



Original article

HLA-G liver expression in chronically HIV/hepatitis C-coinfected individuals.

Fernando Crivelenti Vilar^a, Eduardo Donadi^a, Benedito Antônio Lopes da Fonseca^a,
Janaina Cristiana de Oliveira Crispim Freitas^b, Ana Letícia Gomide Zanin Borducchi^a,
Rodrigo de Carvalho Santana^{a,*}

^a Department of Internal Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

^b Department of Clinical Analysis and Toxicology, Federal University of Rio Grande do Norte, Natal, Brazil

ARTICLE INFO

Article History:

Received 12 April 2024

Accepted 23 October 2024

Available online 2 December 2024

Keywords:

Hepatitis C

HCV

HIV

HLA-G, Liver Disease

HCV/HIV coinfection

ABSTRACT

Introduction and Objectives: Hepatitis C-induced liver disease represents a significant threat to the survival of people living with HIV. HIV/HCV-coinfected individuals have a more rapid progression to cirrhosis and its complications than HCV monoinfected patients. Although the underlying mechanisms remain unclear, HLA-G, a non-classical class I HLA molecule, has a well-recognized property to down-regulate the immune response against viruses and may favor the progression of chronic hepatitis C.

Materials and Methods: We analyzed HLA-G expression in 59 liver specimens of patients harboring chronic HCV and HIV coinfection and stratified the findings according to clinical and histopathological features.

Results: Genotype 1 was the most prevalent (88%); the HLA-G expression was observed in 38 (64%) liver specimens, and it was more frequent in more severe stages than in milder stages of chronic hepatitis (94,1% x 55%; $p < 0.01$). HLA-G expression in the liver was not correlated to antiviral response to hepatitis C therapy with pegylated-IFN- α plus ribavirin.

Conclusions: HLA-G expression in the context of HCV/HIV coinfection is a complex process modulated by many factors. HLA-G expression may play a role in the mechanisms that facilitate disease progression and may contribute to the deterioration of the immune response against HCV in individuals living with HIV.

© 2024 Fundación Clínica Médica Sur, A.C. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

1. Introduction

Hepatitis C virus (HCV) infection is associated with the persistence of viremia in most infected individuals in the presence or absence of progressive liver disease. The coinfection with human immunodeficiency virus (HCV/HIV) has been associated with accelerated progression to severe liver injury compared to HCV monoinfected patients [1]. In the United States and Europe, approximately 33% of all HIV-infected persons are coinfecting with HCV. This percentage may vary depending on the geographical region and mode of acquisition of HIV, being higher among intravenous drug users [2]. Although the underlying mechanisms remain unclear, HIV infection alters the natural history of HCV disease. It is associated with an increased likelihood of chronicity following acute infection, accelerated progression

to end-stage liver disease, and increased risk of liver-related deaths (1). Different pathogenetic factors contribute to liver disease progression in HIV/HCV coinfection, including quantitative decline in CD4⁺ and CD8⁺ T-cells responses to HCV infection, qualitative changes in immune responses as evaluated by cytokine polarization profile and impaired dendritic cell function. In addition, host and virus genetic factors may influence the evolution of progressive liver disease [3].

The classical class I HLA (HLA-A, HLA-B, and HLA-C) molecules play an essential role in presenting viral peptides to cytotoxic CD8⁺ T-cells [4]. Some viruses, including cytomegalovirus, have developed the ability to decrease the expression of these molecules as a powerful evasion mechanism of the lytic activity of T CD8 cells [5]. Virus-infected cells lacking HLA classical class I molecules are usually attacked by cytotoxic NK cells, which detect this membrane abnormality. As a strategy to evade the action of cytotoxic NK cells, some viruses induce the expression of non-classical HLA (HLA-E, HLA-F, and HLA-G) molecules, whose central role is to regulate the function of immune system cells [6]. In this context, HLA-G has a well-recognized property to down-regulate the immune response, binding to inhibitory receptors (ILT-2, ILT-4, KIR2DL4, and others) present on

Abbreviations: HCV, hepatitis C virus; PEG-IFN- α , pegylated interferon alfa; HLA-G, Human leukocyte antigen G; F0, No fibrosis; F1, mild liver fibrosis; F2, moderate liver fibrosis; F3, severe liver fibrosis; F4, cirrhosis; SVR, sustained virologic response; NR, non-responders

* Corresponding author.

E-mail address: santanacrod@fmrp.usp.br (R.d.C. Santana).

<https://doi.org/10.1016/j.aohep.2024.101755>

1665-2681/© 2024 Fundación Clínica Médica Sur, A.C. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

antigen-presenting cells, CD4⁺ and CD8⁺ T lymphocytes, and NK cells. Inhibition of the immune response may be beneficial, as in autoimmune disorders and transplantation, but may produce detrimental effects in malignancies and chronic viral diseases, in which a solid cytotoxic immune response is required to eliminate malignant and chronically virus-infected cells [7].

HLA-G was first detected in cytotrophoblast cells at the maternal-fetal interface, where they maintain a tolerogenic status between the mother and the semiallogenic fetus. In pathological conditions, HLA-G has been detected in several types of tumor, allografts, inflammatory and autoimmune disorders, and virus diseases, including those caused by influenza, rabies, cytomegalovirus, herpes, and HIV [8]. Increased soluble HLA-G level is detected in the very early stage of HIV infection, and its production has been associated with an increased rate of disease progression [9]. Concerning hepatotropic viruses, our group reported a high percentage of HLA-G expression in hepatocyte and biliary epithelial cells of biopsy samples obtained from hepatitis B [10] and HCV-infected patients [11]. Considering that the coinfection of HIV/HCV has been associated with worsening liver damage and that HLA-G down-regulates the immune response against viruses, we hypothesized that HLA-G expression could be associated with impaired liver and patient morbidity. Then, we analyzed HLA-G expression in liver specimens of HIV/HCV-coinfecting subjects and stratified the findings according to clinical and histopathological features.

2. Patients and Methods

2.1. Sampling

It included 59 chronically HCV/HIV-coinfecting patients followed at the School of Medicine of Ribeirão Preto, University of São Paulo (FMRP-USP), São Paulo, Brazil, from 2002 to 2006. Liver biopsies and blood samples were obtained during the follow up. Epidemiologic data clinical and laboratory results were retrieved from the patients' medical records. Fifty patients had been submitted to hepatitis C antiviral therapy with pegylated-IFN- α (PEG-IFN- α) plus Ribavirin, according to recommendations for treatment at that time.

2.2. Histological analyses

Liver tissue samples were obtained by using the percutaneous biopsy of the right hepatic lobe with a 14-gauge Tru-cut needle guided by ultrasound. After performing the biopsy, patients remained in the hospital to monitor vital signs for 6 hours.

Histologic analyses of liver samples were performed by using 5- μ m-thick paraffin-embedded liver sections stained with hematoxylin/eosin, Masson-trichrome, and Prussian blue. The magnitude of fibrosis degree and the histological activity index (HAI) were assessed according to the criteria described by Knodell *et al.* modified by Desmet *et al.* [12], which classify liver involvement according to the necroinflammatory activity (graded 0-18) and severity of fibrosis (stage F0-F4). Iron deposit semi-quantitative analysis was performed using Perl's staining and classified according to Sciot *et al.* [13]. We graded hepatic steatosis according to the method of Patton *et al.* [14]. All biopsies were assessed and classified in a blind protocol by an experienced pathologist specialized in liver diseases.

2.3. HIV viral load and HCV RNA detection

HIV viral load was determined directly by a hybridization sandwich nucleic acid technique using the VERSANT1 HIV-1 RNA 3.0 (bDNA) (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) protocol, according to the manufacturer's instructions. Values less than 50 copies/ml were reported as undetectable. A negative control was included in each run.

HCV-RNA detection was determined by the polymerase chain reaction (PCR) (commercial COBAS AMPLICOR HCV method, v2.0, Roche Molecular Systems, Branchburg, USA); sensitivity limit of 50 IU/mL. Response to HCV therapy, also known as sustained virologic response (SRV), was defined as undetectable HCV-RNA in plasma 6 months after the end of treatment with PEG-IFN- α and Ribavirin.

2.4. Immunohistochemistry assay for HLA-G

Additional 5- μ m-thick sections were placed on organosilane-pre-treated slides for HLA-G immunohistochemical assay (IHA) using two HLA-G monoclonal antibodies: 5A6G7 (EXBIO, Vestec, Czech Republic) against soluble HLA-G5 and HLA-G6 isoforms, and 4H84 (kindly provided by Mc-Master, San Francisco, CA), which recognize all soluble and membrane-bound HLA-G molecules. The IHA was performed as reported elsewhere [15], using an antibody dilution of 1:50. As a positive control, paraffin-embedded sections of trophoblast tissue obtained from spontaneous abortions at approximately 12 gestational weeks were systematically used. A negative control was performed by omitting the primary antibody. The experienced pathologist assessed the magnitude of HLA-G expression in a qualitative way.

2.5. Statistical analyses

Statistical analyses were performed using the Student *t* or non-parametric tests to compare continuous variables. Pearson's χ^2 test corrected by the Yates method and Fisher's exact test were used, when appropriate, to compare proportions between HLA-G-positive and -negative groups. *p* values <0.05 were considered significant.

2.6. Ethical statement

The study protocol was approved by the local Ethics Research Committee (protocol number 4916/2010) and all the participants gave written informed consent to participate. As an exclusion criterion, patients needed to be free of hepatitis B infection.

3. Results

Demographic, laboratory, and histologic findings of patients are shown in Table 1. Genotype 1 was the most prevalent (88%), especially subgenotype 1a (61%), followed by genotype 3a (10%) and genotype 2a (2%).

According to the severity of hepatic fibrosis, 24 cases (41%) were classified as F1, 17 cases (29%) as F2, 13 cases (22%) as F3 and 5 cases (8%) as F4. For statistical analyses, the degree of fibrosis was compared between groups and between mild (F1 and F2) and severe (F3 and F4) forms. Median ages of the patients at liver biopsy were 36.5 years for F1, 37 years for F3 and 38 years for F2 and F4. According to the necroinflammatory activity, 26 (41.1%) patients exhibited no or mild activity (0 to 9), and 33 patients (55.9%) showed moderate or severe (>9) activity.

The profile of HLA-G staining was similar for both monoclonal antibodies used in this study. HLA-G was strongly expressed in all trophoblast specimens used as positive controls. In the HCV/HIV coinfecting patients, HLA-G molecules were detected in 38 cases (64%), and expression was primarily observed in hepatocytes and in biliary epithelial cells. HLA-G expression exhibited both focal and diffuse liver distribution and occurred irrespective of zones 1, 2, and 3 of the hepatic acinus. Figure 1 illustrates different patterns of HLA-G expression in liver biopsy samples.

Table 2 shows the analyses of HLA-G expression in HIV/HCV liver specimens, stratified according to laboratory and histological features, as well as response to PEG-IFN- α plus Ribavirin therapy. Considering the severity of liver fibrosis and comparing the expression or

Table 1

Demographic, HCV genotype and liver histological characteristics of 59 HIV-HCV coinfectd subjects

Characteristics	n	%
Sex		
Male	45	76.3
Female	14	23.7
Population group		
Caucasian	49	83.1
Non-Caucasian	10	16.9
Age (years)		
Mean (\pm SD)	37.7 (\pm 5.53)	-
Median (range)	38 (25-50)	-
HCV Genotype		
1 (not subtyped)	2	3.3
1a	36	61.1
1b	9	15.2
1a/1b	5	8.5
2	1	1.7
3a	6	10.2
Liver fibrosis		
F1	24	40.7
F2	17	28.8
F3	13	22.0
F4	5	8.5
Liver inflammatory activity		
0 – 9	26	44.1
> 9	33	55.9
Liver iron deposition		
No	29	49.2
Yes	30	50.8
Liver Steatosis		
No	43	72.8
Yes	16	27.2

not of HLA-G in each group of fibrosis, the HLA-G expression increased as long as increased the fibrosis degree (45.8% for F1; 64.7% for F2; 92.3% for F3; 80% for F4; $p = 0.03$). Comparing more severe fibrosis stages (F3 and F4) versus milder fibrosis (F1 and F2), HLA-G expression was more prominent in the first group (F3 and F4) (94.1% \times 55%; $p < 0.01$) (not shown in the table). No association was observed between the HLA-G distribution patterns and the severity of liver necroinflammatory activity or response to hepatitis C therapy.

Iron staining and detection of hepatic steatosis, performed in 100% specimens, were positive in 30 (51%) and 16 (27%), respectively. No association was detected between liver HLA-G expression and iron deposits or hepatic steatosis. All HCV/HIV coinfectd patients exhibited detectable HCV viremia. HIV-1 viral load was available for 52 patients (88.1%) and was performed within the last six months before the hepatic biopsy. Twenty-seven patients (51.9%) had an undetectable viral load. No association was observed regarding HLA-G liver expression and age, sex, population group, HCV genotype and HIV viral load.

4. Discussion

In low- and middle-income countries, HCV infection represents a significant problem in persons infected with HIV. With the evolution of antiretroviral therapy for HIV over the years, there has been a progressive decline in mortality from causes related to AIDS, with a proportional increase in mortality from liver disease, mainly due to chronic viral hepatitis B and C [1].

The role of cell-mediated immune response and its relationship to sustained HCV control in the context of HIV infection remains unclear. Available information about the immunological variables associated with the outcome of HIV/HCV-coinfection is scarce. Previous studies revealed that CD4 T cell depletion induced by HIV infection is associated with loss of adaptive HCV-specific immune responses. In fact, in HCV mono-infection, most patients who spontaneously resolve HCV generate and maintain vigorous peripheral lymphoproliferative responses to HCV antigens [16,17].

A more rapid progression of liver disease in HIV/HCV coinfectd patients is well documented in several studies. [1,16,18]. Different factors contribute to the accelerated evolution of severe liver disease. The central among these factors is HIV-induced immunosuppression, which may induce liver modifications of cytokine polarization patterns towards a Th2 response [19]. Also, viruses that infect humans have developed many mechanisms for evading the immune system. For instance, HIV Nef protein differentially down-regulates the expression of HLA-A and HLA-B, but not of HLA-C, HLA-E, or HLA-G molecules, on the surface of infected cells. [20] Moreover, HIV infection leads to increased HLA-E and HLA-G expression, reducing NK cell-mediated cytotoxicity activity [21]. HLA-G expression has been described in cells infected by HIV, cytomegalovirus, rabies, and herpes simplex [7,8].

The present study observed that HLA-G expression occurs primarily in the later stages of fibrosis in the HIV/HCV coinfection. This finding was similar to the other research of our group, which described that the HLA-G expression increased with the progression of liver fibrosis, both in HCV-monoinfected and HIV/HCV-coinfectd patients [22]. Previously, Amiot et al. [23] had described a positive correlation between HLA-G+ cell numbers and fibrosis area in the liver among HCV monoinfected patients.

The presented results suggest that HLA-G expression may play a role in the mechanisms that facilitate disease evolution and may participate in the deterioration of the immune response against HCV in the coinfection. Several observations support this idea: i) increased plasma levels of soluble HLA-G and IL-10, an inducer of HLA-G expression, are reported in patients with chronic HCV infection [24], ii) high levels of soluble HLA-G and IL-10 are reported in HIV rapid progressors [9], iii) cytokines associated with the Th-2 cytokine profile may contribute to liver fibrosis in HIV patients [3], iv) the

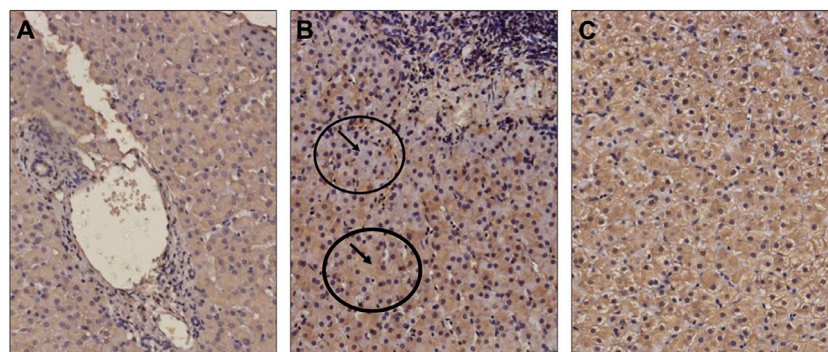


Fig. 1. HLA-G expression in liver biopsy of HIV/HCV coinfectd patient analyzed by immunohistochemistry. (A) sample with no expression of HLA-G. (B) sample with mild HLA-G expression. Thicker circle shows an area with hepatocytes labeled by anti-HLA-G antibody (thick arrow). Thinner circle shows an area with non-labeled hepatocytes (thin arrow). (C) marked and diffuse expression of HLA-G in hepatocytes.

Table 2

Virological and histological features, according to liver HLA-G expression, in HIV-HCV coinfecting subjects

Variables	HLA-G + n (%)	HLA-G – n (%)	p-value*
HCV Genotype			
1(not subtyped)	2 (100)	0	0.42
1a	21 (58.3)	15 (41.7)	
1b	7 (77.7)	2 (22.3)	
1a/1b	4 (80.0)	1 (20.0)	
2	1 (100.0)	0	
3a	3 (50.0)	3 (50.0)	
Liver fibrosis			
F1	11 (45.8)	13 (54.2)	0.03
F2	11 (64.7)	6 (35.3)	
F3	12 (92.3)	1 (7.7)	
F4	4 (80)	1 (20)	
Liver inflammatory activity			
0 – 9	16 (61.5)	10 (38.5)	0.79
> 9	22 (66.6)	11 (33.4)	
Liver iron deposition			
No	18 (62.1)	11 (37.9)	0.42
Yes	20 (66.7)	10 (33.3)	
Liver Steatosis			
No	28 (65.1)	15 (34.9)	0.82
Yes	10 (62.5)	6 (37.5)	
Plasma HIV-1 viral load			
≤ 50	17 (63.0)	10 (37.0)	0.70
> 50	17 (68.0)	8 (32.0)	
Sustained virologic response			
No	27 (54)	11 (22)	0.32
Yes	6 (12)	6 (12)	

* P-value of Yates corrected chi-square

expression of HLA-G has been associated with the shift to a Th2 cytokine polarization [7].

In the present study, HLA-G expression in the liver was not correlated to antiviral response to hepatitis C therapy with PEG-IFN- α plus ribavirin. To our knowledge, this is the first work regarding HLA-G liver expression and response to PEG-IFN- α based therapy. Murdaca et al. [25] observed that basal serum levels of soluble isoforms of HLA-G antigens were similar among HCV positive responders (SVR) and non-responders (NR) to PEG-IFN- α and ribavirin. However, the levels of soluble HLA-G molecules decreased in both groups after three months of HCV therapy but remained higher in NR patients than in SVR patients.

No patient in the present study was treated with the new Direct-Acting Antiviral (DAA) agents, as these medications were unavailable when the patients were treated. DAAs have dramatically changed the panorama of chronic hepatitis C, with cure rates of more than 90% [26]. Initial studies have alerted for increasing rates of hepatocellular carcinoma (HCC) after treatment with DAAs [27–29]. It has been proposed that ceasing HCV infection may induce relevant modifications in the hepatic immune anti-tumor response [28]. However, other more extensive studies have not confirmed these results [30–35]. Amaddeo et al. [36] showed that in patients with compensated cirrhosis who underwent surgical resection for hepatocellular carcinoma and HCV infection cured by therapy with DAAs, intra-hepatic inflammation and immune responses persist after treatment. To our knowledge, no study has described the correlation between HLA-G expression in the liver or serum and response to DAA-based therapy.

5. Conclusions

In conclusion, the present study is one of very few to explore the HLA-G expression profile in the liver microenvironment of HIV/HCV-coinfecting patients, in whom more than half of the biopsies (64%) exhibited HLA-G expression. The presence of HLA-G was significantly associated with later stages of fibrosis. It was not influenced by

patient age, sex, population group, HCV genotype, HIV viral load, iron deposits, and hepatic steatosis.

Author contributions

Fernando Crivelenti Vilar: conceived the study, collected and analysed the data, and wrote the manuscript; Rodrigo de Carvalho Santana: collected and analysed the data, wrote the manuscript; Janaina Cristiana de Oliveira Crispim: data analysis, study design, and draft the manuscript; Ana Letícia Gomide Zanin Borducchi: collected and analysed the data; Eduardo Antonio Donadi: conceived the study, collected and analysed the data, wrote the manuscript; Benedito Antonio Lopes da Fonseca: conceived the study, analysed the data and wrote the manuscript.

All authors have contributed to the critical revision and writing of the paper. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of interests

None.

References

- [1] Weber R, Sabin CA, Friis-Møller N, Reiss P, El-Sadr WM, Kirk O, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med* 2006;166(15):1632–41.
- [2] Rockstroh JK, Mocroft A, Soriano V, Tural C, Losso MH, Horban A, et al. Influence of hepatitis C virus infection on HIV-1 disease progression and response to highly active antiretroviral therapy. *J Infect Dis* 2005;192(6):992–1002.
- [3] Hernandez MD, Sherman KE. HIV/hepatitis C coinfection natural history and disease progression. *Curr Opin HIV AIDS* 2011;6(6):478–82.
- [4] Campbell EC, Antoniou AN, Powis SJ. The multi-faceted nature of HLA class I dimer molecules. *Immunology* 2012;136(4):380–4.
- [5] Lin A, Xu H, Yan W. Modulation of HLA expression in human cytomegalovirus immune evasion. *Cell Mol Immunol* 2007;4(2):91–8.
- [6] Favier B, LeMaout J, Carosella ED. Functions of HLA-G in the immune system. *Tissue Antigens* 2007;69(Suppl 1):150–2.
- [7] Carosella ED, Moreau P, Lemaout J, Rouas-Freiss N. HLA-G: from biology to clinical benefits. *Trends Immunol* 2008;29(3):125–32.
- [8] Favier B, LeMaout J, Rouas-Freiss N, Moreau P, Menier C, Carosella ED. Research on HLA-G: an update. *Tissue Antigens* 2007;69(3):207–11.
- [9] Lajoie J, Fontaine J, Tremblay C, Routy JP, Poudrier J, Roger M. Persistence of high levels of blood soluble human leukocyte antigen-G is associated with rapid progression of HIV infection. *AIDS* 2009;23(11):1437–40.
- [10] Souto FJ, Crispim JC, Ferreira SC, da Silva AS, Bassi CL, Soares CP, et al. Liver HLA-G expression is associated with multiple clinical and histopathological forms of chronic hepatitis B virus infection. *J Viral Hepat* 2011;18(2):102–5.
- [11] de Oliveira Crispim JC, Silva TG, Souto FJ, Souza FF, Bassi CL, Soares CP, et al. Upregulation of soluble and membrane-bound human leukocyte antigen G expression is primarily observed in the milder histopathological stages of chronic hepatitis C virus infection. *Hum Immunol* 2012;73(3):258–62.
- [12] Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994;19(6):1513–20.
- [13] Sciort R, van Eyken P, Facchetti F, Callea F, van der Steen K, van Dijk H, et al. Hepatocellular transferrin receptor expression in secondary siderosis. *Liver* 1989;9(1):52–61.
- [14] Patton HM, Patel K, Behling C, Bylund D, Blatt LM, Vallee M, et al. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. *J Hepatol* 2004;40(3):484–90.
- [15] Crispim JC, Duarte RA, Soares CP, Costa R, Silva JS, Mendes-Junior CT, et al. Human leukocyte antigen-G expression after kidney transplantation is associated with a reduced incidence of rejection. *Transpl Immunol* 2008;18(4):361–7.
- [16] Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 2004;305(5685):872–4.
- [17] Sajadi MM, Shakeri N, Talwani R, Redfield RR. Hepatitis C infection in HIV-1 natural viral suppressors. *AIDS* 2010;24(11):1689–95.
- [18] Tuyama AC, Hong F, Saiman Y, Wang C, Ozkok D, Mosoian A, et al. Human immunodeficiency virus (HIV)-1 infects human hepatic stellate cells and promotes collagen I and monocyte chemoattractant protein-1 expression: implications for the pathogenesis of HIV/hepatitis C virus-induced liver fibrosis. *Hepatology* 2010;52(2):612–22.

- [19] Herbeuval JP, Grivel JC, Boasso A, Hardy AW, Chougnet C, Dolan MJ, et al. CD4+ T-cell death induced by infectious and noninfectious HIV-1: role of type 1 interferon-dependent, TRAIL/DR5-mediated apoptosis. *Blood* 2005;106(10):3524–31.
- [20] Pizzato N, Derrien M, Lenfant F. The short cytoplasmic tail of HLA-G determines its resistance to HIV-1 Nef-mediated cell surface downregulation. *Hum Immunol* 2004;65(11):1389–96.
- [21] Lozano JM, Gonzalez R, Kindelan JM, Rouas-Freiss N, Caballos R, Dausset J, et al. Monocytes and T lymphocytes in HIV-1-positive patients express HLA-G molecule. *AIDS* 2002;16(3):347–51.
- [22] Bertol BC, Dias FC, Debortoli G, Souto BM, Mendonca PB, Araujo RC, et al. HLA-G liver expression and HLA-G extended haplotypes are associated with chronic hepatitis C in HIV-negative and HIV-coinfected patients. *Clin Immunol* 2020;217:108482.
- [23] Amiot L, Vu N, Rauch M, L'Helgoualc'h A, Chalmel F, Gascan H, et al. Expression of HLA-G by mast cells is associated with hepatitis C virus-induced liver fibrosis. *J Hepatol* 2014;60(2):245–52.
- [24] Thibodeau V, Lajoie J, Labbe AC, Zannou MD, Fowke KR, Alary M, et al. High level of soluble HLA-G in the female genital tract of Beninese commercial sex workers is associated with HIV-1 infection. *PLoS One* 2011;6(9):e25185.
- [25] Murdaca G, Contini P, Cagnati P, Marengo S, Pieri G, Lantieri F, et al. Behavior of soluble HLA-A, -B, -C and HLA-G molecules in patients with chronic hepatitis C virus infection undergoing pegylated interferon-alpha and ribavirin treatment: potential role as markers of response to antiviral therapy. *Clin Exp Med* 2017;17(1):93–100.
- [26] Mangia A, Milligan S, Khalili M, Fagiuoli S, Shafran SD, Carrat F, et al. Global real-world evidence of sofosbuvir/velpatasvir as simple, effective HCV treatment: Analysis of 5552 patients from 12 cohorts. *Liver Int* 2020;40(8):1841–52.
- [27] Reig M, Marino Z, Perello C, Inarrairaegui M, Ribeiro A, Lens S, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016;65(4):719–26.
- [28] Ravi S, Axley P, Jones D, Kodali S, Simpson H, McGuire BM, et al. Unusually High Rates of Hepatocellular Carcinoma After Treatment With Direct-Acting Antiviral Therapy for Hepatitis C Related Cirrhosis. *Gastroenterology* 2017;152(4):911–2.
- [29] Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65(4):727–33.
- [30] Nahon P, Layese R, Bourcier V, Cagnot C, Marcellin P, Guyader D, et al. Incidence of Hepatocellular Carcinoma After Direct Antiviral Therapy for HCV in Patients With Cirrhosis Included in Surveillance Programs. *Gastroenterology* 2018;155(5):1436–50 e6.
- [31] Nishibatake Kinoshita M, Minami T, Tateishi R, Wake T, Nakagomi R, Fujiwara N, et al. Impact of direct-acting antivirals on early recurrence of HCV-related HCC: Comparison with interferon-based therapy. *J Hepatol* 2019;70(1):78–86.
- [32] Virlogeux V, Pradat P, Hartig-Lavie K, Bailly F, Maynard M, Ouziel G, et al. Direct-acting antiviral therapy decreases hepatocellular carcinoma recurrence rate in cirrhotic patients with chronic hepatitis C. *Liver Int* 2017;37(8):1122–7.
- [33] Singal AG, Rich NE, Mehta N, Branch A, Pillai A, Hoteit M, et al. Direct-Acting Antiviral Therapy Not Associated With Recurrence of Hepatocellular Carcinoma in a Multicenter North American Cohort Study. *Gastroenterology* 2019;156(6):1683–92 e1.
- [34] Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, Hezode C, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. *Lancet* 2019;393(10179):1453–64.
- [35] Pinero F, Mendizabal M, Ridruejo E, Herz Wolff F, Ameigeiras B, Anders M, et al. Treatment with direct-acting antivirals for HCV decreases but does not eliminate the risk of hepatocellular carcinoma. *Liver Int* 2019;39(6):1033–43.
- [36] Amaddeo G, Nguyen CT, Maille P, Mule S, Luciani A, Machou C, et al. Intrahepatic immune changes after hepatitis C virus eradication by direct-acting antiviral therapy. *Liver Int* 2020;40(1):74–82.