Impact of Recipient HLA-C in Liver Transplant: A Protective Effect of HLA-Cw*07 on Acute Rejection


ABSTRACT: The involvement of the human leukocyte antigen (HLA) in liver graft acceptance is controversial, but the frequency of acute rejection (AR) remains high in spite of the use of the modern immunosuppressive agents. The present study was aimed at determining whether an association exists between liver recipient HLA-C polymorphism and AR development that could influence graft acceptance. Four hundred and forty-six liver recipients and 473 controls were studied within the framework of a collaborative study carried out by the Spanish Transplant Immunotolerance Group (RED-GIT). HLA-A and -B were typed by the standard microlymphocytotoxicity technique, and HLA-C by polymerase chain reaction–sequence-specific oligonucleotide probes (PCR-SSOP). A statistically significant decrease in the HLA-Cw*07 allele frequency was found in liver recipients suffering AR episodes compared to those without AR (NAR). Studies regarding the possible influence of the Asn80 and Lys80 epitopes showed that the Asn80 epitope also could be associated with AR. However, further analysis considering Asn80 alleles other than HLA-Cw*07, confirmed that the apparent protective effect of the Asn80 epitope was actually from the HLA-Cw*07 allele. In conclusion, the HLA-Cw*07 allele carried by the liver recipient is negatively associated with AR development, and could be considered a predictive factor for liver graft acceptance. Human Immunology 68, 51–58 (2007). © American Society for Histocompatibility and Immunogenetics, 2007. Published by Elsevier Inc.

KEYWORDS: HLA-C; Asn80; Lys80; liver transplant; acute rejection

ABBREVIATIONS

AR acute rejection
CI confidence intervals
HBV hepatitis B virus
HCV hepatitis C virus
HLA human leukocyte antigen
KIR killer immunoglobulin-like receptors
NAR non-acute rejection
NK natural killer cells
OR odds ratio
PCR-SSOP polymerase chain reaction–sequence-specific oligonucleotide probes
INTRODUCTION
Several studies have investigated the possible role of the human leukocyte antigen (HLA) system in liver transplantation, although no beneficial effect of HLA compatibility has been firmly established [1, 2]. Moreover, it has been reported that HLA compatibility, in particular partial or total HLA-A and -B class I matching, could have a detrimental effect on liver graft acceptance [3, 4].

To better understand the reduced impact of the HLA system in liver graft alloresponse, a dualistic effect of HLA in liver transplants was initially proposed, whereby HLA matching reduces rejection, but may also trigger restricted mechanisms of liver allograft injury and disease recurrence [5]. However, despite tolerogenic liver behavior and the improvement in graft acceptance obtained with the use of modern immunosuppressive drugs, acute rejection (AR) remains one of the most frequent complications. Acute cellular rejection is commonly present within the first weeks after transplantation, where alloreactive T cells and HLA molecules may play a pivotal role [6].

In this context of reduced alloreactivity the likely impact of the recipient's special immunogenetic status on graft outcome could become more evident. Hence, it could be thought that not only HLA matching but also the HLA molecules from the recipient may be important genetic factors that contribute to liver graft alloresponse [7, 8], thus, influencing graft acceptance or rejection [9].

At present, in the field of HLA studies, there is renewed interest in HLA-C, because molecules encoded by this locus are ligands for killer cell immunoglobulin-like receptors (KIR) expressed on natural killer (NK) cells and in certain subsets of T cells [10, 11]. This function is related to the dimorphism at position 80 in the α1-helix, a position that allows defining of two groups of HLA-C alleles interacting respectively with KIR2DL1/S1 or KIR2DL2/3/S2 inhibitory receptors [12, 13]. Additionally, HLA-C molecules are polymorphic and can function as classical HLA antigens [14–16].

Because of the apparently low impact of HLA-A and -B matching, and because of the few HLA-C studies carried out in organ transplants, the aim of this work was to address the question of whether or not HLA-C molecules carried by the liver recipient are related to early rejection or acceptance in liver grafts. Our findings demonstrate that patients with the HLA-C Asn80 epitope, particularly those bearing HLA-Cw*07 may show better graft acceptance.

PATIENTS AND METHODS
Patients
This is a retrospective study performed as a collaborative work of the Spanish Transplant Immunotolerance Group (RED-GIT, G03-104). A total of 446 liver recipients who underwent orthotopic liver transplants were included in this study. Liver recipients belonged to two geographically distant regions of Spain; 202 of these transplants were carried out at the Juan Canalejo University Hospital in Coruña (Region of Galicia, in the northwest of Spain), whereas the remaining 244 came from the Virgen de la Arrixaca University Hospital in Murcia (Region of Murcia, in the southeast of Spain). Inclusion criteria were: recipients with a first liver graft, liver graft survival higher than a week after transplantation, and availability of adequate samples. Retransplants were excluded from the study.

This series was comprised of 330 males and 116 females with an average age of 51 ± 11 years; the underlying diseases are shown in Table 1. For the analysis recipients were classified into two groups, those showing AR episodes (n = 145), and those without AR (NAR, n = 301). The follow-up comprises a minimum period of time of one year.

Additionally, 473 healthy Caucasian volunteer donors (229 from Galicia and 244 from Murcia) were genotyped as the control population. Informed consent was obtained from each participant and the study protocol was approved by the corresponding institutional ethical committees.

Immunosuppressive Therapy
Immunosuppression consisted of standard triple-drug therapy with cyclosporine A, methylprednisolone, and azathioprine. The initial dose of cyclosporine was at 5–10 mg/kg/day, and the maintenance dose was adapted according to blood concentration measured by radioimmunoassay (range, 300–400 ng/mL for induction and 100–150 ng/mL during the first year after transplant), and to possible clinical complications such as renal function disorder and number of rejection episodes. In the perioperative period, 1 g of methylprednisolone was also...
administered, which was subsequently adjusted at 20 mg/day. Azathioprine was given at 1.5 to 2 mg/Kg/day. Acute rejection episodes were treated with high-doses of methylprednisolone (bolus of 1 g for 3 days), and anti-CD3 therapy was used in patients with histologic evidence of resistance to the standard rejection treatment.

AR Diagnosis

The diagnosis of AR was based on clinical, biochemical, and histologic criteria [17]. Briefly, aminotransferases, bilirubin, alkaline phosphatase, and gamma glutamyltransferase were determined daily. When serum liver enzyme levels increased, Doppler echography was carried out to exclude hepatic ischemia from hepatic artery or portal vein occlusion, and to establish an indication for liver biopsy. Histopathologic diagnosis of AR was based on three main features: 1) mixed, but predominantly mononuclear portal inflammation containing blastic or activated lymphocytes, neutrophils, and eosinophils; 2) subendothelial inflammation of portal and/or terminal hepatic veins; and 3) bile duct inflammation and damage [18]. Once the diagnosis of AR had been established, grading of the severity of AR was based on the Banff Schema for liver allograft rejection [19].

In this series early AR (any grade) was mainly detected within the first six weeks following transplantation, with a mean of 14.3 ± 8.8 days.

Sample Collection and DNA Extraction

Peripheral blood samples were collected in the pre-transplant period using sterile anti-coagulated Vacutainer tubes (Becton-Dickinson, Mountain View CA, USA). Afterwards, a fresh aliquot was used for peripheral mononuclear blood cell separation by a gradient density method. Another aliquot was used for genomic DNA extraction by QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany), as recommended by the manufacturer.

HLA-A, -B Typing

HLA-A and -B class I antigens were determined using the standard microlymphocytotoxicity technique [20, 21]. Sera set used for antigen determination included those generated in our centers, those supplied by participating laboratories in the Spanish Histocompatibility Workshops, well-defined commercial sera, and commercial typing trays (One Lambda, Los Angeles, CA, USA). When needed typing was confirmed by molecular methods.

HLA-C Typing

HLA-C was genotyped using a Dynal RELI SSO HLA-C typing Kit (Dynal Biotech ASA, Oslo, Norway), at a level of resolution that allows us to distinguish the HLA-C dimorphism at position 80 of the α1 helix. According to this, HLA-C alleles could be grouped into two major KIR epitopes: the Asn<sup>80</sup> epitope shared by HLA-Cw*01, *03, *07, *08, *12, *14, *16, alleles and the Lys<sup>80</sup> epitope comprising HLA-Cw*02, *04, *05, *06, *15, *17, *18 alleles.

Statistical Analysis

Demographic data and results of the analysis were collected in a database (Microsoft Access 2.0; Microsoft Corporation, Seattle, WA), and statistical analysis was performed using the SPSS 12.0 software (SPSS Inc., Chicago IL, USA). Frequencies of the HLA class I antigens or alleles were estimated by direct counting and represent the percentage of positive individuals for a certain HLA allele or antigen. Arlequin program version 1.1 was used to estimate maximum-likelihood three-locus haplotype frequencies through an expectation-maximization algorithm Arlequin Program (University of Geneva, Switzerland). The frequencies observed in controls and liver recipients with and without AR were compared using χ<sup>2</sup> and the two-sided Fisher exact test. P-values were corrected (P<sub>c</sub>), multiplying them by the number of antigens or alleles tested within each locus (Bonferroni correction) [22]. Odds ratios (OR) were cal-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Clinical Characteristics of 446 Spanish Liver Recipients Included in This Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics</strong></td>
<td><strong>n (%)</strong></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td><strong>n (%)</strong></td>
</tr>
<tr>
<td>Male</td>
<td>330 (74.0)</td>
</tr>
<tr>
<td>Female</td>
<td>116 (26.0)</td>
</tr>
<tr>
<td><strong>Age at transplant</strong></td>
<td><strong>Mean ± SD</strong></td>
</tr>
<tr>
<td></td>
<td>51 ± 11 years</td>
</tr>
<tr>
<td><strong>Geographic area</strong></td>
<td><strong>n (%)</strong></td>
</tr>
<tr>
<td>Murcia</td>
<td>244 (54.7)</td>
</tr>
<tr>
<td>Galicia</td>
<td>202 (45.3)</td>
</tr>
<tr>
<td><strong>Indications for transplant</strong></td>
<td><strong>n (%)</strong></td>
</tr>
<tr>
<td>Alcoholic cirrhosis</td>
<td>177 (39.7)</td>
</tr>
<tr>
<td>HBV infection</td>
<td>18 (4.0)</td>
</tr>
<tr>
<td>Chronic active hepatitis B</td>
<td>105 (23.5)</td>
</tr>
<tr>
<td>Chronic active hepatitis C</td>
<td>27 (6.1)</td>
</tr>
<tr>
<td>Chronic active hepatitis B and C</td>
<td>5 (1.1)</td>
</tr>
<tr>
<td>HBV, HCV and alcoholic cirrhosis</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Malignant disease</td>
<td>26 (5.8)</td>
</tr>
<tr>
<td>Fulminant hepatitis</td>
<td>11 (2.5)</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>8 (1.8)</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>15 (3.4)</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>7 (1.6)</td>
</tr>
<tr>
<td>Wilsons disease</td>
<td>5 (1.1)</td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>9 (2.0)</td>
</tr>
<tr>
<td>Corinodo Andrade</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Others</td>
<td>26 (5.8)</td>
</tr>
</tbody>
</table>

HLA-C in Liver Recipients
RESULTS

Analysis of the HLA Class I in the Two Spanish Populations Studied

First, the distribution of HLA-A, -B, and -C frequencies was compared in the control populations from Coruña and Murcia Regions, and no statistically significant differences in antigen or allele frequencies were found between both populations (data not shown). These results allow us to add up the data from both populations and use them as a Caucasian control population for the following comparisons with liver recipients.

HLA-C Allele Frequencies in Liver Recipients

Of the 446 liver recipients analyzed, 145 developed AR, whereas the remaining 301 did not. This represents 32.5% of the whole series, with a similar percentage of AR for the two studied series (30.7% in the Murcian vs 34.7% in the Galician transplanted recipients). No association was found between AR frequency and age or gender ($p > 0.05$ in both cases). Comparisons of allele frequencies between groups revealed that HLA-Cw*07 was significantly lower in the liver recipients belonging to the AR group than in those of the NAR group (31.0% vs 47.5%, OR = 0.50; 95% CI: 0.33 to 0.76, $p = 0.001$, $P_0 = 0.01$). Furthermore, the HLA-Cw*07 allele frequency was also lower in liver recipients from the AR group than in controls, but in this case the statistical significance was lost after $P$ value correction (Table 2).

Analysis of the HLA-A and -B Antigens in the AR and NAR Liver Recipients

To discard the possible influence of recipient HLA class I loci other than HLA-C, a total of 425 recipients for HLA-A and 424 for HLA-B were also typed (data not shown). Between the HLA-A and -B antigens, only HLA-A21 antigen was more frequent in NAR than in AR patients (5.9% vs 1.4%, OR = 0.23, CI: 0.05 to 1.02, $p = 0.04$), but without statistical significance after $P$ correction ($P_0 = 0.64$). No other HLA-A or HLA-B antigen associations were found, allowing us to discard the influence of these loci in our study.

HLA class I Incompatibility Between Donor-Recipient Pairs

Similarly, to assess whether HLA class I allele mismatch influenced the early liver graft outcome, HLA class I compatibility was examined in 405 of 446 liver transplants. However, no significant association was found between the HLA class I (HLA-A+B+C) mismatching and the AR frequency (data not shown).

Frequencies of HLA-C Epitopes in Liver Recipients

When the influence of a single HLA-C Asn$^{80}$ or Lys$^{80}$ epitope was also studied in liver recipients, the distribution of both epitopes was significantly different in those

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**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Total controls (n = 473) n (%)</th>
<th>Total Recipients (n = 446) n (%)</th>
<th>Recipients AR Group (n = 145) n (%)</th>
<th>Recipients NAR Group (n = 301) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-Cw*01</td>
<td>29 (6.1)</td>
<td>28 (6.3)</td>
<td>7 (4.8)</td>
<td>21 (7.0)</td>
</tr>
<tr>
<td>HLA-Cw*02</td>
<td>35 (7.4)</td>
<td>49 (11.0)</td>
<td>13 (9.0)</td>
<td>36 (12.0)</td>
</tr>
<tr>
<td>HLA-Cw*03</td>
<td>55 (11.6)</td>
<td>51 (11.4)</td>
<td>19 (13.1)</td>
<td>32 (10.6)</td>
</tr>
<tr>
<td>HLA-Cw*04</td>
<td>133 (28.1)</td>
<td>97 (21.7)</td>
<td>34 (23.4)</td>
<td>63 (20.9)</td>
</tr>
<tr>
<td>HLA-Cw*05</td>
<td>71 (15.0)</td>
<td>78 (17.5)</td>
<td>31 (21.4)</td>
<td>47 (15.6)</td>
</tr>
<tr>
<td>HLA-Cw*06</td>
<td>76 (16.1)</td>
<td>68 (15.2)</td>
<td>29 (20.0)</td>
<td>39 (13.0)</td>
</tr>
<tr>
<td>HLA-Cw*07</td>
<td>206 (43.6)</td>
<td>188 (42.2)</td>
<td>45 (31.0)$^{a,b}$</td>
<td>143 (47.5)</td>
</tr>
<tr>
<td>HLA-Cw*08</td>
<td>55 (11.6)</td>
<td>63 (14.1)</td>
<td>19 (13.1)</td>
<td>44 (14.6)</td>
</tr>
<tr>
<td>HLA-Cw*12</td>
<td>79 (16.7)</td>
<td>60 (13.5)</td>
<td>21 (14.5)</td>
<td>39 (13.0)</td>
</tr>
<tr>
<td>HLA-Cw*14</td>
<td>11 (2.3)</td>
<td>15 (3.4)</td>
<td>3 (2.1)</td>
<td>12 (4.0)</td>
</tr>
<tr>
<td>HLA-Cw*15</td>
<td>35 (7.4)</td>
<td>32 (7.2)</td>
<td>15 (10.3)</td>
<td>17 (5.6)</td>
</tr>
<tr>
<td>HLA-Cw*16</td>
<td>76 (16.1)</td>
<td>78 (17.5)</td>
<td>22 (15.2)</td>
<td>56 (18.6)</td>
</tr>
<tr>
<td>HLA-Cw*17</td>
<td>9 (1.9)</td>
<td>10 (2.2)</td>
<td>4 (2.8)</td>
<td>6 (2.0)</td>
</tr>
<tr>
<td>HLA-Cw*18</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>0 (0.0)</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

Abbreviations: AR, acute rejection; NAR, non-acute rejection.

$p$ Value was determined by two-sided Fisher’s exact test.

$a$ AR group vs NAR group; OR: $0.50$; 95% CI: 0.33 to 0.76, $p = 0.001$; $P_0 = 0.01$.

$^{b}$ AR group vs total controls; OR: $0.58$; 95% CI: 0.39 to 0.87, $p = 0.009$; $P_0 = 0.13$. 

Culculated for the estimation of the relative risk, and their 95% confidence intervals (CI) were calculated by the Cornfield method.
patients suffering AR from those not suffering (Table 3). Comparisons between the NAR and AR groups revealed that the Asn\(^{80}\) epitope frequency was significantly decreased in AR recipients (75.2% vs 85.4%, OR: 0.52, 95% CI: 0.32 to 0.85; \(p = 0.01\)), whereas the frequency of the Lys\(^{80}\) epitope was significantly higher in the AR recipients (69.7% vs 57.8%, OR: 1.68, 95% CI: 1.10 to 2.55; \(p = 0.02\)).

A subsequent analysis was made, according to which patients were classified into three groups: homozygous individuals for HLA-C alleles with the Asn\(^{80}\) epitope, homozygous individuals for HLA-C alleles with the Lys\(^{80}\) epitope, and heterozygous individuals bearing HLA-C alleles with the Asn\(^{80}\) and Lys\(^{80}\) epitopes. The whole distribution of the HLA-C phenotypes according to the Asn\(^{80}\) and Lys\(^{80}\) epitopes was significantly different in patients with AR from those not suffering from it (\(p = 0.009\)). The Asn\(^{80}\)/Asn\(^{80}\) phenotype was significantly under-represented in the AR group of recipients compared with those of the NAR group (30.3% vs 42.2%, OR: 0.60, 95% CI: 0.39 to 0.91; \(p = 0.02\)), whereas the frequency of the Lys\(^{80}\)/Lys\(^{80}\) phenotype was significantly higher in the AR group (24.8% vs 14.6%, OR = 1.93, 95% CI: 1.18 to 3.16; \(p = 0.01\)).

Because a lower frequency of the HLA-Cw\(^*07\) allele was previously observed in the AR recipients, additional comparisons were made to determine whether the effect seen for HLA-C alleles with Asn\(^{80}\) epitope was shared by all the alleles belonging to this group or whether it was mainly from the influence of HLA-Cw\(^*07\). When patients bearing the HLA-Cw\(^*07\) allele were segregated from the total number of recipients, the whole distribution of the HLA-C phenotypes was similar in the two groups of liver recipients (\(p = 0.46\)). Likewise, the distribution of the Asn\(^{80}\) and Lys\(^{80}\) epitopes as well as that of the Asn\(^{80}\)/Asn\(^{80}\) and Lys\(^{80}\)/Lys\(^{80}\) phenotypes was similar in both groups of recipients. These results indicated that the negative association between alleles bearing the Asn\(^{80}\) epitope and the AR group of recipients was mainly mediated by the HLA-Cw\(^*07\) allele, indicating that this allele could exert a protective effect against AR development.

### HLA-Cw\(^*07\) More Frequent Haplotypes

Given that HLA-Cw\(^*07\) may be in linkage disequilibrium with certain HLA-A or -B alleles, an analysis regarding the most frequent HLA-A-B-Cw\(^*07\) extended haplotypes in liver recipients was performed. This analysis revealed that the most frequent haplotypes were, in decreasing order of frequency, HLA-A1-B8-Cw\(^*07\), HLA-A2-B49-Cw\(^*07\), HLA-A1-B49-Cw\(^*07\), and HLA-A2-B7-Cw\(^*07\). Interestingly, none of these haplotypes was significantly over-represented in NAR recipients.

### Study of HLA-C in the Main Indications for Liver Transplant

Finally, to exclude the possible influence of the primary liver disease in our study, HLA-C polymorphism was analyzed in each one of the most frequent causes of liver transplant, such as alcoholic cirrhosis, HCV and HBV infection, malignant disease, and diseases of autoimmune origin. In no patient was any association observed between HLA-C polymorphism and disease susceptibility (data not shown).

### DISCUSSION

The results of this report show, for the first time, the existence of a correlation between the presence of HLA-Cw\(^*07\) allele in liver recipients and protection against the risk of suffering AR. This protective effect was initially associated with the presence of the Asn\(^{80}\) epitope, but disappeared after excluding HLA-Cw\(^*07\) patients from the analysis, indicating that only this allele was responsible for the observed protective effect (Table 3). This HLA-Cw\(^*07\) effect seems not to be influenced by linkage disequilibrium, because in our series no positive or negative associations between any of the most frequent HLA-A-B-Cw\(^*07\) haplotypes and protection against rejection was found. Indeed, this effect appears independent of HLA-A+B+C class I mismatching, as well as from the most frequent liver diseases, as well as age and gender (data not shown).

### TABLE 3  HLA-C Epitope Frequencies in Liver Recipients

<table>
<thead>
<tr>
<th>Epitopes</th>
<th>Controls (n = 473)</th>
<th>Total Recipients (n = 446)</th>
<th>Recipients AR Group (n = 145)</th>
<th>Recipients NAR Group (n = 301)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-C(^{Asn80})</td>
<td>386 (81.6)</td>
<td>366 (82.1)</td>
<td>109 (75.2)(^a)</td>
<td>257 (85.4)</td>
</tr>
<tr>
<td>HLA-C(^{Lys80})</td>
<td>300 (63.4)</td>
<td>275 (61.7)</td>
<td>101 (69.7)(^b)</td>
<td>174 (57.8)</td>
</tr>
</tbody>
</table>

Abbreviations: AR, acute rejection; NAR, non-acute rejection.

\(^{a}\)  AR vs NAR group; OR: 0.52; 95% CI: 0.32 to 0.85, \(p = 0.01\).

\(^{b}\)  AR vs NAR group; OR: 1.68; 95% CI: 1.10 to 2.55, \(p = 0.02\).
The present data partially agree with those previously obtained by us in a shorter series of liver transplants [14], and appear to support the influence of the liver recipient’s HLA-C genotype in the early success of the liver graft. However, they do not definitely confirm the previously suggested deleterious effect of the HLA-Cw*06 allele for liver graft acceptance. These findings also agree with the observations of Oertel et al. [23], about the potential absence of association between NK allostereactivity and AR. Unlike the results of Bishara et al. [24], which suggest an impact of the HLA-C epitope disparity in liver transplants, we find an influence on the part of the recipient HLA-C, although this discrepancy could be explained by the different sizes of the series in these two studies.

The importance of the liver recipients HLA-C could be considered in the light of two potential explanations that are linked to different functions: 1) the potential ability of HLA-C alleles to present peptides in the context of the indirect pathway to stimulate alloreactive T cells, and 2) the likelihood that they could act as ligands for KIR receptors.

Accordingly to the first possibility, it has been demonstrated that HLA-C molecules present peptides [25–27], although this function could be restricted by their low level of expression on the cell surface and their limited polymorphism in the peptide-binding groove [28, 29]. Because associations of the HLA-Cw*0602 and HLA-Cw*0701 alleles with autoimmune diseases have been described [30–32], it might be thought that in transplant situations, HLA-C alleles carried from recipients could also bind and present allopeptides, inducing specific alloresponses. Nevertheless, the HLA-Cw*07 protective effect cannot be explained by this capability, unless HLA-Cw*07 peptide presentation results in insufficient signals, leading to the anergization or tolerization of effector allospecific T cells.

From the second point of view, it is known that cognate HLA-KIR ligation can modify NK and T cell cytotoxic programs and contributes to self-tolerance [11, 33–35], and it is reasonable to think that these HLA-C-KIR interactions could also mediate in allotolerance. In fact, some studies support that not only HLA but also KIR gene repertoires can influence the stem cell transplant outcome [36–38], and that in these transplants HLA class I could dictate the frequency of cells expressing KIRs, whereas KIR repertoires mainly control the level of KIR expression [34, 39].

Because CD8+ T cells mostly express inhibitory rather than activating KIRs [13, 35], the possible recognition of autologous HLA-C molecules loaded with allopeptides by CD8+ T cells expressing KIR2D receptors might provide a complementary mechanism to down-modulate the cytotoxic function of these T cells once they are specifically alloactivated [11, 40]. In this setting, our findings postulate that in liver recipients, Asn80 alleles, and mostly HLA-Cw*07, could have a higher capacity to interact with their cognate KIR2DL2/3 receptors expressed on alloreactive cytotoxic CD8+ T lymphocytes, and to modulate the alloreactivity of these cells. This effect could be supported by the fact that the promoter region of the HLA-Cw*07 gene has special characteristics that differentiate it from the other HLA-C promoters. Consequently, HLA-Cw*07 genes are transcribed twice more than other HLA-C genes [40], leading to a higher expression of these molecules, which, in turn, could increase their capacity to interact with cognate inhibitory receptors. Furthermore, the overexpression of HLA-Cw*07 could improve their capacity to interact with non-MHC restricted alloactivated NK-like T lymphocytes, such as occurs in some subsets of CD4+ T lymphocytes, in which the cytotoxic activity is regulated by HLA-Cw*07-mediated inhibition [41]. Taking this into consideration, it is also possible that HLA-Cw*07 amplify T cell inhibitory signals, over-riding those provided by other HLA-KIR combinations such as the Lys80–KIR2DL1 interactions.

In the same way, there has been evidence that KIR2D triggering by the corresponding HLA-C ligand can interfere with the early TCR signaling, preventing completion of the T cell activation programs [42]. Therefore, it is plausible that higher HLA-Cw*07 antigen levels on membrane surfaces could also increase the interference with the TCR-activating signals. On the other hand, as HLA-KIR interactions are complex, the mediation of additional signals other than those provided by HLA-C and KIR2D ligation cannot be discarded. Even so, and independently of the complexity of HLA-C/KIR interactions, our study of the HLA polymorphism shows that Asn80 HLA-C alleles from liver recipients could play a role in protection against AR.

Finally, although classically alloactivated effector T cells dominate in the alloresponse, NK cells, at least, could mediate in the alloresponse of donor-recipient pairs where the HLA-C-ligands are missing in donors. However, in our series, such intervention is unlikely, because no relation with HLA incompatibility was demonstrated.

In summary, these findings suggest that liver recipient HLA-C alleles, particularly HLA-Cw*07, could play a role in the control of T lymphocyte alloactivation and liver graft allotolerance, and also might lead to support an influence of the recipient immunogenetic status on graft acceptance or rejection. Although more extensive studies including a major effort to type KIR gene repertoires would be of interest, our data show that the single analysis of HLA-C polymorphism may be of help in clinical follow-up and prognosis, and for the indication of individualized therapies in an attempt to avoid
the negative side effects of the immunosuppressive standard treatments.

Acknowledgments
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