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HLA-C and KIR Genes in Hepatitis C Virus Infection

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ABSTRACT Natural killer (NK) cells are key components of the innate antiviral immune response. NK cell function is regulated by the interaction of major histocompatibility complex class I molecules with NK inhibitory receptors. The aim of this study was to investigate the role of the HLA-C/KIR pair in hepatitis C virus clearance in our population. A total of 196 hepatitis C virus-infected patients (65 resolved and 131 with persistent infection) were included in the study. Genotyping of HLA-C was carried out using polymerase chain reaction followed by a reverse sequence-specific oligonucleotide probe detection system. NK receptor-specific polymerase chain reaction typing of KIR2DL1, KIR2DL2, and KIR2DL3 was performed on the same patient group. Frequencies of the KIR2DL2 gene and the KIR2DL2/KIR2DL2 genotype were lower among patients with persistent infection (32.3% vs 45.4% among resolved, $P = 0.01$, OR = 0.57, 95% CI = 0.36–0.91; and 16.2% vs 32.3% among resolved, $P = 0.02$, OR = 0.41, 95% CI = 0.19–0.87). Nevetheless, the frequency of the KIR2DL3 gene was higher among patients with persistent infection (66.9% vs 54.6% among resolved $P = 0.02$, OR = 1.68, 95% CI = 1.07–2.65). Trends toward lower frequencies of the HLA-C2C2 genotype and NK-HLA interactions with strong and moderate affinity among the patients with persistent infection were also observed. *Human Immunology 66, 1106–1109 (2005).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: HLA-C; KIR; hepatitis C virus

ABBREVIATIONS

HbsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HLA	human leukocyte antigen
KIR	killer cell immunoglobulin-like receptors
MHC	major histocompatibility complex
NK	natural killer

INTRODUCTION

Natural killer (NK) cells are key components of the innate antiviral immune response. NK cell function is regulated by the interaction of the major histocompatibility complex (MHC) class I molecules with inhibitory receptors [1, 2]. Effector functions occur only when activating signals overcome inhibitory signals [3, 4]. The family of killer cell immunoglobulin-like receptors (KIR) is composed of activating and inhibitory receptors of NK cells. At present, 15 KIR genes and 2 pseudogenes have been described in humans [5], but there are only four KIRs with clearly defined human MHC (HLA) specificities: KIR2DL2 and KIR2DL3 bind HLA-C molecules with Asn80 (HLA-C group 1), KIR2DL1 recognizes HLA-C molecules with Lys80 (HLA-C group 2), and KIR3DL1 binds with the HLA-Bw4 epitope [6]. KIR haplotypes vary in number and type of genes, but, in general, KIR haplotypes could be divided into two groups, A and B, according to the genes they contain. Group A is simpler than group B and contains the

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KIR2DL3 gene. Group B could be subdivided into different groups, the majority of which contain the KIR2DL2 gene [7]. KIR genes map in chromosome 19, whereas their ligands map in chromosome 6; thus, some individuals lack specific receptor–ligand pairs. The presence or absence of KIR genes is usually associated with gain or loss of specificity. That the lack of specificity caused by the absence of a determinate KIR gene cannot be functionally compensated by the presence of others results in functional holes in the repertoire [8]. The population diversity and rapid evolution of the KIR genes strongly suggest that they are under pathogen-mediated selection [9, 10].

Hepatitis C virus (HCV) is a common infection worldwide, causing cirrhosis and hepatocellular carcinoma. HCV infection leads to chronic disease in 50–70% of infected individuals; 15–35% remain asymptomatic, and only about 15% of infected people spontaneously clear the virus [11, 12]. The factors that determine the development of chronic hepatitis have not been clarified, but several factors, including route of infection, size of inoculum, and viral genotype, could be involved [13, 14]. Association between the human MHC polymorphism and spontaneous clearance or self-limited infection has also been reported in HCV infection (see a recent review in [15]). Recently, the direct influence of KIR2DL3 and its ligand HLA-C1 in the resolution of HCV infection has been described, suggesting that diminished inhibitory NK responses confer protection against HCV [16]. The aim of this study was to investigate the role of the HLA-C/KIR pair in HCV clearance in our population.

MATERIAL AND METHODS

Enrolled in this study were 131 Caucasian Spanish patients (81 males, 50 females) with biopsy-proven chronic hepatitis C with compensated liver disease followed in the outpatient clinic of Hospital Universitario Virgen del Rocío (Seville), Hospital Universitario de Valme (Seville), Hospital Virgen Macarena (Seville), Hospital General de Valencia (Valencia), and Hospital Virgen de la Victoria (Malaga). All patients were HbsAg-negative, HIV-negative, and anti-HCV-positive with raised ALT levels and positive HCV RNA in serum. Anti-HCV, HbsAg, and HIV were determined by commercially available methods. Sixty-five Caucasian Spanish patients (28 males, 37 females) who were anti-HCV-positive and HCV RNA-negative constituted the group with spontaneous viral clearance. Patients agreed to a blood examination according to the guidelines of the hospital bioethics committee.

The HLA-C locus was genotyped using the RELI SSO HLA-C SSP Typing Kit (Dynal Biotech, Bromborough, UK). Ambiguities were resolved by sequencing analysis. Individuals from the different groups (persistent and resolved) were categorized as HLA-C1 and HLA-C2 according their genotyping data.

NK receptor-specific polymerase chain reaction (PCR) typing of KIR2DL1, KIR2DL2, and KIR2DL3 was performed with a pair of sense and antisense primers, each possessing a 3' residue matching a polymorphic position on a given NK receptor gene, as previously described [9]. Data from individuals with negative results for the three loci were discarded from the analysis.

RESULTS

There were more HLA-C2C2 individuals in the group with resolved infection (23.1%) than in the group with persistent infection (13.7%), although this difference was not statistically significant ($p = 0.1$) (Table 1). With respect to the genotyping of KIR genes KIR2DL1, KIR2DL2, and KIR2DL3 (Table 2), only one sample obtained from one individual with persistent infection did not amplify in any loci; this sample was eliminated from the analysis. The frequency of KIR2DL3 was higher in patients with persistent infection (66.9%) than in patients with resolved infection (54.6%, $p = 0.02$, OR = 1.68, 95% CI = 1.07–2.65). On the contrary, the frequency of both the KIR2DL2 gene and the KIR2DL2/KIR2DL2 genotype was lower in patients with persistent infection (32.3 and 16.2%, respectively) than in patients with resolved infection (45.4%, $p = 0.01$, OR = 0.57, 95% CI = 0.36–0.91; and 32.3%, $p = 0.02$, OR = 0.41, 95% CI = 0.19–0.87 respectively). We tested the hypothesis that no receptor–ligand or weaker receptor–ligand interaction would be protective. Samples were divided into two groups on the basis of genotyping results for both HLA-C1/C2 and KIR genes: samples having strong or intermediate interactions (presence of KIR2DL1 and C2 or KIR2DL2 and C1) and samples having weak or no interactions (the remainder). Results are summarized in Table 2. Although no significant differences in the distribution of frequencies were observed, strong or intermediate interactions were more frequent in the group with resolved infection (87.7%)

TABLE 1 Frequency of the HLA-C1/C2 genotypes in patients with resolved and persistent HCV infection

HLA-C group genotype	Persistent infection ($n = 131$)	Resolved infection ($n = 65$)	p	OR (95% CI)
C1C1	40 (30.5%)	18 (27.7%)	0.6	1.15 (0.57–2.34)
C1C2	73 (55.7%)	32 (49.2%)	0.4	1.30 (0.69–2.46)
C2C2	18 (13.7%)	15 (23.1%)	0.1	0.53 (0.23–1.22)