiency virus-infected patients?" J Infect Dis **177**(6): 1723-6.

Hunt, P. W., J. Brenchley, 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**(1): 126-33.

Hunt, P. W., J. N. Martin, et al. (2003). "T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy." J Infect Dis **187**(10): 1534-43.

Katayama, K., T. Kageyama, et al. (1998). "Full-length GBV-C/HGV genomes from nine Japanese isolates: characterization by comparative analyses." Arch Virol **143**(6): 1063-75.

Kaufman, T. M., J. H. McLinden, et al. (2007). "The GBV-C envelope glycoprotein E2 does not interact specifically with CD81." Aids **21**(8): 1045-8.

Kovacs, J. A., R. A. Lempicki, et al. (2001). "Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV." J Exp Med **194**(12): 1731-41.

Lampe, E., F. L. Saback, et al. (1998). "Age-specific prevalence and genetic diversity of GBV-C/hepatitis G virus in Brazil." J Med Virol **56**(1): 39-43.

Lampe, E., F. L. Saback, et al. (1997). "Infection with GB virus C/hepatitis G virus in Brazilian hemodialysis and hepatitis patients and asymptomatic individuals." J Med Virol **52**(1): 61-7.

Lane, H. C., H. Masur, et al. (1983). "Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome." N Engl J Med **309**(8): 453-8.

Lau, D. T., K. D. Miller, et al. (1999). "Hepatitis G virus and human immunodeficiency virus coinfection: response to interferon-alpha therapy." J Infect Dis **180**(4): 1334-7.

Leary, T. P., A. S. Muerhoff, et al. (1996). "Sequence and genomic organization of GBV-C: a novel member of the flaviviridae associated with human non-A-E hepatitis." J Med Virol **48**(1): 60-7.

Lefrere, J. J., C. Ferec, et al. (1999). "GBV-C/hepatitis G virus (HGV) RNA load in immunodeficient individuals and in immunocompetent individuals." J Med Virol **59**(1): 32-7.

Lefrere, J. J., F. Roudot-Thoraval, et al. (1999). "Carriage of GB virus C/hepatitis G virus RNA is associated with a slower immunologic, virologic, and clinical progression of human immunodeficiency virus disease in coinfected persons." J Infect Dis **179**(4): 783-9.

Levi, J. E., D. G. Contri, et al. (2003). "High prevalence of GB virus C/hepatitis G virus RNA among Brazilian blood donors." Rev Inst Med Trop Sao Paulo **45**(2): 75-8.

Linnen, J., J. Wages, Jr., et al. (1996). "Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent." Science **271**(5248): 505-8.

Lisco, A., J. C. Grivel, et al. (2007). "Viral interactions in human lymphoid tissue: Human herpesvirus 7 suppresses the replication of CCR5-tropic human immunodeficiency virus type 1 via CD4 modulation." J Virol **81**(2): 708-17.

Liu, Z., W. G. Cumberland, et al. (1997). "Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression." J Acquir Immune Defic Syndr Hum Retrovirol **16**(2): 83-92.

Loureiro, C. L., R. Alonso, et al. (2002). "High prevalence of GB virus C/hepatitis G virus genotype 3 among autochthonous Venezuelan populations." J Med Virol **68**(3): 357-62.

Moaven, L. D., C. A. Hyland, et al. (1996). "Prevalence of hepatitis G virus in Queensland blood donors." Med J Aust **165**(7): 369-71.

Muerhoff, A. S., G. J. Dawson, et al. (2006). "A previously unrecognized sixth genotype of GB virus C revealed by analysis of 5'-untranslated region sequences." J Med Virol **78**(1): 105-11.

Muerhoff, A. S., T. P. Leary, et al. (1995). "Genomic organization of GB viruses A and B: two new members of the Flaviviridae associated with GB agent hepatitis." J Virol **69**(9): 5621-30.

Muerhoff, A. S., J. N. Simons, et al. (1996). "Sequence heterogeneity within the 5'-terminal region of the hepatitis GB virus C genome and evidence for genotypes." J Hepatol **25**(3): 379-84.

Muerhoff, A. S., D. B. Smith, et al. (1997). "Identification of GB virus C variants by phylogenetic analysis of 5'-untranslated and coding region sequences." J Virol **71**(9): 6501-8.

Mukaide, M., M. Mizokami, et al. (1997). "Three different GB virus C/hepatitis G virus genotypes. Phylogenetic analysis and a genotyping assay based on restriction fragment length polymorphism." FEBS Lett **407**(1): 51-8.

Naito, H., K. M. Win, et al. (1999). "Identification of a novel genotype of hepatitis G virus in Southeast Asia." J Clin Microbiol **37**(4): 1217-20.

Nattermann, J., H. D. Nischalke, et al. (2003). "Regulation of CC chemokine receptor 5 in hepatitis G virus infection." Aids **17**(10): 1457-62.

Nishiya, A. S., G. Ribeiro-dos-Santos, et al. (2003). "Genotype distribution of the GB virus C in citizens of Sao Paulo City, Brazil." Rev Inst Med Trop Sao Paulo **45**(4): 213-6.

Nunnari, G., L. Nigro, et al. (2003). "Slower progression of HIV-1 infection in persons with GB virus C co-infection correlates with an intact T-helper 1 cytokine profile." Ann Intern Med **139**(1): 26-30.

O'Brien, T. R. and J. J. Goedert (1998). "Chemokine receptors and genetic variability: another leap in HIV research." JAMA **279**(4): 317-8.

Okamoto, H., H. Nakao, et al. (1997). "The entire nucleotide sequences of two GB virus C/hepatitis G virus isolates of distinct genotypes from Japan." J Gen Virol **78 (Pt 4)**: 737-45.

Okoye, A., M. Meier-Schellersheim, et al. (2007). "Progressive CD4+ central memory T cell decline results in CD4+ effector memory insufficiency and overt disease in chronic SIV infection." J Exp Med **204**(9): 2171-85.

Oliveira, L. A., R. M. Martins, et al. (2002). "Prevalence and genotypes of GB virus C/hepatitis G virus among blood donors in Central Brazil." Mem Inst Oswaldo Cruz **97**(7): 953-7.

Pantaleo, G. and A. S. Fauci (1996). "Immunopathogenesis of HIV infection." Annu Rev Microbiol **50**: 825-54.

Pavesi, A. (2000). "Detection of signature sequences in overlapping genes and prediction of a novel overlapping gene in hepatitis G virus." J Mol Evol **50**(3): 284-95.

Pereira, L. M., V. Spinelli, et al. (2002). "Chronic hepatitis C infection: influence of the viral load, genotypes, and GBV-C/HGV coinfection on the severity of the disease in a Brazilian population." J Med Virol **67**(1): 27-32.

Pinho, J. R., M. L. Capacci, et al. (1996). "Hepatitis G virus/GB virus C in Brazil. Preliminary report." Rev Inst Med Trop Sao Paulo **38**(3): 243-6.

Pinho, J. R. and L. C. da Silva (1996). "GB virus C/hepatitis G virus and other putative hepatitis non A-E viruses." Rev Inst Med Trop Sao Paulo **38**(6): 441-50.

Pinho, J. R., P. M. Zanotto, et al. (1999). "High prevalence of GB virus C in Brazil and molecular evidence for intrafamilial transmission." J Clin Microbiol **37**(5): 1634-7.

Puig-Basagoiti, F., M. Cabana, et al. (2000). "Prevalence and route of transmission of infection with a novel DNA virus (TTV), hepatitis C virus, and hepatitis G virus in patients infected with HIV." J Acquir Immune Defic Syndr **23**(1): 89-94.

Ramos Filho, R., M. A. Carneiro, et al. (2004). "GB virus C/hepatitis G virus infection in dialysis patients and kidney transplant recipients in Central Brazil." Mem Inst Oswaldo Cruz **99**(6): 639-43.

Rey, D., S. Fraize, et al. (1999). "High prevalence of GB virus C/hepatitis G virus RNA in patients infected with human immunodeficiency virus." J Med Virol **57**(1): 75-9.

Ribeiro-dos-Santos, G., A. S. Nishiya, et al. (2002). "Prevalence of GB virus C (hepatitis G virus) and risk factors for infection in Sao Paulo, Brazil." Eur J Clin Microbiol Infect Dis **21**(6): 438-43.

Rodriguez, B., I. Woolley, et al. (2003). "Effect of GB virus C coinfection on response to antiretroviral treatment in human immunodeficiency virus-infected patients." J Infect Dis **187**(3): 504-7.

Sawayama, Y., J. Hayashi, et al. (1999). "Heterosexual transmission of GB virus C/hepatitis G virus infection to non-intravenous drug-using female prostitutes in Fukuoka, Japan." Dig Dis Sci **44**(10): 1937-43.

Seifried, C., M. Weber, et al. (2004). "High prevalence of GBV-C/HGV among relatives of GBV-C/HGV-positive blood donors in blood recipients and in patients with aplastic anemia." Transfusion **44**(2): 268-74.

Seipp, S., M. Scheidel, et al. (1999). "Hepatotropism of GB virus C (GBV-C): GBV-C replication in human hepatocytes and cells of human hepatoma cell lines." J Hepatol **30**(4): 570-9.

Simmonds, P. (2001). "The origin and evolution of hepatitis viruses in humans." J Gen Virol **82**(Pt 4): 693-712.

Simons, J. N., T. P. Leary, et al. (1995). "Isolation of novel virus-like sequences associated with human hepatitis." Nat Med **1**(6): 564-9.

Simons, J. N., T. J. Pilot-Matias, et al. (1995). "Identification of two flavivirus-like genomes in the GB hepatitis agent." Proc Natl Acad Sci U S A **92**(8): 3401-5.

Smith, D. B., M. Basaras, et al. (2000). "Phylogenetic analysis of GBV-C/hepatitis G virus." J Gen Virol **81**(Pt 3): 769-80.

Souza, I. E., W. Zhang, et al. (2006). "Effect of GB virus C on response to antiretroviral therapy in HIV-infected Brazilians." HIV Med **7**(1): 25-31.

Spellberg, B. and J. E. Edwards, Jr. (2001). "Type 1/Type 2 immunity in infectious diseases." Clin Infect Dis **32**(1): 76-102.

Stapleton, J. T. (2003). "GB virus type C/Hepatitis G virus." Semin Liver Dis **23**(2): 137-48.

Stark, K., U. Bienzle, et al. (1996). "Detection of the hepatitis G virus genome among injecting drug users, homosexual and bisexual men, and blood donors." J Infect Dis **174**(6): 1320-3.

Supapol, W. B., R. S. Remis, et al. (2008). "Reduced mother-to-child transmission of HIV associated with infant but not maternal GB virus C infection." J Infect Dis **197**(10): 1369-77.

Suzuki, Y., K. Katayama, et al. (1999). "Slow evolutionary rate of GB virus C/hepatitis G virus." J Mol Evol **48**(4): 383-9.

Tacke, M., K. Kiyosawa, et al. (1997). "Detection of antibodies to a putative hepatitis G virus envelope protein." Lancet **349**(9048): 318-20.

Tacke, M., S. Schmolke, et al. (1997). "Humoral immune response to the E2 protein of hepatitis G virus is associated with long-term recovery from infection and reveals a high frequency of hepatitis G virus exposure among healthy blood donors." Hepatology **26**(6): 1626-33.

Thomas, D. L., D. Vlahov, et al. (1998). "Association of antibody to GB virus C (hepatitis G virus) with viral clearance and protection from reinfection." J Infect Dis **177**(3): 539-42.

Tillmann, H. L., H. Heiken, et al. (2001). "Infection with GB virus C and reduced mortality among HIV-infected patients." N Engl J Med **345**(10): 715-24.

Tillmann, H. L., M. P. Manns, et al. (2004). "GB virus C infection and quality of life in HIV-positive patients." AIDS Care **16**(6): 736-43.

Toyoda, H., Y. Fukuda, et al. (1998). "Effect of GB virus C/hepatitis G virus coinfection on the course of HIV infection in hemophilia patients in Japan." J Acquir Immune Defic Syndr Hum Retrovirol **17**(3): 209-13.

Tucker, T. J., H. Smuts, et al. (1999). "Molecular characterization of the 5' non-coding region of South African GBV-C/HGV isolates: major deletion and evidence for a fourth genotype." J Med Virol **59**(1): 52-9.

Tucker, T. J., H. E. Smuts, et al. (2000). "Evidence that the GBV-C/hepatitis G virus is primarily a lymphotropic virus." J Med Virol **61**(1): 52-8.

Valdez, H. and M. M. Lederman (1997). "Cytokines and cytokine therapies in HIV infection." AIDS Clin Rev: 187-228.

Van der Bij, A. K., N. Kloosterboer, et al. (2005). "GB virus C coinfection and HIV-1 disease progression: The Amsterdam Cohort Study." J Infect Dis **191**(5): 678-85.

Vargas, H. E., T. Laskus, et al. (1997). "Hepatitis G virus coinfection in hepatitis C virus-infected liver transplant recipients." Transplantation **64**(5): 786-8.

Wenner, M. (2008). "Virology: the battle within." Nature **451**(7177): 388-9.

Williams, C. F., D. Klinzman, et al. (2004). "Persistent GB virus C infection and survival in HIV-infected men." N Engl J Med **350**(10): 981-90.

Xiang, J., K. J. Daniels, et al. (1999). "Visualization and characterization of GB virus-C particles: evidence for a nucleocapsid." J Viral Hepat **6 Suppl 1**: 16-22.

Xiang, J., S. L. George, et al. (2004). "Inhibition of HIV-1 replication by GB virus C infection through increases in RANTES, MIP-1alpha, MIP-1beta, and SDF-1." Lancet **363**(9426): 2040-6.

Xiang, J., D. Klinzman, et al. (1998). "Characterization of hepatitis G virus (GB-C virus) particles: evidence for a nucleocapsid and expression of sequences upstream of the E1 protein." J Virol **72**(4): 2738-44.

Xiang, J., J. H. McLinden, et al. (2008). "Characterization of a peptide domain within the GB virus C NS5A phosphoprotein that inhibits HIV replication." PLoS ONE **3**(7): e2580.

Xiang, J., J. H. McLinden, et al. (2006). "An 85-aa segment of the GB virus type C NS5A phosphoprotein inhibits HIV-1 replication in CD4+ Jurkat T cells." Proc Natl Acad Sci U S A **103**(42): 15570-5.

Xiang, J., S. Wunschmann, et al. (2001). "Effect of coinfection with GB virus C on survival among patients with HIV infection." N Engl J Med **345**(10): 707-14.

Xiang, J., S. Wunschmann, et al. (2000). "Full-length GB virus C (Hepatitis G virus) RNA transcripts are infectious in primary CD4-positive T cells." J Virol **74**(19): 9125-33.

Yeo, A. E., A. Matsumoto, et al. (2000). "Effect of hepatitis G virus infection on progression of HIV infection in patients with hemophilia. Multicenter Hemophilia Cohort Study." Ann Intern Med **132**(12): 959-63.

8 ANEXOS

8.1 MANUSCRITO 4

Título e autores

Freqüência do polimorfismo CCR5Δ32 em coorte de indivíduos recém infectados pelo HIV-1 em São Paulo, Brasil.

Thais Rodrigues, Helena Tomiyama, Sabri Sanabanni, Maria Cecília Araripe Sucupira, Ester C. Sabino, Ricardo S. Diaz, Esper G. Kallas, María Teresa Maidana Giret.

Resumo

Um dos fator genético que influencia a infecção pelo Vírus da Imunodeficiência Humana tipo 1 (HIV-1) e progressão de doença é a deleção de 32 pares de base do gene que codifica o receptor de superfície CCR5. Existem poucos casos de indivíduos hozigotes para esta deleção, infectados pelo HIV no mundo e indivíduos heterozigote para CCR5Δ32 apresentam um risco menor de progressão do estado inicial de infecção para um estado avançado da imunodeficiência. Como a população brasileira é caracterizada por uma mistura de etnia e São Paulo, o epicentro da epidemia, fomos investigar a freqüência do polimorfismo de CCR5 na população de indivíduos recém infectados pelo HIV pela técnica de PCR. Neste trabalho não identificamos nenhum indivíduo homozigoto para essa deleção e encontramos uma freqüência de CCR5Δ32 de 8.7%. Nesse grupo de pacientes houve uma tendência leve, mas não significativo do número de células T CD4+ e diminuição na carga viral do HIV assim como uma tendência de sobrevida maior. Estes resultados podem ser interessantes clinicamente considerando que descrevemos a prevalência do polimorfismo do receptor CCR5 (CCR5Δ32) e associamos aos parâmetros de progressão da doença em uma população recém infectada pelo HIV-1

Frequency of CCR5Δ32 polymorphism in a cohort of recently HIV-1 infected subjects in Sao Paulo, Brazil.

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Summary (190 words)

Background:

One of the genetic factor that influences the infection and disease progression of the human immunodeficiency virus type 1 (HIV-1) is the deletion of 32 base pairs of the gene encoding the cell surface receptor CCR5. Only a few cases of HIV-infected homozygous for this deletion are known and individuals CCR5Δ32 heterozygous have a reduced risk for progressing from initial HIV-1 infection to the development of AIDS. As Brazilian population is characterized by a mixture of ethnicity and Sao Paulo, epicenter of the epidemic, we wanted to investigate the frequency of the CCR5 polymorphism in the group of recently HIV-1 infected patients. In this population, a total of 8.7% were heterozygous, while 91.3% were the wild type for the deletion and none of the infected subjects were homozygous for the Δ 32 allele. A slight but not significant increase in the CD4+Tcells count and lower HIV-1 viral load was found in the CCR5Δ32 subjects as well as a tendency of better survival. This may be important clinically, since we describe the prevalence of CCR5 polymorphism and association with parameters of disease progression in a population of recently HIV-1 infected patients.

INTRODUCTION

Human Imunodeficiency Type 1 Virus (HIV-1) infects the host cells by binding to the CD4 molecule and then interacting in most cases with either chemokine (C-C motif) receptor 5 (CCR5) or the CXC chemokine receptor 4 (CXCR4). After the identification that HIV-1 uses chemokine receptors for cell binding and entry, variants in the genes encoding such receptors and their natural ligands have been shown to modify the risk for infection and disease progression. Homozygosity for a 32-bp deletion in the CCR5 allele (CCR5Δ32) results in an inactive CCR5 gene product, lack of CCR5 expression on the cell surface, and, consequently, confers high resistance against HIV-1 acquisition (1). The CCR5Δ32 heterozygosity has been shown to be significantly associated with slower HIV-1 disease progression (2, 3).

The HIV-1 infection in CCR5Δ32 homozygotes is exceedingly rare, with a total of twelve cases reported worldwide so far (4-14). A very interesting recent case report described a better outcome in a HIV infected patient after stem-cell transplantation from a donor who was homozygous for CCR5Δ32 (15).

The frequency of the allele CCR5Δ32 has been estimated in various ethnic groups (16). This mutation is common among caucasians of Western European descent with a heterozygote frequency close to 20%, and approximately 1% of this population being homozygous for the allele (17, 18). In contrast, the deleted allele is rare among African, Asian and Hispanic and absent in black Africans and the Japanese population (18). For North American caucasians the frequency of CCR5Δ32 heterozygosity was

21.7%, albeit much less common in Afro-Americans (5.8%), hispanics of North American origin (6.9%), and Asian-Americans (0.6%) (19-21). Only a few preliminary studies have been completed in South America, including Argentina (22) and Chile (23), where a not significant difference was observed between HIV-1 infected and non-infected (4%), as well as in hispanic Mexican population (4.4%), but was absent in indigenous Mexican populations (20). The frequency for heterozygosis was found to be 7% in a Brazilian urban population (24) or in a admixed population of healthy individuals defined as white, black and brown at 6.8%, 3.8% and 6.4%, respectively (25). Other study have shown a higher frequency, with 12.5% heterozygotes among HIV-uninfected individuals and 11.5% in HIV-1-infected patients (26).

As the Brazilian population is characterized by a mixture of etnicity and São Paulo constitutes the epicenter of the Brazilian epidemic, the aims of this study were to determine the frequency of CCR5Δ32 in recently HIV-1-infected individuals in São Paulo and explore possible associations of the polimorphism with laboratory parameters such as CD4+ and CD8+ T lymphocytes and HIV-1 viral load.

MATERIALS AND METHODS

Volunteers

Two hundred and twenty nine recently HIV-1-positive patients were analyzed from the Outpatient Clinic at the Federal University of São Paulo in São Paulo, Brazil. These samples were obtained accordance to the ethics standars of the Ethics Committee of the Federal University of Sao Paulo.

T lymphocyte counts and HIV-1 viral loads

CD4+ and CD8+ T cell counts were performed using a lymphocyte marking technique with CD3, CD4 and CD8 conjugated monoclonal antibodies (TriTest, BD Biosciences, San Diego, California, USA). The plasma RNA measurements were performed using the Amplicor HIV-1 Monitor test, version 1.5 (Roche Diagnostics, Indianapolis, IN, USA) until January 2007, which was subsequently substituted by the bDNA (branched DNA) (Versant® - bDNA HIV-1 RNA 3.0 ASSAY, Bayer Health Care LLC Tarrytown, NY). Both assays were performed according to manufacturer protocols.

CCR5 polymorphism

We obtained genomic DNA samples extracted from 300µl of buffy coat using a QIAamp Blood Kit (QIAGEN Inc, CA), using the methodology indicated by the manufacter. The presence of CCR5Δ32 allele was determined by polymerase chain

reaction (PCR) and subsequent gel electrophoresis. The reaction mix of 20μ volume using 0,375 mM of both forward (5' – TCAAAAAGAAGGTCTTCACACC- 3') and reverse (5'- AGCCCAGAAGAGAAAATAAACAATC- 3') primers, 20ng of genomic DNA, 1 unit of Amplitaq Gold (Roche Molecular Systems), and 23,4Mm DNTP's (Roche) in a 1,5 mM MgCl 2 buffer. Thermalcycling commenced by incubating for 10 minutes at 94ºC, and was followed by 40 cycles of melting for 45 seconds at 94ºC, annealing for 45 seconds at 58ºC, and extending for 45 seconds at 72ºC. Subsequently, amplified products were separeted with electrophoresis in a 3% agarose gel for 40 minutes at 110mV and visualized with ethidium bromide under ultraviolet light. The expected PCR product size was 241bp for the wild- type and 209 bp for the CCR5Δ32 alleles (Figure1).

Prediction of HIV-1 coreceptor

Seventy six DNA sequences from the V3 loop region were extracted from an ongoing HIV-1 full genome-sequencing project (Sanabani et al., unpublished results). The data were subsequently translated into amino acids and selected for phenotypic tropism determination using the web-based service geno2pheno [coreceptor] (http://www.geno2pheno.org) which considers all V3 mutational patterns, and not only changes of arginine or lysine at positions 11 or 25.

Statistical analysis.

Comparisons between groups were carried out using two-sided Student's *t* tests or Mann-Whitney non-parametric tests. To perform the Mann-Whitney tests and *logrank* tests for the comparison of Kaplan-Meier survival curves we used GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA). Values of $P < 0.05$ were considered statistically significant.

RESULTS

A total of 229 patient were studied in the Brazilian cohort of recently HIV-1 infected individuals (27) to determine the point prevalence of CCR5Δ32 alleles. This cohort is composed, in the maiority, by men (91.1%) and 83.8% refer to have had sex with men (MSM).

No one in this group was homozygous for the CCR5Δ32 alleles. We found 20 (8.7%) patients heterozygous (CCR5Δ32/CCR5), whereas 209 (91,3%) were homozygous for the wild-type CCR5 allele. Among the heterozygous for the deletion allele (CCR5Δ32/CCR5), 10 (50%) were white, 2 (10%) were black, 5 (25%) were mulattos, and 3 (15%) related to other groups (Table 1).

The median CD4+ T lymphocyte in both CCR5Δ32/CCR5 and CCR5/CCR5 patients were 593 cells/μL (interquartile range 25-75% [IQR], 419-745) and 519 cells/μL (IQR, 392-698) (p=0.4486), respectively; median CD8+ T lymphocyte count was 746 cells/μL (IQR, 609-1066) and 915 cells/μL (IQR, 624-1246) (p=0.2666), and the median viral load of 6,845 copies/mL (IQR, 1,078-47,536) and 19,873 copies/mL (IQR 4,263-71,325) (p=0.1987) as shown in Table 1

The laboratory characteristics did not differ significantly between CCR5Δ32/CCR5 and CCR5/CCR5 patients at the enrollment and also there was no significant difference between those groups regarding age, sex, and ethnicity.

Survival rates after the early visit did not differ significantly between CCR5Δ32/CCR5 and CCR5/CCR5 patients when we set the outcomes at CD4+ T cell count dropping under 300 cells/uL (p=0.2998), but a trend of better outcome was observed in the heterozygous group (Figure 2).

We also repeated the comparative analysis in a small group of patients based in the prediction of HIV-1 coreceptor used and any significant difference was observed in terms of HIV-1 viral load (p= 0.4112), CD4+ T lymphocyte (p= 0.1275) and CD8+ T lymphocyte couts (p= 0.6870)

DISCUSSION

Host and microbial genetics are important determinants of infection and disease outcome. Several single nucleotide polymorphisms (SNPs) within the CC chemokine receptor 5 coding regulatory region, as the promoter region, seem to affect disease progression in HIV-infected subjects (16, 28).

This cohort was primarily constituted by ethnically diverse young men who have sex with men, with no intravenous drug users (27, 29) and predominantly infected by clade B virus in the city of São Paulo (30), which is at the epicenter of the Brazilian epidemic. In this study, none of the subjects are homozygous for the 32 base pair deletion. A considerably frequency of heterozygosis for CCR5Δ32 (8.7%) was observed, but are still lower than that described in other HIV-1-infected and -uninfected populations in Brazil, at 11.5% and 12.5% respectively (26). On the other hand, it is quite similar to the previous funding in the healthy Brazilian population (7%) (24) and other Hispanics of North American origin (6.9%), whereas the same study have shown a frequency of (0.6%) in Asian-Americans (21), slightly lower than in Asian and Indian population (1.3%) described in our cohort. The observed frequencies now considering the white, black and mulatto subjects (4.3%, 0.87%, and 2.1%) were consistent with other Brazilian healthy population (6.8%, 3.8%, and 6.4%), suggesting an European lineage interference.

There is a relationship between genetic polymorphism in host cell co-receptors and disease progression. The onset of AIDS seems to be postponed in individuals

heterozygous for the CCR5Δ32 allele (16, 31, 32). Based on our analyses we were able to detect a trend for CCR5Δ32 positive subjects to display a slighly lower level of HIV-1 viral load than CCR5/CCR5 wild type subjects, as well as higher numbers of CD4+T lymphocytes. These observations are in accordance with previous results from CCR5Δ32 heterozygotes with lower viremia early in the course of the disease (33). Also a trend for better survival has been suggested. Taking together these findings, we can speculate that the presence of CCR5Δ32 may have a rather modest influence in the disease progression, as suggested by others (2, 34).

To better assess the influence of CCR5Δ32 deletion in the HIV-1 susceptibility as well as the frequency, a large number of individuals at different groups of risk would be necessary, considering that the majority of the HIV-1 transmission in homozygous for this deletion was by heterosexual contact (4-8) and, actually, this risk group represent the greater number in the cohorts studied.

This survey has a significant importance, because this is the first description of the prevalence of CCR5 polymorphism and association with parameters of disease progression in recently HIV-1-infected subjects in Brazil. It should also to be noted that different selection criteria exist for the various populations studied in different countries and we cannot consider them as fully representative of the respective source populations. Therefore, we believe that the frequency found in this study has the potential to be a better representation of subjects who are acquiring HIV-1 infection in Brazil.

REFERENCES

1. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell1996 Aug 9;86(3):367-77.

2. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, et al. 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 Study. Science1996 Sep 27;273(5283):1856-62.

3. Mulherin SA, O'Brien TR, Ioannidis JP, Goedert JJ, Buchbinder SP, Coutinho RA, et al. Effects of CCR5-Delta32 and CCR2-64I alleles on HIV-1 disease progression: the protection varies with duration of infection. Aids2003 Feb 14;17(3):377-87.

4. Balotta C, Bagnarelli P, Violin M, Ridolfo AL, Zhou D, Berlusconi A, et al. Homozygous delta 32 deletion of the CCR-5 chemokine receptor gene in an HIV-1-infected patient. Aids1997 Aug;11(10):F67-71.

5. Biti R, Ffrench R, Young J, Bennetts B, Stewart G, Liang T. HIV-1 infection in an individual homozygous for the CCR5 deletion allele. Nat Med1997 Mar;3(3):252-3.

6. Djin-Ye Oh JH. CCR5 Delta 32 Genotypes in a German HIV-1 Seroconverter Cohort and Report of HIV-1 Infection in a CCR5 Delta 32 Homozygous Individual. Plos One2008.

7. Gorry PR, Zhang C, Wu S, Kunstman K, Trachtenberg E, Phair J, et al. Persistence of dual-tropic HIV-1 in an individual homozygous for the CCR5 Delta 32 allele. Lancet2002 May 25;359(9320):1832-4.

8. Gray L, Churchill MJ, Keane N, Sterjovski J, Ellett AM, Purcell DF, et al. Genetic and functional analysis of R5X4 human immunodeficiency virus type 1 envelope glycoproteins derived from two individuals homozygous for the CCR5delta32 allele. J Virol2006 Apr;80(7):3684-91.

9. Heiken H, Becker S, Bastisch I, Schmidt RE. HIV-1 infection in a heterosexual man homozygous for CCR-5 delta32. Aids1999 Mar 11;13(4):529-30.

10. Iversen AK, Christiansen CB, Attermann J, Eugen-Olsen J, Schulman S, Berntorp E, et al. Limited protective effect of the CCR5Delta32/CCR5Delta32 genotype on human immunodeficiency virus infection incidence in a cohort of patients with hemophilia and selection for genotypic X4 virus. J Infect Dis2003 Jan 15;187(2):215-25.

11. Kuipers H, Workman C, Dyer W, Geczy A, Sullivan J, Oelrichs R. An HIV-1-infected individual homozygous for the CCR-5 delta32 allele and the SDF-1 3'A allele. Aids1999 Feb 25;13(3):433-4.

12. O'Brien TR, Winkler C, Dean M, Nelson JA, Carrington M, Michael NL, et al. HIV-1 infection in a man homozygous for CCR5 delta 32. Lancet1997 Apr 26;349(9060):1219.

13. Sheppard HW, Celum C, Michael NL, O'Brien S, Dean M, Carrington M, et al. HIV-1 infection in individuals with the CCR5-Delta32/Delta32 genotype: acquisition of syncytium-inducing virus at seroconversion. J Acquir Immune Defic Syndr2002 Mar 1;29(3):307-13.

14. Theodorou I, Meyer L, Magierowska M, Katlama C, Rouzioux C. HIV-1 infection in an individual homozygous for CCR5 delta 32. Seroco Study Group. Lancet1997 Apr 26;349(9060):1219-20.

15. Hutter G, Nowak D, Mossner M, Ganepola S, Mussig A, Allers K, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med2009 Feb 12;360(7):692-8.

16. Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, et al. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter

AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. Science1997 Aug 15;277(5328):959-65.

17. Paxton WA, Martin SR, Tse D, O'Brien TR, Skurnick J, VanDevanter NL, et al. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. Nat Med1996 Apr;2(4):412-7.

18. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature1996 Aug 22;382(6593):722-5.

19. Martinson JJ, Chapman NH, Rees DC, Liu YT, Clegg JB. Global distribution of the CCR5 gene 32-basepair deletion. Nat Genet1997 May;16(1):100-3.

20. Salas-Alanis JC, Mellerio JE, Ashton GH, McGrath JA. Frequency of the CCR5 gene 32-basepair deletion in Hispanic Mexicans. Clin Exp Dermatol1999 Mar;24(2):127-9.

21. Zimmerman PA, Buckler-White A, Alkhatib G, Spalding T, Kubofcik J, Combadiere C, et al. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. Mol Med1997 Jan;3(1):23-36.

22. Mangano A, Prada F, Roldan A, Picchio G, Bologna R, Sen L. Distribution of CCR-5 delta32 allele in Argentinian children at risk of HIV-1 infection: its role in vertical transmission. Aids1998 Jan 1;12(1):109-10.

23. Desgranges C, Carvajal P, Afani A, Guzman MA, Sasco A, Sepulveda C. Frequency of CCR5 gene 32-basepair deletion in Chilean HIV-1 infected and non-infected individuals. Immunol Lett2001 Mar 1;76(2):115-7.

24. Passos GA, Jr., Picanco VP. Frequency of the delta ccr5 deletion allele in the urban Brazilian population. Immunol Lett1998 Apr;61(2-3):205-7.

25. Vargas AE, Marrero AR, Salzano FM, Bortolini MC, Chies JA. Frequency of CCR5delta32 in Brazilian populations. Braz J Med Biol Res2006 Mar;39(3):321-5.

26. Munerato P, Azevedo ML, Sucupira MC, Pardini R, Pinto GH, Catroxo M, et al. Frequency of polymorphisms of genes coding for HIV-1 co-receptors CCR5 and CCR2 in a Brazilian population. Braz J Infect Dis2003 Aug;7(4):236-40.

27. Kallas EG, Bassichetto KC, Oliveira SM, Goldenberg I, Bortoloto R, Moreno DM, et al. Establishment of the serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion (STARHS) strategy in the city of Sao Paulo, Brazil. Braz J Infect Dis2004 Dec;8(6):399-406.

28. Kostrikis LG, Huang Y, Moore JP, Wolinsky SM, Zhang L, Guo Y, et al. A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation. Nat Med1998 Mar;4(3):350-3.

29. Barbour JD, Sauer MM, Sharp ER, Garrison KE, Long BR, Tomiyama H, et al. HIV-1/HSV-2 co-infected adults in early HIV-1 infection have elevated CD4+ T cell counts. PLoS ONE2007;2(10):e1080.

30. Sa-Filho D, Kallas EG, Sanabani S, Sabino E, Sucupira MC, Sanchez-Rosa AC, et al. Characterization of the full-length human immunodeficiency virus-1 genome from recently infected subjects in Brazil. AIDS Res Hum Retroviruses2007 Sep;23(9):1087-94.

31. Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, He T, et al. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat Med1996 Nov;2(11):1240-3.

32. Ioannidis JP, Contopoulos-Ioannidis DG, Rosenberg PS, Goedert JJ, De Rossi A, Espanol T, et al. Effects of CCR5-delta32 and CCR2-64I alleles on disease progression of perinatally HIV-1-infected children: an international meta-analysis. Aids2003 Jul 25;17(11):1631-8.

33. Ioannidis JP, Rosenberg PS, Goedert JJ, Ashton LJ, Benfield TL, Buchbinder SP, et al. Effects of CCR5-Delta32, CCR2-64I, and SDF-1 3'A alleles on HIV-1 disease

progression: An international meta-analysis of individual-patient data. Ann Intern Med2001 Nov 6;135(9):782-95.

34. Arenzana-Seisdedos F, Parmentier M. Genetics of resistance to HIV infection: Role of co-receptors and co-receptor ligands. Semin Immunol2006 Dec;18(6):387-403.

Table 1: Baseline characteristics of the HIV-1 infected patients

	N (%)	**CCR5Δ32/CCR5 N (%)**	**CCR5/CCR5 N (%)**
Sex			
male	208 (90,8)	19 (95)	189 (91)
female	21(9,2)	1 (5)	2 (9)
Ethnicity			
White	133 (59,3)	10 (4,3)	123 (54,9)
Mulatto	43 (19,1)	5 (2,1)	38 (16,9)
Black	17 (7,5)	2 (0,8)	15 (6,7)
Others*	31 (13,8)	3 (1,3)	28 (12,5)
Exposure			
MSM**	190 (83,7)	18 (7,8)	172 (83,1)
Heterossexual	37 (16,3)	2 (0,87)	35 (16,9)
Age (Years)			
Mean	30,79		
IC 25%-75%	24,83-36,30		
CD4+ T cells count (cells/ml)			
Mean		593	519
IC 25%-75%		419-745	392-698
CD8+ T cells count (cells/ml)			
Mean		746	915
IC 25%-75%		609-1066	624-1246
Plasma HIV RNA ($\mathbf{log_{10}}$ copies/mL)			
		6845	19873
Mean		1078-47536	4263-71325
IC 25%-75%			

* Indian and Asian
** Man who make sex with man
IC: Interquartile range

Figure Legends

Figure1: Gel electrophoresis of amplified genomic DNA for CCR5Δ32 detection. In lanes 1 and 2 the PCR products show a single band 241 bp in size representing the wild-type CCR5 and in line 3 is present one of 241 bp and the other 209 bp in size, indicating that this individual is heterozygous for one wild-type CCR5 allele and one CCR5Δ32 allele. The molecular weight marker is also presented.

Figure 2: Kaplan-Meier analysis of HIV-1 infected patients showing a relation of CCR5Δ32 genotype with a CD4+ t cell count <300 cells/µL. Estimates of survival among HIV-1 infected patients heterozygous for the deletion allele (CCR5Δ32/CCR5) and homozygous for the wild-type CCR5 allele comparison the outcomes at the CD4+ T cell count drop under 300 cells/µL. The difference between both groups implied by the Kaplan-Meier diagram is reflected in a statistical trend (p=0.2998).

Figure 1

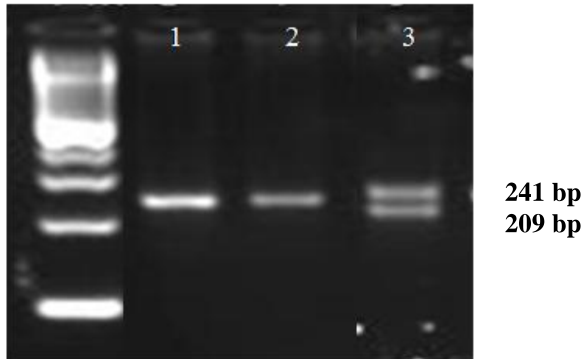
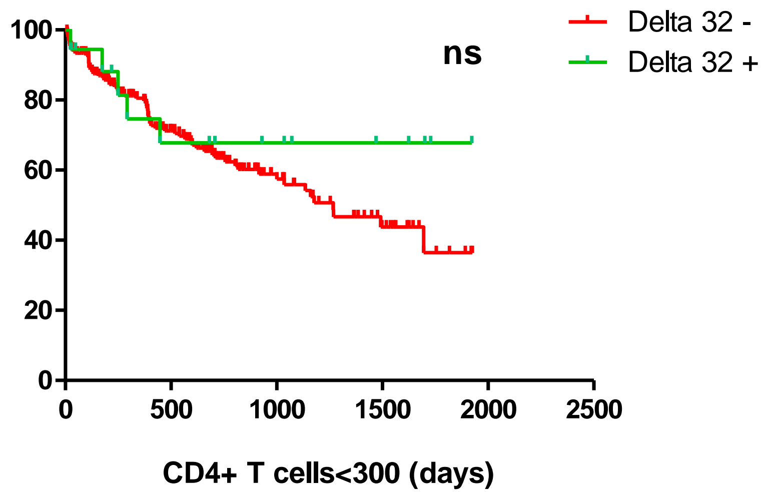


Figure 2



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