Original Article

HIV-1 and GBV-C co-infection in Venezuela

Anny Karely Rodríguez[1,7], Domingo José Garzaro[1], Carmen Luisa Loureiro[1], Cristina R Gutiérrez[2], Gladys Ameli[2], Rossana Celeste Jaspe[1], Leticia Porto[3], Francisca Monsalve[3], Ángela Pozada[4], Luzmary Vásquez[5], Miguel E Quiñones-Mateu[6], Flor Helene Pujol[1], Héctor Rafael Rangel[1]

[1] *Laboratorio de Virología Molecular, Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela*
[2] *Instituto Nacional de Higiene Rafael Rangel, Caracas, Venezuela*
[3] *Laboratorio Regional de Referencia Virológica, LUZ, Maracaibo, Venezuela*
[4] *Escuela de Bioanálisis Universidad Central de Venezuela, Caracas, Venezuela*
[5] *Hospital Central de Barquisimeto, Barquisimeto, Venezuela*
[6] *Department of Pathology, Case Western Reserve University, Cleveland, Ohio, United States*
[7] *Escuela de Matemáticas, Universidad Sergio Arboleda, Bogotá, Colombia*

Abstract

Introduction: Co-infection with GB virus C (GBV-C) in patients infected with human immunodeficiency virus 1 (HIV-1) has been associated with prolonged survival. The aim of this study was to evaluate the prevalence of GBV-C infection among HIV-1-infected patients in Venezuela, and to determine the effects of the co-infection on the levels of relevant cytokines.

Methodology: Plasma samples were collected from 270 HIV-1-seronegative and 255 HIV-1-seropositive individuals. GBV-C infection was determined by RT-PCR of the NS5 region and genotyped by sequence analysis of the 5´UTR region. HIV-1 strains were characterized by sequence analysis of *pol*, *vif*, *env*, and *nef* genes. Selected cytokines were evaluated by ELISA.

Results: Ninety-seven of 525 (18.5%) plasma samples tested positive for GBV-C RNA. A significantly higher prevalence of GBV-C was found among HIV-1 patients compared to HIV-1-seronegative individuals (67/255, 26% versus 30/270, 11%; p < 0.001). Statistical difference was observed in the viral load between HIV-1+GBV-C+ and HIV-1+GBV-C- (p = 0.014), although no differences in CD4$^+$ cell counts were found between both groups. TNFα concentration was higher in HIV-1$^+$GBV-C$^-$ than in HIV-1$^+$GBV-C$^+$ patients (25.9 pg/mL versus 17.3 pg/mL; p = 0.02); RANTES expression levels were more variable in GBV-C co-infected patients and more frequently elevated in HIV-1 mono-infected patients compared to patients co-infected with GBV-C.

Conclusions: The previously observed beneficial effect of co-infection with HIV-1 and GBV-C on disease progression is complex and might be due in part to a change in the cytokine environment. More studies are required to understand the interaction between both viruses.

Introduction

Human immunodeficiency virus 1 (HIV-1) and GB virus C (GBV-C), a member of the *Flaviviridae* family originally thought to be associated with hepatitis, share common routes of transmission; thus, the co-infection prevalence is usually high [1]. It has been shown in several studies that the natural course of HIV-1 can be modulated by the presence of viral, bacterial, or parasitic co-infections. For example, while tuberculosis infection exacerbates HIV-1 progression to acquired immunodeficiency syndrome (AIDS) [2], GBV-C co-infection seems to exert a beneficial effect, reducing the HIV-1 viral load and thus delaying the progress of AIDS [3-5].

Several factors may be involved in the apparent delay in disease progression caused by GBV-C. It has been proposed that GBV-C regulates the Th1 cytokine profile [6]. *In vitro* GBV-C infection increases the production of certain chemokines such as RANTES, macrophage inflammatory protein (MIP)-1α, MIP-1β, and SDF-1, while it reduces CCR5 expression on the cell surfaces [7]. *In vivo*, contradictory results have shown higher [7] or lower [8] levels of RANTES in HIV-1$^+$GBV-C$^+$ co-infected patients compared to non-co-infected individuals. Nevertheless, some of these factors have been shown to reduce susceptibility to HIV-1 infection [9]. HIV-1 genetic factors, as drug resistance mutation or mutation in the *env* or *vif* genes,

may affect viral replication [10-13], and thus viral load; a selective pressure of GBV-C on HIV-1 genes may be not discarded.

It is estimated that 0.5% of the population, around 150,000 from a range of 65,000 to 230,000 people, are infected by HIV-1 in Venezuela [14]. On the other hand, few studies have been conducted in Venezuela in relation to GBV-C; these studies indicate that the prevalence is relatively high among the general population in Venezuela, *i.e.*, 7% active infection among blood donors, and particularly in Amerindians, where the Asian GBV-C genotype 3 circulates [15]. The aim of this study was to evaluate the prevalence of GBV-C infection among HIV-1-infected patients in Venezuela, and to determine the effect of HIV-1 and GBV-C co-infection on the levels of relevant cytokines, which may provide clues to understand the mechanism by which GBV-C interferes with HIV-1 progression.

Methodology

Patient samples

All the samples were obtained after patients provided their informed consent. The protocol was approved by the bioethical committee of the Instituto Venezolano de Investigaciones Científicas (IVIC). HIV-1-negative samples were obtained from blood donors, and HIV-1-positive samples were obtained from patients attending the Instituto Nacional de Higiene Rafael Rangel and Hospital Central de Barquisimeto for viral load diagnostics. The samples consisted of 270 HIV-1-seronegative individuals corresponding to blood donors from IVIC (n = 79), from the military (n = 70), and from a prison (n = 121). The 255 HIV-1-infected patients were naïve (n = 126), defined as patients who never received treatment for HIV-1 and experienced, defined as patients who received at least one treatment regimen (n = 129). For cytokine determination, only HIV-1-infected naïve patients were analyzed. Viral load (bDNA, Siemens Philadelphia, USA) and CD4$^+$ cell count (BD FACS Count, Becton Dickinson, Franklin Lakes, USA) were determined in HIV-1-infected patients as described by the manufacturers.

GBV-C molecular characterization

GBV-C active infection was determined by RT-PCR amplification of the NS5 region; genotype was determined by sequence analysis of the 5´non-coding region as previously described [15]. The sequences were deposited in GenBank under accession numbers JX494176 to JX494226.

HIV-1 molecular characterization

Partial or complete coding regions of HIV-1 *pol, vif, env*, and *nef* genes were amplified and sequenced as previously described [12,16]. For the *pol* gene, only antiretroviral-naïve patients were selected for sequence analysis.

Cytokine profile determination

Plasma levels of a series of cytokines (*i.e.*, RANTES, SDF-α, Leptin, MIP-β, and MCSF) were determined using a commercial ELISA (R&D Systems Minneapolis, USA), according to the manufacturer's instructions.

Results

A total of 525 plasma samples from HIV-1-seronegative or HIV-1-infected individuals were tested for the presence of GBV-C RNA, and 97 (18.5%) were found to be positive. Among the HIV-1-seronegative individuals, a slightly higher prevalence was found in inmates, although the difference was not statistically significant (Table 1). A significantly higher prevalence of GBV-C was found among HIV-1-infected patients compared to uninfected individuals (27% versus 11%, p < 0.001). GBV-C genotype could be determined in 51 out of the 97 GBV-C-positive plasma samples. The most frequent genotype was genotype 2 (31/51, 61%), followed by genotype 3 (17/51, 33%) and genotype 1 (3/51, 6%). No difference was observed in the GBV-C genotype distribution between the HIV-1$^+$GBV-C$^+$ co-infected patients compared to HIV-1$^-$GBV-C$^+$ individuals (Table 1).

Plasma viral load was available for the 126 antiretroviral-naïve HIV-1 patients. A significant difference was observed in the viral load of HIV-1$^+$GBV-C$^-$ patients versus HIV-1$^+$GBV-C$^+$ individuals (p = 0.014) (Table 2). In addition, a higher number in HIV-1$^+$GBV-C$^+$ co-infected patients was found to harbor viral load below 55,000 copies/mL, the threshold suggested in the past by some authors to start treatment [17], compared to non-co-infected HIV-1$^+$GBV-C$^-$ patients: 43/96 (45%) versus 21/30 (70%) of HIV-1$^+$GBV-C$^+$ (p = 0.03). No significant difference between both groups was observed in the mean CD4$^+$ cell count or time since diagnosis (Table 2).

Table 1. GBV-C prevalence in healthy donors and HIV-1-seropositive patients

| Population groups	GBV-C prevalence (%)	GBV-C genotype[1]
Blood donors		
Civil	7/79 (8.8%)	
Military	6/70 (8.6%)	
Inmate	16/121 (13%)	
Total	29/270 (11%)[2]	1 G1, 4 G2, 2 G3
HIV-1	68/255 (27%)[2]	2 G1, 27 G2, 15 G3

[1]GBV-C genotype is shown for samples that could be amplified in the 5´NC region; [2]$p < 0.001$

Table 2. Virological parameters relative to HIV-1 infection in patients co-infected or not with GBV-C.

HIV-1 naive patients (n = 126)	Age (years)	Time since diagnosis (HIV-1) (months)	Viral load (copies/mL)	CD4$^+$ cell count (cells/mL)	Gene mutations	
					Vif[1]	*Nef* deletions
HIV-1+GBV-C+	29.4±5.6	17.9±12.1	54,371±76,185	430.9±317.1	8/21 R132S	0/23
HIV-1+GBV-C-	33.6±8.7	21.2±23.9	146,617±224,546	449.2±252.4	10/26 R132S	2/23[2]
	p = 0.009	NS	p = 0.014	NS	NS	NS

[1]*Vif* substitution R132S, associated with lower viral load in previous studies [18]; [2]Two strains carried *Nef* deletions, one a premature stop codon at position 177 and the other a gross deletion complemented in the quasispecies with the wild-type strain

Figure 1. Levels of cytokines in different population groups.
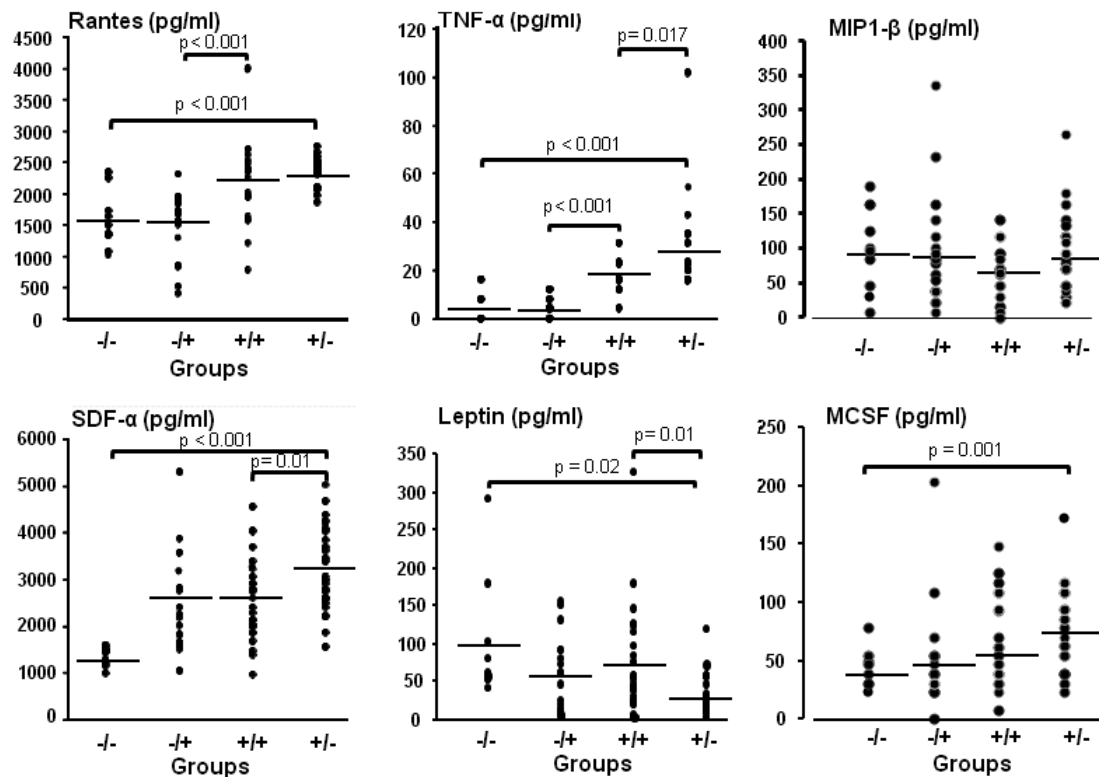
-/-: individuals negative for HIV-1 and GBV-C infection, -/+: GBV-C infected individuals, +/-: HIV-1 infected patients, +/+: HIV-1 and GBV-C co-infected patients. Bars represent the average of each cytokine evaluated. p values are show between the groups where statistical differences were found.

Four HIV-1 genes (*pol, vif, env,* and *nef*) were sequenced from patients co-infected (HIV-1$^+$GBV-C$^+$) or not co-infected (HIV-1$^+$GBV-C$^-$) in order to identify potential signatures associated with GBV-C co-infection. All HIV-1 strains in each particular genomic region were classified as subtype B. No primary resistance mutation was detected and no differences were observed in the frequency of secondary mutation associated with drug resistance between *pol* sequences obtained from HIV-1-positive patients co-infected or not with GBV-C. In *vif*, the amino acid substitution R132K has been associated with lower viral load in patients harboring viruses with this polymorphism compared to patients carrying viruses with wild type *vif* [12,18]. Although this substitution was observed in 18/47 (38.3%) of the sequenced viruses, no statistically significant difference was observed in the frequency of this polymorphism between HIV-1$^+$GBV-C$^+$ and HIV-1$^+$GBV-C$^-$ (Table 2). In addition, two samples exhibited the *nef* gene with deletion in co-infected patients, but the prevalence of this deletion was not significantly higher in co-infected patients (Table 2). Finally, no relevant differences were found in *env* genes between the two groups. In summary, no signatures in the four HIV-1 genomic regions analyzed were found to be associated with GBV-C co-infection.

The levels of six cytokines were determined in the plasma of HIV-1-negative and HIV-1-positive individuals, co-infected or not with GBV-C (Figure 1). HIV-1$^+$GBV-C$^-$ patients exhibited RANTES levels significantly higher than those of the HIV-1$^-$GBV-C$^-$ individuals (mean 2,386 versus 1,578; Student's *t* test $p < 0.0001$). HIV-1$^+$GBV-C$^+$ patients showed a higher degree of variation in RANTES levels compared to the other groups. A significant difference in the frequency of patients exhibiting levels of RANTES over the mean of negative controls plus one standard deviation was observed between HIV-1mono-infected patients (25/27) compared to patients infected with GBV-C (15/24, $p < 0.01$). TNF-α levels were also significantly higher in HIV-1$^+$GBV-C$^-$ patients compared to HIV-1$^+$GBV-C$^+$ and HIV-1$^-$GBV-C$^-$ individuals (28 versus 19, $p = 0.01$ and 28 versus 6, $p = 0.0001$, respectively). SDF-1 expression was significantly higher for the patients infected with at least one of the two viruses, compared to uninfected persons ($p < 0.001$). The difference between HIV-1 patients co-infected or not with GBV-C was, however, not significant ($p = 0.07$). Leptin concentrations were lower in HIV-1 mono-infected patients compared with all the other groups ($p = 0.005$), and were significantly

lower than the levels observed in HIV-1 patients co-infected with GBV-C ($p = 0.001$). No significant differences were observed in MIP-1β and MCSF concentrations among the different groups (Figure 1).

Discussion

GBV-C active infection was determined in selected population groups in Venezuela to evaluate the effect of HIV-1 co-infection in GBV-C infection. GBV-C prevalence in civil and military personnel was similar to the prevalence previously described in other urban population groups in Venezuela [19]. Although a slightly higher GBV-C prevalence was observed among imprisoned individuals, this difference was not statistically significant, perhaps due to the limited number of patients studied. It has been shown that bloodborne hepatitis viruses are common among prison inmates with higher GBV-C prevalence [20,21]. Anti-E2 antibodies, which could allow for detection of differences in exposure among these groups, as found in other countries [22], were not tested in this study. A higher GBV-C frequency of active infection was found among HIV-1-infected patients (27%) compared to HIV-1-negative individuals (11%), in agreement with previous reports [23,24].

Previous studies have shown lower plasma HIV-1 loads and higher CD4$^+$ cell counts in patients co-infected with GBV-C [25]. In his study, a significant reduction in the mean HIV-1 load was observed in GBV-C co-infected patients, without differences in CD4$^+$ cell counts (Table 2). A negative correlation between plasma GBV-C and HIV-1 load has been reported [8]; however, this aspect was not evaluated in the current study.

Several *in vitro* studies have shown an increase in the level of selected cytokines as a consequence of GBV-C infection (*e.g.*, RANTES and SDF-1, among others), which correlates with a decreased rate of HIV-1 infection [7]. This *in vitro* observation was not corroborated in the present study. Instead, RANTES and TNF-α levels were frequently lower in HIV-1$^+$GBV-C$^+$ co-infected patients compared to HIV-1$^+$GBV-C$^-$ individuals, similar to a report by Hattori *et al*. [8], who found lower RANTES levels in HIV-1$^+$GBV-C+ co-infected patients compared to non-co-infected HIV-1 patients. Interestingly, GBV-C co-infection appeared to restore leptin levels in HIV-1-infected patients (Figure 1). A negative correlation between HIV-1 load and leptin concentration has been previously reported [26], suggesting that this cytokine may affect HIV-1 replication. No correlation was found in this study between HIV-1 load and leptin

levels, neither in HIV-1-infected patients, nor in GBV-C co-infected patients (data not shown). Based on this, the possible interference exerted by GBV-C on HIV-1 replication might be more complex than initially thought.

It has been suggested that the beneficial effect of GBV-C in HIV-1-infected patients might be genotype related; genotype 2 has been proposed to be responsible for the beneficial effect [27]. However, *in vitro* studies have failed to identify any differences in HIV-1 inhibition between different genotypes of GBV-C NS5A, the protein proposed to be responsible of the interference [29]. Indeed, GBV-C genotype 2 was the most common genotype found in this study and in previous studies in Venezuela [10]. However, we did not found any difference in $CD4^+$ levels among patients co-infected or not with GBV-C. A possible explanation for the discrepancies between different studies may be the time of infection of HIV-1 infected patients. Some studies have reported a beneficial effect of GBV-C co-infection in advanced HIV-1 disease [30], which was not the case in our study.

Mutations in the HIV-1 genome may affect its viral replication [10-13]. Four different HIV-1 genomic regions (*pol, vif, env,* and *nef* genes) were analyzed to detect potential signatures related to the selective pressure of GBV-C. However, no differences were found between both HIV-1 patients co-infected or not with GBV-C.

In conclusion, the prevalence of GBV-C infection was significantly higher in HIV-1 co-infected patients, with no difference in the distribution of GBV-C genotypes. No differences were identified in HIV-1 genes between co-infected or not co-infected patients, providing no evidence of selective pressure of GBV-C on HIV-1. GBV-C may somehow affect the cytokine environment in which HIV-1 replicates, which might affect HIV-1 viral load in a variable way. The co-infection seems to occur in a complex interplay; the mechanisms associated are not fully understood, which might be the reason for contradictory reports in the literature.

Acknowledgements

References

1. Rey D, Vidinic-Moularde J, Meyer P, Schmitt C, Fritsch S, Lang JM, Stoll-Keller F (2000) 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 19: 721-724.
2. Sharma SK, Mohan A, Kadhiravan T (2005) HIV-TB co-infection: epidemiology, diagnosis & management. Indian J Med Res 121: 550-567.
3. Xiang J, Wünschmann S, Diekema DJ, Klinzman D, Patrick KD, George SL, Stapleton JT (2001) Effect of coinfection with GB virus C on survival among patients with HIV infection. N Eng J Med 345: 707-714.
4. Maidana MT, Sabino EC, Kallas EG (2005) GBV-C/HGV and HIV-1 coinfection. Braz J Infect Dis 9: 122-125.
5. Vahidnia F, Petersen M, Stapleton JT, Rutherford GW, Busch M, Custer B (2012) Acquisition of GB virus type C (HGV) and lower mortality in patients with advanced HIV disease. Clin Infect Dis 55: 1012-1019.
6. Nunnari G, Nigro L, Palermo F, Attanasio M, Berger A, Doerr HW, Pomerantz RJ, Cacopardo B (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: 26-30.
7. Xiang J, George SL, Wünschmann S, Chang Q, Klinzman D, Stapleton JT (2004) Inhibition of HIV-1 replication by GB virus C infection through increases in RANTES, MIP-1alpha, MIP-1beta, and SDF-1. Lancet 363: 2040-2046.
8. Hattori J, Okumura N, Yamazaki Y, Uchiyama M, Hamaguchi, M Nishiyama Y, Kaneda T (2007) Beneficial effect of GB virus C co-infection in Human Immunodeficiency Virus type 1 infected individuals. Microbiol Immunol 51: 193-200.
9. Marmor M, Hertzmark K, Thomas SM, Halkitis PN, Vogler M (2006) Resistance to HIV infection. J Urban Health 83: 5-17.
10. Weber J, Rangel HR, Chakraborty B, Marotta ML, Valdez H, Fransen K, Florence E, Connick E, Smith KY, Colebunders RL, Landay A, Kuritzkes D, Lederman, MM Vanham G, Quiñones-Mateu ME (2003) Role of baseline *pol* genotype in human immunodeficiency virus type 1 fitness evolution. JAIDS 33: 448-460.
11. Rangel HR, Weber J, Chakraborty B, GutierrezA, Marotta ML, Mirza M, Kiser P, Martinez MA, Este JA, Quiñones-Mateu ME (2003) Role of the human immunodeficiency virus type 1 envelope gene in viral fitness. J Virol 77: 9069-9073.
12. Rangel HR, Garzaro D, Rodríguez AK, Ramírez AH, Ameli G, Gutiérrez CR, Pujol FH (2009) Deletion insertion and stop codon mutations in Vif genes of HIV-1 infecting slow progressor patients. J Infect Dev Cties 3: 531-538. doi:10.3855/jidc.471.
13. Peng J, Ao Z, Matthews C, Wang X, Ramdahin S, Chen X, Li J, Chen L, He J, Ball B, Fowke K, Plummer F, Embree J, Yao X (2013) A naturally occurring Vif mutant (I107T) attenuates anti-APOBEC3G activity and HIV-1 replication. J Mol Biol 425: 2840-2852.
14. UNAIDS (2011) AIDS info Epidemiological Status. Available: http://www.unaids.org/en/dataanalysis/datatools/aidsinfo/ Accessed 30 July 2013.
15. Loureiro CL, Alonso R, Pacheco BA, Uzcátegui MG, Villegas L, León G, de Saéz A, Liprandi F, López JL, Pujol FH (2002) High Prevalence of GB virus C/Hepatitis G virus

genotype 3 among autochthonous Venezuelan populations. J Med Virol 68: 357-362.

16. Rangel HR, Garzaro D, Gutiérrez CR, Vásquez L, Guillen G, Torres JR, Pujol FH (2009) HIV diversity in Venezuela: predominance of HIV type 1 subtype B and genomic characterization of non-B variants. AIDS Res Hum Retroviruses 25: 347-350.

17. Dybul M, Fauci AS, Bartlett JG, Kaplan JE, Pau AK; Panel on Clinical Practices for Treatment of HIV (2002) Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. Ann Intern Med 137: 381-433.

18. Hassaïne G, Agostini I, Candotti D, Bessou G, Caballero M, Agut H, Autran B, Barthalay Y, Vigne R (2000) Characterization of human immunodeficiency virus type 1 vif gene in long-term asymptomatic individuals. Virology 276: 169-180.

19. Pujol FH, Khudyakov YE, Devesa M, Cong ME, Loureiro CL, Blitz L, Capriles F, Beker S, Liprandi F, Fields HA (1998) Hepatitis G virus infection in Amerindians and other Venezuelan high-risk groups. J Clin Microbiol 36: 470-474.

20. Butler T, Spencer J, Cui J, Vickery K, Zou J, Kaldor J (1999) Seroprevalence of markers for hepatitis B, C and G in male and female prisoners-NSW, 1996. Aust N Z J Public Health 23: 377-384.

21. Christensen PB, Fisker N, Mygind LH, Krarup HB, Wedderkopp N, Varming K, Georgsen J (2003) GB virus C epidemiology in Denmark: different routes of transmission in children and low- and high-risk adults. J Med Virol 70: 156-162.

22. Anastassopoulou CG, Paraskevis D, Sypsa V, Psichogiou M, Katsoulidou A, Tassopoulos N, Skoutelis A, Malliori M, Hatzakis A (1998) Prevalence patterns and genotypes of GB virus C/hepatitis G virus among imprisoned intravenous drug users. J Med Virol 56: 246-252.

23. Lau DT, Miller KD, Detmer J, Kolberg J, Herpin B, Metcalf JA, Davey RT, Hoofnagle JH (1999) Hepatitis G virus and human immunodeficiency virus coinfection: response to interferon-alpha therapy. J Infect Dis 180: 1334-1337.

24. Giret MT, Miraglia JL, Sucupira MC, Nishiya A, Levi JE, Diaz RS, Sabino EC, Kallas EG (2011) Prevalence, incidence density, and genotype distribution of GB virus C infection in a cohort of recently HIV-1-infected subjects in Sao Paulo, Brazil. PLoS One 6: e18407.

25. Tillmann HL, Heiken H, Knapik-Botor A, Heringlake S, Ockenga J, Wilber JC, Goergen B, Detmer J, McMorrow M, Stoll M, Schmidt RE, Manns MP (2001) Infection with GB virus C and reduced mortality among HIV-infected patients. N Engl J Med 345: 715-724.

26. Azzoni L, Crowther NJ, Firnhaber C, Foulkes AS, Yin X, Glencross D, Gross R, Kaplan MD, Papasavvas E, Schulze D, Stevens W, van der Merwe T, Waisberg R, Sanne I, Montaner LJ (2010) Association between HIV replication and serum leptin levels: an observational study of a cohort of HIV-1-infected South African women. J Int AIDS Soc 13: 33-41.

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

28. Xiang J, McLinden JH, Chang Q, Kaufman TM, Stapleton JT (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: 15570-15575.

29. Schwarze-Zander C, Neibecker M, Othman S, Tural C, Clotet B, Blackard JT, Kupfer B, Luechters G, Chung RT, Rockstroh JK, Spengler U (2010) GB virus C coinfection in advanced HIV type-1 disease is associated with low CCR5 and CXCR4 surface expression on CD4(+) T-cells. Antivir Ther 15: 745-752.

Corresponding author
Héctor Rafael Rangel
Laboratorio de Virología Molecular, Centro de Microbiología y Biología Celular
Instituto Venezolano de Investigaciones Científicas
Apdo 20632, Caracas 1020-A, Venezuela
Email: hrangel2006@gmail.com

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