Increased frequencies of activating natural killer receptors are associated with liver injury in individuals who do not eliminate hepatitis C virus

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Abstract

This study was designed to investigate the role of killer immunoglobulin-like receptor (KIR) genes in the outcome of hepatitis C virus (HCV) infection. In patients who cleared the virus (HCV RNA−) we found a decrease of 2DL2 ($P = 0.04$), and 2DS2 ($P = 0.014$) accompanied by an increase of 2DS5 ($P = 0.04$). Those RNA+ patients with elevated levels of hepatic transaminases (HCV RNA+ elevated alanine aminotransferase) showed an increased frequency of 2DS3 ($P = 0.018$). Additionally, in cirrhotic patients we found an increased frequency of individuals having two copies of 3DS1 and HLA-Bw4 ($P = 0.016$). We conclude that higher natural killer cytotoxicity might be associated with a worse progression of the HCV infection.

In hepatitis C virus (HCV) infection about 80% of the patients develop a chronic disease, which may progress to cirrhosis and hepatocellular carcinoma (1, 2). The mechanisms whereby HCV causes acute liver injury and initiates the cascade of events leading to the establishment of persistent infection and development of chronic liver disease are not clearly established.

A strong natural killer cell and T helper 1 cell-mediated immune response in the acute phase of the disease seems to be a key factor in the protection against HCV infection (3, 4).

It was recently reported that a decrease of inhibitory signals in individuals with two copies of killer immunoglobulin-like receptor (KIR) 2DL3 and its ligand human leukocyte antigen (HLA)-C1 homozygosity favors the viral clearance in HCV infection. Additionally, KIR3DS1 + HLA-Bw4 were also associated with clearance (3) but little is known on the role of the interaction of KIR–HLA in the susceptibility to progressive forms of the disease. The present study was designed to characterize KIR genes associated with progressive forms of HCV infection and analyze it in combination with their respective HLA ligands.

This retrospective study included 339 healthy controls (HC) and 257 anti-HCV positive individuals (HCV patients) derived from the Hepatology Units of the Infectious Diseases Hospital 'F. J. Muñiz', the Gastroenterology Hospital 'Dr C. Bonorino Udaondo' and Buenos Aires Italian Hospital, Buenos Aires, Argentina. The ethnicity of this population is known as Latin American Caucasoid.

The clinical features of HCV patients included in this study are described in Table 1. Within the different groups of HCV patients, we found no significant differences between age at which patients became infected, viral genotype, serum HCV

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Table 1 Clinical features of anti-HCV positive patients

		Anti-HCV positive patients[a]					
	Anti-HCV positive patients[a]	HCV RNA negative[b,c]	HCV RNA positive[b]	HCV RNA positive patients[b]			
				Normal ALT[d]	Elevated ALT[d]	Noncirrhotic[e]	Cirrhotic[e]
n	257	23	234	54	180	125	109
Age (years), mean (range)	50 (22–77)	40 (27–61)	51 (22–77)	54 (22–76)	50 (25–77)	49 (25–77)	57 (27–76)
Gender (M:F)*	134:123	8:15	129:105	19:35	108:72	68:57	66:43
HCV RNA (10^3 IU/ml), mean (range)[f]	—	—	1.769 (1.6–20.000)	1.748 (126–6.324)	1.771 (1.6–20.000)	1.960 (1.6–20.000)	1.837 (10–10.417)
Genotype (% genotype 1)[f]	—	—	73%	NA	72%	63%	85%

[a] Anti-HCV antibodies detected by a third generation enzyme-linked immunosorbent assay (4.0 Murex-Abbott).
[b] Circulating HCV RNA detected by qualitative PCR analysis (AMPLICOR HCV test, version 2.0).
[c] Anti-HCV antibodies detected by confirmatory test LIA 3 supplementary test (INNO-LIA HCV-AbIII, Inogenetics).
[d] ALT levels persistently elevated between 1.2 and 7 times over the upper normal limit.
[e] Degree of liver fibrosis (F0–4) assessed by Metavir system (5) in liver biopsies of 168 HCV RNA+ patients. In the other 66 patients, the diagnosis of cirrhosis was performed by clinic and ultrasonographic studies.
[f] Percentage obtained from data available (HCV RNA: $n = 45$, genotype: $n = 82$).
* Gender (M:F): HCV RNA− *vs* HCV RNA+: $P = 0.01$, OR = 3 (1.3–7.3); HCV RNA+ normal ALT *vs* HCV RNA+ elevated ALT: $P = 0.003$, OR = 2.5 (1.4–4.4). HCV: hepatitis C virus; ALT: alanine aminotransferase; elevated ALT: patient with elevated serum level of ALT; normal ALT: patient with normal serum level of ALT; IU: International Units; NA: not available; CI: confidence interval; OR: odds ratio.

RNA level, or alcohol consumption. Patients were negative for human immunodeficiency virus (HIV) markers and other causes of liver disease like hepatitis B virus (HBV) infection, metabolic disease, drug-induced hepatitis, or autoimmune hepatitis. All HC were tested and found to be negative for the presence of infectious diseases (HIV, HBV, HCV, cytomegalovirus, and Chagas disease). Informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of each author's institution.

Table 2 shows the frequencies of KIR genes observed in HCV patients according to the outcome of the infection.

In self-limited HCV patients (RNA−) we detected a decrease in the frequency of the inhibitory 2DL2 KIR gene when compared with HC [$P = 0.047$, odds ratio (OR) = 2.5, confidence interval (CI) = 1.04–5.9] and with RNA+ patients ($P = 0.04$, OR = 2.6, CI = 1.09–6.3; Table 2). This decrease of 2DL2 implies an increase in the frequency of 2DL3/2DL3. However, our cohort had a small number of patients that cleared the virus. Thus, we were not able to confirm the increase of this genotype in combination with HLA-C1 homozygosity as previously reported (3). Additionally, RNA− patients also showed a decreased frequency of 2DS2 when compared with RNA+ patients ($P = 0.014$, OR = 3, CI = 1.3–7.5; Table 2). It is known that 2DL2 and 2DS2 are commonly present in the same haplotype. This is supported in this study by the finding that there was

Table 2 KIR phenotype frequencies in HCV patients and controls

		KIR activators genes						KIR inhibitors genes					
KIR genes	*n*	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	2DL1	2DL2	2DL3	2DL5	3DL1	2DP1
Healthy controls[a]	339	0.45	0.58	0.29	0.95	0.35	0.40	0.95	0.61	0.85	0.55	0.95	0.95
HCV	257	0.40	0.61	0.35	**0.88****	0.36	0.47	0.96	0.61	0.86	0.58	0.91	0.96
HCV RNA+	234	0.43	0.62	0.35	**0.88****	0.35	0.46	0.96	0.63	0.87	0.57	**0.90***	0.96
HCV RNA−	23	0.62	**0.38**$^{\#}$**	0.29	0.90	**0.57****	0.57	0.95	**0.38***$^{\#}$**	0.90	0.67	0.95	0.95
HCV RNA+ elevated ALT	180	0.42	0.65	**0.40***	**0.89***	0.30	0.44	0.97	0.66	0.88	0.55	0.90	0.97
HCV RNA+ normal ALT	54	0.47	0.56	0.30	0.88	0.42	0.51	0.93	0.60	0.81	0.58	0.93	0.93
Noncirrhotic	125	0.42	0.58	0.30	0.92	0.32	0.48	0.96	0.65	0.91	0.55	0.94	0.96
Cirrhotic	109	0.42	0.64	0.37	**0.86****	0.31	0.42	0.97	0.57	0.85	0.55	**0.86****	0.97

The typing of killer immunoglobulin-like receptor (KIR) genes by PCR-sequence-specific oligonucleotide probing was performed as previously described. (6). Two-sided Fisher's exact test for 2 × 2 tables was used to compare the KIR gene frequencies between the groups of patients and controls. Odds ratio with a 95% confidence interval was calculated to evaluate the relative risk in each patient's group. HCV: hepatitis C virus; ALT: alanine aminotransferase; elevated ALT: patient with elevated serum level of ALT; normal ALT: patient with normal serum level of ALT.
Values in bold: $^{*}P < 0.05$, $^{**}P < 0.01$ compared with healthy controls; $^{\#}P < 0.05$ compared with PCR-positive patients.
[a] A. C. Flores et al. manuscript in preparation.

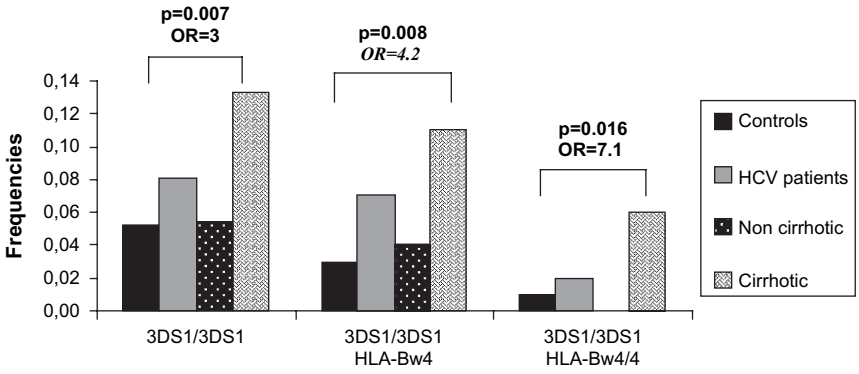

Figure 1 Frequency of 3DS1 and human leukocyte antigen (HLA)-Bw4 in cirrhotic hepatitis C virus patients. The typing of killer immunoglobulin-like receptor and HLA genes was performed by the PCR-sequence-specific oligonucleotide probing technique (6–9). Two-sided Fisher's exact test for 2 × 2 tables was used to compare the frequencies between the groups of patients and controls. Odds ratio with a 95% confidence interval was calculated to evaluate the relative risk in each patient's group.

a complete correlation between presence and absence of 2DL2 and 2DS2 in the RNA− patients.

The frequency of the 2DS3 gene was higher in RNA+ patients with elevated levels of hepatic transaminases [RNA+ elevated alanine aminotransferase (ALT)] compared with HC ($P = 0.018$, OR = 1.6, CI = 1.1–2.3, Table 2). In normal ALT patients the frequency of 2DS3 was similar to the one observed in controls. Thus, the lack of significance between RNA+ elevated ALT and normal ALT patients might merely reflect the small number of patients in the last group. These results might suggest that the cytotoxic activity promotes the tissue injury.

The analysis of 2DS4 shows a significant decrease when we compared the whole population of HCV patients with controls ($P = 0.002$, OR = 0.37, CI = 0.2–0.7). This decrease was also found in RNA+ patients ($P = 0.004$, OR = 0.43, CI = 0.24–0.76), RNA+ with elevated ALT ($P = 0.02$, OR = 0.46, CI = 0.25–0.87), and in cirrhotic patients ($P = 0.01$, OR = 0.4, CI = 0.2–0.8). The lack of significance between HC and RNA− or RNA+ normal ALT patients might also reflect the small number of patients in these groups. Interestingly, those patients who cleared the virus (RNA negative) showed an increase in 2DS5 ($P = 0.04$, OR = 0.4, CI = 0.17–0.96) suggesting that an increase of this activating receptor might favor the virus elimination (Table 2).

We also observed a decrease of KIR3DL1 in RNA+ patients ($P = 0.01$, OR = 0.43, CI = 0.23–0.84) and cirrhotic patients ($P = 0.007$, OR = 0.34, CI = 0.16–0.7; Table 2). As depicted in Figure 1, this decrease is accompanied by an increased homozygosity of 3DS1 (determined by the absence of 3DL1) in cirrhotic patients ($P = 0.007$, OR = 3, CI = 1.4–6.3). Additionally, an increase of 3DS1/3DS1 was observed in patients who also carried at least one allele of HLA-Bw4 ($P = 0.008$, OR = 4.2, CI = 1.5–12) or both genes (3DS1 and Bw4) in homozygosity ($P = 0.016$, OR = 7.1, CI = 1.5–33).

In summary, this study was designed to evaluate the role of KIR genes in the outcome of HCV infection. We found a decrease of 2DL2 and 2DS2 and an increase of 2DS5 in patients who cleared the virus (HCV RNA− patients). The small number of self-limited HCV patients included in this cohort did not allow us to confirm the previously reported role of KIR in the HCV clearance (3). In patients who did not eliminate the virus (HCV RNA+ patients) we found an increase of several activating KIRs, suggesting that higher cytotoxic activity might be associated with a worst progression of HCV infection, in particular in those with elevated transaminases levels and with progression to cirrhosis.

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Conflict of Interest Statement

All authors have declared no conflicts of interests.

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