

Impact of HLA-C and Bw Epitopes Disparity on Liver Transplantation Outcome

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ABSTRACT: The occurrence of graft rejection episodes after orthotopic liver transplantation (OLT) despite the use of immunosuppressive drugs designed to suppress T lymphocyte functions, indicates the involvement of other types of cells in this process. The activity of natural killer cells and their killer immunoglobulin-like receptors (KIR) is regulated by human leukocyte antigen (HLA) class I determinants; C and Bw epitopes. Because recipient/donor pairs are usually HLA mismatched, recipient natural killer alloreactivity may be the mediating factor in rejection. In this retrospective study, we have analyzed rejection occurrence and outcome in 66 OLT recipients, 42 with and 24 without C or Bw epitope disparity in the rejection direction. Recipients transplanted from donors with no C epitope disparity had significantly fewer rejection episodes in the first year after transplantation compared with recipients transplanted across C epitope dis-

parity ($p = 0.0002$). Moreover, this effect was more pronounced when the outcome was analyzed in OLT recipients across negative crossmatching for the anti-HLA class I and II antibodies. In contrast, Bw epitope disparity did not affect the outcome. In conclusion, C epitopes disparity between recipients and donors in the rejection direction appears to influence posttransplant liver outcome. This finding may be helpful in the choice of appropriate liver donor and planning immune suppression. *Human Immunology* 66, 1099–1105 (2005). © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: Liver transplantation; rejection; killer inhibitory receptors; NK alloreactivity; HLA C; Bw epitopes

ABBREVIATIONS

CDC complement-dependent cytotoxicity
CM crossmatch
GVH graft versus host
HLA human leukocyte antigen

KIR killer immunoglobulin-like receptor
MHC major histocompatibility complex
NK natural killer cells
OLT orthotopic liver transplantation

INTRODUCTION

Human leukocyte antigen (HLA) matching reduces renal and heart allograft rejection episodes and prolongs survival. It was therefore suggested that allograft rejection might be mediated by T-lymphocytes, with allorecognition by CD4⁺ and execution by CD8⁺ [1, 2]. Consequently, immunosuppressive agents were designed to suppress T-lymphocyte functions [3, 4]. In the case of

orthotopic liver transplants (OLT), however, the beneficial role of HLA matching is still controversial and inconclusive [5, 6]; indeed, HLA class I mismatch, mainly in the A locus, appears to correlate significantly with the incidence of acute rejection and the development of the vanishing bile duct syndrome [5]. Moreover, although, conventional immunosuppressant therapies have reduced the occurrence of graft rejection, it remains the major complication after OLT. These data indicate that immunologic mechanisms other than the classical antigen-presenting cell pathways are involved in hepatic allograft rejection [7, 8]. This study was therefore undertaken to investigate if natural killer (NK) cells, through their inhibitory receptors and their ligands, are involved in graft rejection after liver transplantation.

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Soon after reperfusion of the grafted liver, donor leukocytes are released into the bloodstream of the host, and uptake of the host leukocytes into the graft "influx." The influx of NK and NK-like cells may induce apoptosis of the host alloreactive T-lymphocytes, thus inducing tolerance and acceptance of the graft [9, 10]. Activity of NK cells is regulated by killer immunoglobulin-like receptors (KIR) expressed on their surface. These receptors have both inhibitory and activating potential that are triggered by inhibitory or activating motifs presented in their cytoplasmic tail [9, 11, 12]. Among the inhibitory receptors, some are specific for different groups of major histocompatibility complex (MHC) class I alleles, whereas others are "orphan" receptors [11, 12]. Inhibitory KIRs recognize three loci of self-HLA class I determinants: HLA-A-, B-, and Cw, but C and Bw epitopes dominate their activity. C epitopes are recognized by KIR 2DL1 and 2DL2 based on dimorphism in amino acids at positions 77 and 80 [13, 14]. Bw4 is recognized by KIR 3DL1 [14], whereas KIR 3DL2 recognizes A3 and A11 [15]. Activating KIRs 2DS1 and 2DS2 also recognize the C epitopes [12]. In general, KIRs recognize self-cells via specific loci of the class I antigen. Killing only occurs when target cells are either HLA class I negative or deficient. Inhibitory activity, as described in the missing self theory, dominates the activation activity; hence, net NK activity is a balance of both inhibitory and activating receptor activity [16, 17].

The effect of KIR epitope or gene disparity on the outcome of hematopoietic stem cell transplantation from mismatched donors has been assessed [18, 19]. However, the involvement of NK cells in solid-organ transplantation has not yet been elucidated. Based on the HLA profiles of recipients and donors and the missing self theory, we might speculate that NK alloreactivity after liver transplantation would lead to graft rejection [20]. However, although OLT recipients with potential NK alloreactivity in the direction of rejection showed an increased number of NK cells and higher NK activity, this was not associated with a high incidence of acute rejection [21]. Using a liver allograft rejection rat model, it was demonstrated that NK cells of recipient origin infiltrate the allografts, produce cytokines, and express cytolytic mediators such as granzyme-B and Fsa-L that are part of the rejection process [22]. Further indication that NK cells may be involved in solid-organ allograft rejection comes from cardiac allografts. These grafts have been shown to activate recipient NK cells that killed their targets with specificity and potency comparable to cytotoxic T-lymphocytes [23].

In this study, we analyze the acute rejection episodes and other complications in 66 OLT recipients in relation to their potential NK alloreactivity in the rejection direction. Our aim is to clarify whether C and Bw epitopes

disparity have an impact on OLT outcome, thereby improving the selection of suitable donors and planning immune suppression treatment.

PATIENTS AND METHODS

Patients, Immunosuppression, and Transplantation Regimens

Sixty-six consecutive adult OLT recipients were included in this study. All were transplanted at the Hadassah-Hebrew University Medical Center (Jerusalem, Israel) between January 1992 and June 2004. Sixteen patients received cyclosporine-based triple immunosuppressive therapy, consisting of cyclosporine (blood trough levels aimed for 150 ng/ml), azathioprine (1–2 mg/kg/day), or mycophenolate mofetil (2 g twice/day) (calculated per kg/m²), and prednisolone (starting with 1 mg/kg/day, tapering to 7.5 mg/day). The other 50 patients received an immunosuppressive regimen consisting of tacrolimus (whole blood trough levels 7–15 ng/ml) and prednisolone. All recipients had more than 3 months post-transplant follow-up and were evaluated for rejection episodes and posttransplant complications. Acute rejection episodes were diagnosed clinically (raised serum aminotransferase and bilirubin values, secretion of pale bile) in the absence of signs of infection (negative bacterial cultures, no evidence of active hepatitis B virus, hepatitis C virus, or cytomegalovirus infection), and proven by core biopsy in all cases and by the response to antirejection treatment. Acute rejection was defined by Snovir's triad of portal hepatitis, endothelialitis, and lymphocytic cholangitis, and was graded as mild, moderate or severe [24]. Chronic rejection was also confirmed by histology. Antirejection therapy consisted of three to five pulses of methylprednisolone. None of the patients had steroid-resistant rejection. Demographic characteristics of the recipients are summarized in Table 1.

HLA Typing and Definition of Epitope Disparity

Recipient/donor pairs were typed for HLA class I and class II by serologic and molecular methods [25]. Molecular HLA-Cw typing was performed in all cases of undetectable HLA-Cw related serology. Based on typed HLA-Cw alleles, recipients and donors were classified as belonging to HLA C1 (alleles with Asn⁷⁷-Lys⁸⁰) or HLA C2 (alleles with Ser⁷⁷-Asn⁸⁰) [14]. Classification of HLA B alleles was based on the presence or absence of the Bw4 epitope. Because there is no universal agreement as to which HLA A alleles interact with the KIR3DL2 receptor, HLA A alleles were not considered in this cohort. Based on recipient/donor HLA profile, patients were subdivided into two major histocompatibility states: those with epitope disparity in rejection direction when HLA C1/C2 or Bw4 epitopes was absent in the donor but

TABLE 1 Clinical and demographic characteristics of the study group in relation to no and epitope disparity

	No epitope disparity <i>n</i> = 24	C or Bw disparity <i>n</i> = 42	Statistics (<i>p</i>) ^a
Median age (years)	41	46	0.47
Male/female	10/14	26/16	0.13
Diagnosis: hepatitis B virus	6	11	1
Hepatitis C virus	6	11	1
Primary sclerosing cholangitis	3	6	1
Cryptogenic	4	7	1
Others	4	7	0.75
Cyclosporine A ^b	6	10	1
FK506 ^b	18	32	1
Positive crossmatch	6	9	1
Negative crossmatch	18	33	

^a Fishers' exact test.

^b Immunosuppressant regimens combined with prednisone.

present in the recipient [19]. All other recipient/donor combinations were considered as epitope matched; lacking potential NK alloreactivity in the rejection direction. Crossmatches (CM) and immunologic evaluation for the presence of HLA class I and class II antibodies were performed in all cases. Antidonator-specific CM was performed using the complement-dependent cytotoxicity (CDC) method [26].

Statistical Analysis

The rejection episodes of the groups studied were compared by means of Fisher's exact tests and Student's *t*-tests [27].

RESULTS

Effect of NK Epitope Mismatching on Outcome

Of the 66 liver recipients, 42 (64%) were mismatched with their donors in the rejection direction. Twenty-five donors of the 42 were lacking HLA-C1/C2 (mismatched

for C epitopes), 14 lacking the Bw4 epitope (mismatched for Bw epitopes), and 3 lacking C and Bw epitopes (these 3 were analyzed with the group that were mismatched for C epitope [*n* = 28]). The remaining 24 patients (36%) were matched with their donors for C and Bw epitopes. There were no significant differences in age, sex, diagnosis, and immunosuppressive treatment between the matched and mismatched groups (Table 1). We analyzed the number of rejection episodes in the first year after OLT, of the recipients that survived longer than 1 year, and in the three groups; the results are presented in Table 2.

The results presented in Table 2 clearly demonstrate that recipients transplanted from donor without epitope disparity had significantly better outcome than the group transplanted from a donor with C-epitope disparity. Table 2 demonstrates that 50% of the no-disparity group had a rejection-free outcome as compared with only 4% in the C-epitope disparity group. The percentage of recipients that developed a single rejection episode was similar in both groups. Moreover, the portion of recipients that developed two or more rejection episodes was significantly higher in the C-epitope mismatched groups: 70% as compared with 33% in the group lacking disparity (*p* = 0.0046).

The outcome of recipients in the group that was transplanted across Bw epitope disparity was very similar to that of the group without epitope disparity, indicating that the Bw epitope disparity had no impact on outcome.

Effect on OLT Outcome of C-Epitope Disparity Across Negative CM

Some of the C-epitope-mismatched recipients were transplanted across positive CM because of the presence of HLA class I and class II antibodies. In an attempt to isolate the effect of C epitope disparity on OLT outcome, the outcome was analyzed in the group that was transplanted across negative CM and survived longer than 1 year.

Results presented in Table 3 confirm those of the whole group and even demonstrate a more profound

TABLE 2 Rejection episodes in the first year after orthotopic liver transplantation in relation to human leukocyte antigen-C or Bw epitope disparity

Rejection episodes	Epitope disparity in rejection direction				
	None <i>n</i> = 22 ^a	C epitope <i>n</i> = 27 ^a	Statistics <i>p</i> ^b	Bw epitope <i>n</i> = 14 ^a	Statistics <i>p</i> ^b
None	11 (50%)	1 (4%)	0.00021	5 (36%)	0.50
One	5 (28%)	6 (22%)	1	2 (14%)	0.68
Two	5 (28%)	13 (48%)	0.08	4 (29%)	0.71
Three or more	1 (5%)	6 (22%)	0.11	0	1
Graft loss	1	2	1	3	0.277

^a Number of recipients who survived longer than 1 year.

^b Fischer's exact test.

TABLE 3 Rejection episodes in the first year after orthotopic liver transplantation in relation to human leukocyte antigen-C or Bw epitope disparity in recipients transplanted across negative crossmatch

Rejection episodes	Epitope disparity in rejection direction				
	None $n = 17^a$	C epitope $n = 22^a$	Statistics p^b	Bw epitope $n = 10^a$	Statistics p^b
None	11 (65%)	1 (5%)	0.00007	5 (50%)	0.69
One	4 (24%)	6 (27%)	1	2 (20%)	1
Two	2 (11%)	11 (50%)	0.017	3 (30%)	0.33
Three or more	0	4 (18%)	0.11	0	1
Graft loss	0	1	1	0	1

^a Number of recipients who survived longer than 1 year.

^b Fischer's exact test.

effect of C-epitope disparity on outcome: 68% of the recipients in the group with C epitope disparity developed two or more rejection episodes as compared with only 11% in the group with no epitope disparity ($p = 0.0008$). These results were as expected because the percentage of transplants across positive CM was similar in the C epitope matched and mismatched groups (Table 1).

Of the 17 recipients with no epitope disparity in the rejection direction, 7 were mismatched in graft-versus-host (GVH) direction and 10 with no epitope mismatching in any direction. Analyzing the outcome in relation to mismatching at GVH revealed that 71% of the mismatched group and 60% of the group with no epitope disparity were rejection free by the end of the first year after OLT ($P = 1$). Bw epitope disparity did not influence the outcome in this analysis.

Because the group that was mismatched only for Bw epitopes was matched for C epitopes, we combined these groups and compared the outcome with that of the group that was mismatched for C epitopes. The results are presented in Table 4 and clearly demonstrate that C epitope disparity has major effect on outcome: only 5% of the recipients were rejection free and 58% had two or

more rejection episodes as compared with 59% and 19% in the group with no disparity for C epitopes in the rejection direction. These differences are of high significance: $p = 0.000054$ and $p = 0.00057$, respectively.

The effect of C epitope disparity on outcome was therefore observed to be dominant in recipients of OLT across negative CM and in the overall picture.

Effect of C and Bw Epitope Disparity on Post-OLT Complications

To analyze the effect of C and Bw epitope disparity on post-OLT complications, the analysis was performed on all recipients who were transplanted across negative CM and the complications were analyzed in relation to no epitope disparity and to C or Bw epitope disparity. The results are presented in Table 5. Hepatic artery thrombosis was more frequent in the C epitope-mismatched groups. There were no significant differences in the other complications between these groups, though the total number of complications was higher in the C epitope-mismatched group and the difference was significant ($p = 0.025$). Also in this analysis, the disparity for Bw epitope did not have major impact on the occurrence of post-OLT complications.

In general, the number of recipients in each group in this analysis is rather small, a factor that will affect and make the statistical evaluation more difficult.

TABLE 4 Rejection episodes in the first year after orthotopic liver transplantation in relation to no C and C epitope disparity in recipients transplanted across negative crossmatch

Rejection episodes	No C epitope $n = 27^a$	C epitope $n = 22^a$	Statistics p^b
None	16 (59%)	1 (5%)	0.000054
One	6 (22%)	6 (27%)	0.74
Two	5 (19%)	11 (50%)	0.042
Three or more	0	4 (18%)	0.034
Graft loss	0	1	0.45

^a Number of recipients who survived longer than 1 year.

^b Fischer's exact test.

DISCUSSION

The results presented in this article suggest that mismatching HLA class I epitopes that control NK activity via ligation with inhibitory receptors are related to a high incidence of acute rejection after OLT from cadaver donors. The results also demonstrate a dominant influence of C epitopes over Bw epitopes in a hierarchy of inhibitory effects mediated by class I loci [28]. In a previous report, we demonstrated a deleterious effect of positive CM on post-OLT outcome [29]. In an attempt

TABLE 5 Post OLT complications in relation to Epitope disparity in the rejection direction

Complications	Epitope disparity in rejection direction				
	None <i>n</i> = 18	C epitope <i>n</i> = 23	Statistics <i>p</i> ^a	Bw <i>n</i> = 10	Statistics <i>p</i> ^a
Hepatic artery thrombosis	1	5	0.2	1	1
Hepatitis C virus reinfection	2	3	1	2	0.6
Hepatitis B virus reinfection	0	1	1	0	1
Cytomegalovirus infection	0	0	1	1	0.35
Chronic rejection	0	1	1	0	1
Retransplantation	0	3	0.24	0	1
Patient death	1	1	1	0	1
Total	4	14	0.025	4	0.7

^a Fischer's exact test.

to isolate the effect of epitope disparity on the outcome, the outcome was analyzed in relation to epitope disparity in recipients transplanted across negative CM. Results evidenced the deleterious effect of C epitope disparity in the negative CM group as well, indicating that it is an independent risk factor in acute rejection episodes. Post-OLT complications such as hepatic artery thrombosis tend to be more frequent in OLT recipients across C epitope disparity. Our results contradict those of Oertel *et al.* [21], who did not find increased rates of allograft rejection in OLT recipients mismatched for C and Bw epitopes, although they did find a high frequency of NK cells and increased cytotoxic activity against NK targets in the mismatched patients.

The ability of NK cells to reject transplants has been demonstrated in hematopoietic stem cell transplantation, an example being the hybrid resistance phenomenon [30, 31]. In the case of solid-organ rejection, it has been demonstrated that recipient NK cells infiltrate the transplanted organ before T lymphocytes, but their role in rejection has still not been fully elucidated [20]. In a rat model, it has been demonstrated that Wistar Furth heart allografts transplanted to Brawn Norway recipients are able to activate alloreactive NK cells that are capable of killing their allogeneic targets with specificity, similar to that of cytotoxic T-lymphocytes [23]. Also, in a rat liver allograft model, an activating receptor, RNKp30, homologous to the human NKp30 activating receptor, was identified, which may play a role in liver rejection [32]. As for human liver allografts, immediately after revascularization there is an "influx" of recipient NK and NK-like cells into the transplanted organ, which may cause apoptosis of the alloreactive recipient T lymphocytes, thus inducing acceptance of the liver. On the other hand, acute rejection occurs despite immunosuppressive drug therapy. This suggests that liver allograft acceptance or, alternatively, rejection, is a complex process that involves multiple pathways in the innate and acquired immune systems [33, 34].

To prevent allograft rejection, two main strategies are presently employed: (1) the prevention of allorecognition by means of donor/recipient MHC matching and (2) immunosuppressive therapy, designed to suppress recipient immune response. Both of these approaches have proved effective in reducing allograft rejection and increasing survival in kidney and heart allograft recipients. In contrast, the effect of HLA matching on OLT outcome is still controversial [35, 36] and, although immunosuppressive therapy has been shown to be effective in the prevention and treatment of acute rejection, acute rejections remain a main complication after OLT.

The results of our study demonstrate the impact of mismatched C and Bw epitopes, which regulate NK alloreactivity, and perhaps also T-lymphocyte alloreactivity, on OLT outcome. Moya-Quiles *et al.* [37] demonstrated that OLT recipients without acute rejection episodes had fewer HLA-Cw*06 alleles than those with acute rejection episodes, or the controls, and also observed that the frequency of acute rejection episodes decreased when there were fewer HLA-C mismatches. The same group reported that graft survival rates increased when there were fewer mismatches in both HLA-C and NK alloantigens [38]. In our cohort of OLT patients, there was no significant difference in survival between those with and without NK alloreactivity potential. The impact of CW*06 expression on outcome was not analyzed because of the small number of rejection-free recipients, and there were no significant differences in the number of HLA antigen shared between the recipients and donors in the group with and without potential NK alloreactivity in the rejection direction.

OLT recipients matched with the donors for C epitopes in the rejection direction might therefore be mismatched for HLA-Cw alleles; thus, allorecognition by T lymphocytes may emerge also. This might explain the occurrence of rejection episodes in recipients matched for C epitopes with their donors. Alternatively, in this group with no C epitope mismatch in the rejection direction, a

mismatch in GvH direction may occur and NK cells in the transplanted liver may eliminate donor specific T lymphocyte, thus preventing rejection [18]. In this study, there were only 7 recipients mismatched in the GVH direction and 10 with no mismatch in this direction. There were no significant differences in the outcome between these groups.

Both the cyclosporine-based and FK506-based regimens of immunosuppressants used affect interleukin-2 production, on which T and NK lymphocytes depend for clonal expansion [39, 40]. However, it was demonstrated that NK cells and Antibody-dependent cell-mediated cytotoxicity (ADCC) activity are resistant to FK506 [41], and Vampa *et al.* [42] demonstrated in a direct *ex vivo* setting that recipient NK antidonor cytotoxicity was increased 3 days after transplantation despite quadruple immunosuppression therapy. Recipients exhibiting increased NK cytotoxicity against their donors after transplantation were found to possess more activating KIR genes specific for donor class I MHC than those in whom killing activity did not increase.

Acute rejection episodes may be overcome by increasing the doses of the immunosuppressant agents; however, such action results in an increased incidence and severity of side effects such as nephrotoxicity, neurotoxicity, osteoporosis, obesity, and glucose intolerance [40].

The results presented in this article have shown that disparity for C epitopes between recipient and donor in the rejection direction may increase the risk of acute rejection episodes and other post-OLT complications. Consequently, matching for C epitopes may facilitate the choice of more suitable liver donors, thereby reducing the need for post-OLT immunosuppressant agents and improving the quality of life for transplanted patients.

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