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ORIGINAL ARTICLE

HLA variants related to primary sclerosing cholangitis influence rejection after liver transplantation

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Abstract

AIM: To investigate influence of human leukocyte antigen (HLA) and killer immunoglobuline-like receptor (KIR) genotypes on risks of acute rejection (AR) after liver transplantation (LTX).

METHODS: In this retrospective study we included 143 adult donor-recipient pairs with a minimum of 6 mo follow-up after LTX for whom DNA was available from both donor and recipients. Clinical data, all early complications including episodes and severity of AR and graft/patient survival were registered. The diagnosis of AR was based on clinical, biochemical and histological criteria. All suspected episodes of AR were biopsy confirmed. Key classical HLA loci (*HLA-A*, *HLA-B*, *HLA-C* and *HLA-DRB1*) were genotyped using Sanger sequencing. 16 KIR genes were genotyped using a novel real time PCR approach which allows for determination of the diploid copy number of each KIR gene. Immunohistochemical staining for T (CD3), B (CD20) and natural killer (NK) cells (CD56 and CD57) were performed on liver biopsies from 3 different patient groups [primary sclerosing cholangitis (PSC), primary biliary cirrhosis and non-autoimmune liver disease], 10 in each group, with similar grade of AR.

RESULTS: Fourty-four (31%) patients were transplanted on the basis of PSC, 40% of them had AR *vs* 24% in the non-PSC group (*P* = 0.04). No significant impact of donor-recipient matching for HLA and KIR genotypes was detected. In the overall recipient population an increased risk of AR was detected for HLA-B*08 (*P* = 0.002, OR = 2.5; 95%CI: 1.4-4.6), HLA-C*07 (*P* = 0.001, OR = 2.4; 95%CI: 1.4-4.0) and HLA-DRB1*03 (*P* = 0.03, OR = 1.9; 95%CI: 1.0-3.3) and a decreased

risk for HLA-DRB1*04 (*P* = 0.001, OR = 0.2; 95%CI: 0.1-0.5). For HLA-B*08, HLA-C*07 and DRB1*04 the associations remained evident in a subgroup analysis of non-PSC recipients (*P* = 0.04, *P* = 0.003 and *P* = 0.02, respectively). In PSC recipients corresponding *P* values were 0.002, 0.17 and 0.01 for HLA-B*08, HLA-C*07 and DRB1*04, respectively. A dosage effect of AR prevalence according to the PSC associated HLA alleles was also notable in the total recipient population. For HLA-B*08 the frequency of AR was 56% in HLA-B*08 homozygous recipients, 39% in heterozygous recipients and 21% in recipients lacking HLA-B*08 (*P* = 0.02). The same was observed for the HLA-C*07 allele with AR in 57%, 27% and 18% in recipients being homozygous, heterozygous and lacking HLA-C*07 respectively (*P* = 0.003). Immunohistochemical analysis showed similar infiltration of T, B and NK cells in biopsies with AR in all three groups.

CONCLUSION: We found significant associations between the PSC-associated HLA-B*08, HLA-C*07, HLA-DRB1*03 and HLA-DRB1*04 alleles and risk of AR in liver transplant recipients.

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Key words: Liver transplantation; Primary sclerosing cholangitis; Acute rejection; Human leukocyte antigen; Killer immunoglobulin-like receptor

Core tip: Patients undergoing liver transplantation on the basis of primary sclerosing cholangitis (PSC) have a higher frequency of acute cellular rejections than non-PSC patients. Recent studies have determined the genetic susceptibility to PSC, of which genetic variants in the human leukocyte antigen complex represent the strongest risk factors. In the present report we show that these variants also influence risk of acute cellular rejection after liver transplantation in PSC. Moreover, we show that the same variants also involve in risk of acute cellular rejection in non-PSC recipients.

Fosby B, Næss S, Hov JR, Traherne J, Boberg KM, Trowsdale J, Foss A, Line PD, Franke A, Melum E, Scott H, Karlsen TH. HLA variants related to primary sclerosing cholangitis influence rejection after liver transplantation. *World J Gastroenterol* 2014; 20(14): 3986-4000 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i14/3986.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i14.3986

INTRODUCTION

Liver transplantation (LTX) is the only curative treatment for end-stage liver disease[1]. Although both graft and patient survival have improved dramatically over the last two decades, acute rejection (AR) still represents a significant clinical problem[2-5]. Numerous studies over the years have shown different frequencies (range 11%-80%) of AR after LTX, but consistently the incidence of AR in patients transplanted on the basis of primary sclerosing cholangitis (PSC) is increased (range 17%-100%) compared to other groups[3-6]. Patients treated for non-autoimmune liver diseases (*e.g.*, alcoholic liver disease or fulminant hepatic failure from paracetamol intoxication) seem to have a low risk of AR[2,6-8]. Analyses of variables affecting AR risk after LTX have been inconsistent, and whether matching of donor and recipient for human leukocyte antigen (HLA) influences AR occurrence after LTX remains controversial[9-12]. The increased risk of AR in PSC patients after LTX is clinically important because increased immunosuppression is needed with concomitant side effects. Rejection episodes in PSC patients may also increase the risk for recurrent PSC in the liver allograft[5,13-15].

HLA class Ⅰ molecules interact specifically with T-cell receptors (TCR) upon binding of antigenic peptides, and they (HLA-B and HLA-C in particular) can also act as ligands for killer-cell immunoglobulin-like receptors (KIR) expressed on natural killer (NK) cells and subsets of T-cells[16-18]. The interaction between the genetically determined HLA-C2 variant and its corresponding KIRs delivers a more potent inhibitory signal to NK cells than HLA-C1[16]. The potential benefit of incorporating genetic killer immunoglobulin-like (KIR) variation when assessing the impact of HLA matching on AR risk is not clear[19-23]. The major biological role of the HLA-KIR interaction is to influence activation status in various contexts of NK cell and T cell function (*e.g.*, cytotoxicity)[16]. The HLA-C/KIR interactions have been studied extensively in HLA-related[24,25] and unrelated[26,27] hematopoietic stem cell transplantation (HSCT).

There is evidence to suggest that the influence of HLA on AR risk is not uniform in all recipients and that stratification for the presence of underlying autoimmune liver diseases with known HLA associations may influence results[12,28]. PSC is strongly HLA-associated[29], with the peak association signal mapping to the HLA class Ⅰ region (HLA-B*08 in particular), but there are also significant residual effects within class Ⅱ, likely represented by several HLA-DRB1 alleles (*i.e.*, DRB1*13:01, DRB1*03:01, DRB1*04, DRB1*07 and DRB1*11)[30-33], or variants in linkage disequilibrium with these. Two studies also suggest that genetic variation affecting the binding of class Ⅰ molecules to KIRs contribute to the HLA associations in PSC[34,35].

In this study we aimed to explore the influence of HLA and KIR genotypes on AR risk after LTX with particular emphasis on patients transplanted for PSC compared to recipients with other underlying liver diseases.

MATERIALS AND METHODS

Patients

All patients undergoing LTX between 1996 and 2008 at Oslo University Hospital, Rikshospitalet, Norway were included in a retrospective design. A total of 520 LTX

were performed during this period and for 198 donor/recipient pairs archival DNA samples were available for HLA and KIR genotyping. We included all patients above 12 years that received a first liver graft from a deceased donor and with a minimum follow-up period of six months (*n* = 176). Patients with early graft loss (< 30 d) resulting from primary dysfunction or vascular complications without evidence of AR were excluded (*n* = 7). Patients receiving ABO incompatible liver or those with a combined liver/kidney transplantation were also excluded (*n* = 4). To minimize the impact of confounders, we excluded patients with uncertain or inconclusive biopsy results and patients treated for AR based on clinical/biochemical suspicion only, without a confirmatory biopsy (*n* = 17; 8 PSC, 3 autoimmune hepatitis, 1 hepatitis C cirrhosis, 2 hepatitis C cirrhosis, 2 alcoholic cirrhosis, 1 primary biliary cirrhosis). Finally we also excluded 5 patients who received immunosuppressive regimens according to obsolete protocols (calcineurin inhibitor in combination with azathioprine and prednisolone).

The final study population comprised 143 adult recipients (82 males, 61 females) with a median age of 52 years (range 13 to 73 years). PSC patients constituted the largest group of liver recipients (*n* = 45). The diagnosis of PSC was in all cases made based on standard diagnostic criteria[36]. The study protocol received prior approval by the regional ethics committee (reference number S-08873b). Written informed consent concerning the use of biopsy/DNA material was obtained from all recipients still alive. The research ethics committee granted exemption from consent for recipients deceased at time of study initiation. Furthermore, the Norwegian Health Directorate approved the utilization of DNA samples from the deceased donors (reference number 08/10827).

Clinical data

By thorough investigation of all patient records, blood tests, radiology results and histology assessments, we determined indications for LTX, number and severity of episodes of AR [Banff rejection activity index (RAI) scoring, steroid responsive/non-responsive], graft survival and overall patient survival. Immunosuppression consisted of standard triple-drug therapy with tacrolimus, prednisolone and mycophenolate mofetil. Tacrolimus (or cyclosporin) was given on the first day after LTX and adjusted according to daily blood concentrations. Methylprednisolone was administered perioperatively at a dosage of 500 mg, subsequently tapered to 20 mg/d during the first week and gradually to 5 mg/d within 6 mo. Mycophenolate was given at an initial dose of 1000 mg twice daily.

The diagnosis of AR was based on clinical, biochemical and histological criteria. According to routine practice at our center, all patients were monitored by daily blood samples and any increase in liver enzymes or bilirubin resulted in examination with liver sonography with doppler to determine the presence of circulatory or anatomical complications. After clinical exclusion of circulatory and surgical causes of detected abnormalities in liver enzymes and/or bilirubin, a liver biopsy was carried out. All suspected episodes of AR included in this study were biopsy confirmed and classified by rejection activity index (RAI) according to the Banff 1997 standard[37]. All but two biopsies, which showed severe AR, were classified as either mild (RAI 3-4) or moderate (RAI 5-6). Ten patients (5 PSC, 2 primary biliary cirrhosis (PBC), 1 hepatocellular carcinoma, 1 cholangiocarcinoma and 1 hepatitis C infection) had steroid-resistant AR (after 3-4 pulses of methylprednisolone over 3-4 consecutive days, total dose of 2-2.5 g) and were subsequently treated with antithymocyte globuline (ATG) according to protocol.

HLA-genotyping

HLA-A, *-B*, *-C* (class Ⅰ) and *-DRB1* (class Ⅱ) typing was performed using previously described sequencing-based typing protocols at Institute for Immunology, Oslo University Hospital, Rikshospitalet, Norway[34]. Ambiguous HLA types were excluded from analysis (1 patient HLA-B, 1 patient *HLA-C*).

KIR-genotyping

KIR genotyping was performed using a novel method allowing accurate determination of copy number variation (CNV) of each *KIR* gene based on a triplex real-time PCR approach[38]. 14 KIR-genes and 2 pseudogenes were genotyped for each sample in quadruplicate; samples with ≤ 1 successful replicate were excluded. Calling of copy numbers was verified manually in CopyCaller v.1.0 (Applied Biosystems, Foster City, CA, United States) and results imported into Microsoft Excel (Redmond, WA, United States). Copy numbers > 4 were excluded and all 0 copies were verified by amplification of the reference gene.

Immunohistochemistry

To expand on the genetic analysis of KIR genes, also considering the conflicting results in previously published experiments[19-23], an immunohistochemical determination of the number of NK cells as compared with T- and B cells in liver allograft biopsies with AR were performed. Archival biopsy material from 10 patients with PSC, 10 patients with PBC and 10 patients with non-immunological liver diseases (3 alcoholic cirrhosis, 1 polycystic liver disease, 2 cryptogenic cirrhosis, 1 Budd Chiari and 3 with colorectal liver metastases) were examined. All selected biopsies had similar severity of rejection characterized according to the Banff criteria[37], with a median RAI score of 5, 4.5 and 5 for PSC, PBC and the non-immunological group, respectively.

Immunohistochemical staining procedure: Antigen retrieval: heat treatment in Tris EDTA, pH9.0 buffer. Primary antibodies: Monoclonal anti CD20, dilution 1:200 and anti CD56, dilution 1:100, both from Daco, Glostrup, Denmark; monoclonal anti CD57, dilution 1/50, Nova Castra, Newcastle, United Kingdom; polyclonal

Table 1 Distribution and frequency of the underlying liver disease of 143 liver transplant recipients

Indication for transplantation	*n* (%)
Autoimmune etiology	65 (45.5)
Primary sclerosing cholangitis	44 (30.8)
Primary biliary cirrhosis	14 (9.8)
Autoimmune hepatitis	7 (4.9)
Viral hepatitis	20 (13.9)
Hepatitis B	5 (3.5)
Hepatitis C	15 (10.5)
Other indications	65 (43.9)
Hepatocellular carcinoma	13 (8.8)
Secondary liver tumours	7 (4.7)
Acute hepatic failure	8 (5.4)
Alcoholic cirrhosis	17 (12.2)
Cholangiocarcinoma	10 (6.8)
Budd-Chiari	2 (1.4)
Cryptogenic cirrhosis	9 (6.1)
Metabolic liver disease	1 (0.5)
Polycystic liver disease	2 (1.4)

rabbit anti CD3, dilution 1/150, Neo Markers, Fremont CA, United States. Diluent: Ventana Antibody Diluent, Ventana Medical System, Tucson AZ. Incubation time: 30 min at 37 C. Detection system: Ultra View Universal DAB- or Alkaline Phosphatase Red-Detection Kits. Double stainings: Sequential stainings, one primary antibodies followed by one of the detection kits, a second primary antibody and the other detection kit. Since NK cell specific monoclonal antibodies could not be used in formalin fixed material, we stained the biopsy samples for CD56 and CD57 for detection of putative NK- and NKT cells.

We calculated the B cell percentage (CD20/CD20 + CD3) in the portal infiltrates based on counting of more than 400 B and T cells in the portal areas. Since the numbers of CD56 and CD57 positive cells turned out to be very low, we decided to use the absolute number per square millimeter. Double immunostaining of CD56 and CD3 was performed in order to distinguish between classical NK cells ($CD3^-CD56^+$) and natural killer T (NKT) cells ($CD3^+CD56^+$), which represent a distinct lineage of T cells and share several surface markers with NK cells.

Statistical analysis

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL). *P* values less than 0.05 were considered significant. The prevalence of AR in the different groups was compared using the χ^2 test and, where appropriate, the Fisher's exact test. OR and corresponding 95%CI were calculated using Woolf's formula with Haldane's correction. As have been demonstrated in previous studies[29,43], there is no significant population stratification in the Norwegian population. Comparison of means for KIR gene copy number correlated to AR was performed using independent sample *t* test (after assessment of normal distribution). For analysis of NK cell infiltration in biopsies with AR, the one way ANOVA test was used. All *P* values from the KIR association analysis were corrected for multiple testing according to Bonferroni (*n* = 68).

RESULTS

The disease indications for LTX are shown in Table 1. The overall frequency of histologically confirmed AR in the study population was 28%, with significantly higher frequency noted in patients with PSC as compared with patients transplanted on the basis of other liver diseases (40% *vs* 24%, OR = 2.2, 95%CI: 1.0-4.6, *P* = 0.04). All episodes of AR occurred within the first 6 weeks after LTX, with a median of 10 days (range 5-40 d). PSC patients with concurrent IBD (82%), showed tendency to an increased risk of AR compared to PSC patients without IBD (43% *vs* 25%), but this was not statistically significant (*P* = 0.34). We found no statistically significant influence from age or gender on risk of acute rejection (data not shown). For further analyses, recipients were grouped according to the occurrence of AR or non-AR and subgrouped according to underlying PSC or not.

PSC-associated HLA alleles confer risk for AR independently of underlying disease

Given the significantly higher frequency of AR in patients with PSC, we specifically analyzed the total study population for differences in frequencies of PSC associated *HLA-A*, *-B*, *-C* and *-DR* alleles between patients with AR and without AR. From the HLA alleles previously reported to associate with increased PSC susceptibility, an increased risk of AR was detected for recipients positive for HLA-B*08 (*P* = 0.002, OR = 2.5, 95%CI: 1.4-4.6), HLA-C*07 (*P* = 0.001; OR = 2.4; 95%CI: 1.4-4.0) and HLA-DRB1*03 (*P* = 0.03, OR = 1.9, 95%CI: 1.0-3.3) (Tables 2-4). For the HLA-DRB1*04 allele, previously shown to associate with decreased risk of PSC, reduced risk of AR in the overall study population was found (*P* = 0.001, OR = 0.2, 95%CI: 0.1-0.5) (Table 4). Notably, for HLA-B*08, HLA-C*07 and HLA-DRB1*04 these associations remained evident in a subgroup analysis of non-PSC recipients only (*n* = 99) (*P* = 0.04, *P* = 0.003 and *P* = 0.02, respectively) (Tables 5-7). In PSC recipients (*n* = 44) corresponding *P* values were 0.002, 0.17 and 0.03 for HLA-B*08, HLA-C*07 and DRB1*04, respectively.

A dosage effect of AR prevalence according to the PSC-associated HLA alleles was also notable in the total recipient population. For HLA-B*08, the frequency of AR was 56% in HLA-B*08 homozygous recipients, 39% in heterozygous recipients and 21% in recipients lacking HLA-B*08 (*P* = 0.02). The same was observed for the HLA-C*07 allele with AR in 57%, 27% and 18% of recipients being homozygous, heterozygous and lacking HLA-C*07 respectively (*P* = 0.003). For HLA-DRB1*04 the situation was reversed, with the frequency of AR being 0% for DRB1*04 homozygous recipients, 12% for heterozygous and 35% in recipients lacking the DRB1*04 allele (*P* = 0.009).

Table 2 Frequencies of human leukocyte antigen-B alleles in the acute cellular rejection group (*n* = 40) and the non-acute cellular rejection group (*n* = 102) in the total population (missing *n* = 1)

HLA-B allele	Acute rejection		No acute rejection		OR	95%CI	Uncorrected
	n (alleles)	(%)	*n* (alleles)	(%)			*P* value
*05	0	(0)	8	(4)	0.1	0.0-1.1	0.070
*07	10	(13)	23	(11)	1.2	0.6-2.5	0.690
*08	27	(34)	34	(17)	2.5	1.4-4.6	0.002
*12	12	(14)	27	(13)	1.2	0.6-2.3	0.700
*13	1	(1)	5	(2)	1.1	0.3-4.6	1.000
*14	2	(2)	4	(2)	1.1	0.3-4.6	1.000
*15	6	(7)	28	(14)	0.5	0.2-1.2	0.150
*16	1	(1)	4	(2)	0.7	0.1-3.4	0.510
*17	1	(1)	6	(3)	0.6	0.1-2.8	0.410
*18	1	(1)	6	(3)	0.4	0.1-2.0	0.240
*21	0	(0)	2	(1)	0.5	0.1-4.8	0.360
*22	3	(3)	2	(1)	3.6	0.8-16.4	0.120
*27	4	(5)	10	(5)	1.1	0.4-3.2	1.000
*35	4	(6)	19	(9)	0.7	0.3-1.8	0.230
*37	4	(5)	4	(2)	2.6	0.7-9.1	0.180
*40	4	(5)	19	(9)	0.6	0.2-1.5	0.220
*41	1	(1)	2	(1)	1.5	0.3-9.1	0.870
*47	0	(0)	1	(0.5)	0.8	0.1-9.2	0.530

OR and corresponding 95%CI were calculated using Woolf's formula with Haldane's correction; uncorrected *P* value: calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen; AR: Acute cellular rejection.

Table 3 Frequencies of human leukocyte antigen-C alleles in the acute cellular rejection group (*n* = 40) and the non-acute cellular rejection group (*n* = 102) in the total population (missing *n* = 1)

HLA-C allele	Acute rejection		No acute rejection		OR	95%CI	Uncorrected
	n (alleles)	(%)	*n* (alleles)	(%)			*P* value
*01	2	(2.5)	8	(3.9)	0.6	0.3-2.9	0.43
*02	2	(2.5)	11	(5.4)	0.5	0.1-1.8	0.24
*03	10	(12.5)	43	(21.1)	0.7	0.3-1.1	0.09
*04	8	(10.0)	24	(11.8)	1.1	0.4-2.2	0.89
*05	8	(10.0)	18	(8.8)	1.0	0.5-2.3	0.76
*06	6	(7.5)	16	(7.8)	1.2	0.5-2.8	0.82
*07	41	(51.2)	62	(30.4)	2.4	1.4-4.0	0.001
*08	2	(2.5)	4	(2.0)	1.1	0.3-4.7	0.54
*12	0	(0)	8	(4.2)	0.2	0.02-1.1	0.07
*14	0	(0)	1	(0.5)	0.9	0.08-9.4	0.72
*15	0	(0)	4	(2.0)	0.3	0.03-2.5	0.26
*16	0	(0)	3	(1.5)	0.4	0.04-3.2	0.31
*17	1	(1.2)	4	(2.0)	0.8	0.2-4.3	0.57

OR and corresponding 95%CI were calculated using Woolf's formula with Haldane's correction; uncorrected *P* value: calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen.

For all other recipient class Ⅰ and class Ⅱ alleles, no significant differences between the groups with and without AR were detected.

Effect of HLA class Ⅰ and class Ⅱ mismatching on the risk of AR

HLA-class Ⅰ or class Ⅱ recipient-donor mismatching (0 *vs* 1-2 mismatches for *HLA-A*, *HLA-B*, *HLA-DR* and up to 4 *vs* 5-6 mismatches for all three epitopes combined, respectively) had no significant effects on AR incidence (Table 8), neither when all patient-donor pairs were analyzed together nor when subgrouped according to PSC/non-PSC status (Table 9).

KIR ligand (i.e., HLA-C and Bw4) disparity does not affect the risk of AR after LTX

According to the established differences in KIR binding strength for HLA-C1 and HLA-C2[21,39,40], genotyped HLA-C alleles were classified as either HLA-C1 (Asn[80]) or HLA-C2 (Lys[80]). The donor HLA-C ligand group (*i.e.*, C1 or C2) did not significantly influence the risk of AR after LTX. AR was observed in 44% of LTX with a HLA-C2 homozygote donor and in 22% in the heterozygous group compared to 31% in the HLA-C1 homo-

Table 4 Frequencies of human leukocyte antigen-DRB1 alleles in the acute cellular rejection group (n = 40) and the non-AR group (n = 103) in the total population

HLA-DRB1	Acute rejection		No acute rejection		OR	95%CI	Uncorrected
allele	n (alleles)	(%)	n (alleles)	(%)			P value
*01	10	(12.5)	23	(11.2)	1.2	0.5-2.5	0.75
*02	15	(18.8)	31	(15.0)	1.3	0.7-2.6	0.44
*03	25	(31.3)	40	(19.4)	1.9	1.0-3.3	0.03
*04	4	(5.0)	46	(22.3)	0.2	0.1-0.5	0.001
*07	7	(8.8)	16	(7.8)	1.2	0.5-2.8	0.78
*08	2	(2.5)	12	(5.8)	0.5	0.1-1.8	0.20
*09	0	(0)	1	(0.5)	0.9	0.1-9.5	0.72
*10	1	(1.3)	4	(1.9)	0.8	0.2-4.4	0.57
*11	1	(1.3)	6	(2.9)	0.6	0.1-2.9	0.37
*12	1	(1.3)	3	(1.5)	1.1	0.2-6.1	0.69
*13	13	(16.3)	21	(10.2)	1.7	0.8-3.6	0.16
*14	1	(1.3)	3	(1.5)	1.1	0.2-6.1	0.69

OR and corresponding 95%CI were calculated using Woolf's formula with Haldane's correction; uncorrected P value is calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen.

Table 5 Comparison of the frequencies of human leukocyte antigen-B alleles in the acute cellular rejection group (n = 23) and the non-acute cellular rejection group (n = 76) in the non-primary sclerosing cholangitis population

HLA-B	Acute rejection		Non acute rejection		OR	95%CI	Uncorrected
allele	n (alleles)	(%)	n (alleles)	(%)			P value
*05	0	(0)	4	(2.6)	0.3	0.1-2.9	0.34
*07	6	(13.0)	15	(9.9)	1.4	0.5-3.1	0.54
*08	12	(26.0)	20	(13.2)	2.1	0.8-4.6	0.04
*12	9	(19.6)	20	(13.2)	1.6	0.6-3.4	0.36
*13	1	(2.2)	4	(2.6)	1.0	0.2-5.4	0.67
*14	2	(4.3)	4	(2.6)	1.8	0.5-5.81	0.43
*15	5	(10.9)	21	(13.8)	0.8	0.3-2.1	0.51
*16	1	(2.2)	3	(2.6)	1.0	0.2-5.34	1.00
*17	0	(0)	4	(2.6)	0.3	0.1-2.9	0.63
*18	1	(2.2)	6	(3.9)	0.6	0.1-2.0	1.00
*21	0	(0)	2	(1.3)	0.7	0.1-2.5	1.00
*22	0	(0)	2	(1.3)	0.7	0.11-2.5	0.71
*27	2	(4.3)	7	(4.6)	1.0	0.3-4.0	1.00
*35	4	(8.7)	15	(9.9)	0.8	0.3-2.5	1.00
*37	2	(4.3)	3	(1.9)	2.4	0.4-10.3	0.34
*40	2	(4.3)	17	(11.2)	0.4	0.1-1.4	0.25
*41	1	(2.2)	2	(1.3)	1.9	0.3-11.3	0.56
*47	0	(0)	1	(0.7)	1.0	0.1-11.4	1.00

OR and corresponding 95%CIs were calculated using Woolf's formula with Haldane's correction. Uncorrected P value is calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen.

zygous group (P = 0.15). For analysis of HLA-C KIR ligand disparity between recipient and donor, we assessed the following possibilities; (1) HLA-C ligand type present in the recipient and absent in the donor (*i.e.*, "missing self" model); (2) ligand present in the donor and absent in the recipient (*i.e.*, "non-self" model); and (3) donor and recipients homozygous for HLA-C1 and HLA-C2, respectively (*i.e.*, complete disparity). We did not detect any significant influence from these types of HLA-C/KIR ligand disparity on risk of AR, either in the total cohort nor in an analysis stratified according to PSC (P > 0.05 in all cases).

Classification of the *HLA-B* alleles was done according to whether they determined the Bw4 (that binds to KIR) or Bw6 (that does not bind KIR). We found no influence of donor HLA-Bw genotype on AR risk, *i.e.*, AR occurred in 29% of LTX with a HLA-Bw4 homozygote donor, in 28% in the heterozygote group and in 35% in the HLA-Bw6 homozygote group (P = 0.15). Ligand disparity was defined as recipient carriage of KIR3DL1 and Bw4 (*i.e.*, presence of inhibition *via* KIR3DL1-Bw4 interaction), with Bw4 absent in the donor (*i.e.*, no inhibition *via* KIR3DL1-Bw4 interaction). There was no significant difference in the frequency of AR between disparate (25%) and non-disparate individuals (29%), P = 0.70.

Impact of KIR genes on risk of AR

No significant associations between KIR genotype of

Table 6 Comparison of the frequencies of human leukocyte antigen-C alleles in the acute cellular rejection group (*n* = 23) and the non-acute cellular rejection group (*n* = 76) in the non-primary sclerosing cholangitis population

HLA-C	Acute rejection		No acute rejection		OR	95%CI	Uncorrected
allele	*n* (alleles)	(%)	*n* (alleles)	(%)			*P* value
*01	1	(2.2)	5	(3.3)	0.9	0.16-4.27	1.00
*02	1	(2.2)	7	(4.6)	0.6	0.11-2.53	0.68
*03	4	(8.7)	34	(22.4)	0.4	0.13-0.93	0.06
*04	7	(15.2)	21	(13.8)	1.2	0.45-2.61	0.81
*05	6	(13.0)	12	(7.9)	1.8	0.55-3.74	0.38
*06	3	(6.5)	12	(7.9)	0.9	0.27-2.74	1.00
*07	23	(50.0)	40	(26.3)	2.8	1.42-5.42	0.003
*08	2	(4.3)	4	(2.6)	1.9	0.34-5.89	0.63
*12	0	(0)	6	(3.9)	0.2	0.02-1.62	0.34
*15	0	(0)	4	(2.6)	0.4	0.04-2.94	0.58
*16	0	(0)	3	(2.0)	0.5	0.05-3.97	0.32
*17	1	(2.2)	4	(2.6)	1.1	0.19-5.46	1.00

OR and corresponding 95%CI were calculated using Woolf's formula with Haldane's correction. Uncorrected *P* value calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen.

Table 7 Comparison of the frequencies of human leukocyte antigen-DRB1 alleles in the acute cellular rejection group (*n* = 23) and the non-acute cellular rejection group (*n* = 76) in the non-primary sclerosing cholangitis population

HLA-DRB1	Acute rejection		No acute rejection		OR	95%CI	Uncorrected
allele	*n* (alleles)	(%)	*n* (alleles)	(%)			*P* value
*01	7	(15.2)	18	(11.8)	1.4	0.6-3.4	0.55
*02	8	(17.4)	23	(15.1)	1.2	0.5-2.8	0.46
*03	13	(28.2)	24	(15.8)	2.5	1.9-5.4	0.05
*04	3	(6.5)	35	(23.0)	0.3	0.1-0.9	0.02
*07	6	(13.0)	12	(7.9)	1.8	0.7-4.8	0.29
*08	2	(4.3)	10	(6.6)	0.7	0.2-2.7	0.73
*10	0	(0)	3	(2.0)	0.5	0.1-4.2	0.59
*11	1	(2.2)	6	(3.9)	0.7	0.1-3.7	1.00
*12	1	(2.2)	3	(2.0)	1.4	0.2-7.9	0.93
*13	7	(15.2)	15	(9.9)	1.7	0.7-4.2	0.31
*14	0	(0)	3	(2.0)	0.5	0.1-4.2	1.00

OR and corresponding 95%CIs were calculated using Woolf's formula with Haldane's correction. Uncorrected *P* value is calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen.

Table 8 Data on human leukocyte antigen mismatches on the risk of acute cellular rejection after liver transplantation *n* (%)

Locus	MM (*n*)	Non-AR group (*n* = 102)	AR group (*n* = 41)	*P* value
HLA-A	1-2	86 (84.3)	38 (92.7)	0.18
	0	16 (15.7)	3 (7.3)	
HLA-B	1-2	97 (95.1)	41 (100)	0.15
	0	5 (4.9)	0 (0)	
HLA-DR	1-2	94 (92.2)	40 (97.6)	0.21
	0	8 (7.8)	1 (2.4)	
A, B, DR	0-4	62 (60.8)	19 (47.3)	0.12
	5-6	40 (39.2)	22 (53.7)	

Uncorrected *P* values were calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen; MM: Mismatches; AR: Acute cellular rejection.

patients and risk of AR were detected (Tables 2 and 10). There was a trend toward the 22 bp deletion variant of the *KIR2DS4* gene in recipients experiencing AR. This was the case in both the overall population and in the PSC-stratified analysis, but this finding was not significant after correction for the number of comparisons made in the KIR analysis (Bonferroni factor 68, see Tables 10-12). There was no significant influence of recipient copy number of each individual KIR gene and risk of AR (Table 12).

Immunohistochemical analysis of biopsies with AR according to indication for liver transplantation

Based on the previous suggestions that HLA *vs* KIR genotype may influence the risk of AR[22,23,41,42], we assessed the frequency of NK cells in the inflammatory infiltrates in AR in PSC and non-PSC patients. We used CD56 and CD57 as markers to detect the number and distribution of possible NK cells, along with CD3 and CD20 for the counting of T-and B cells in the formalin fixed biopsies. Both epithelial cells in small bile ducts and scattered spindle shaped cells and lymphocyte-like cells were CD56-

Table 9 Data on human leukocyte antigen mismatches on the risk of acute cellular rejection after liver transplantation, according to primary sclerosing cholangitis or non-primary sclerosing cholangitis *n* (%)

Locus	MM (*n*)	Non-AR group (PSC, *n* = 26) (Non-PSC, *n* = 76)	AR group (PSC) *n* = 17) (Non-PSC) *n* = 24)	*P* value
HLA-A				
(PSC)	1-2	20 (57.1)	15 (42.9)	0.35
	0	6 (75.0)	2 (25.0)	
(non-PSC)	1-2	65 (73.9)	23 (26.1)	0.18
	0	11 (92.7)	1 (7.3)	
HLA-B				
(PSC)	1-2	25 (59.5)	17 (40.5)	0.41
	0	1 (100.0)	0 (0.0)	
(non-PSC)	1-2	73 (75.3)	24 (24.7)	0.32
	0	3 (100.0)	0 (0.0)	
HLA-DR				
(PSC)	1-2	26 (61.9)	16 (38.1)	0.44
	0	0 (0.0)	1 (100.0)	
(non-PSC)	1-2	67 (74.4)	23 (25.6)	0.27
	0	9 (90.0)	1 (10.0)	
A, B, DR				
(PSC)	5-6	12 (54.5)	10 (45.5)	0.42
	0-4	14 (66.7)	7 (33.3)	
(non-PSC)	5-6	29 (67.4)	14 (32.6)	0.08
	0-4	47 (82.4)	10 (17.6)	

Uncorrected *P* values were calculated by the χ^2 test or the Fisher's exact test where appropriate. HLA: Human leukocyte antigen; PSC: Primary sclerosing cholangitis; MM: mismatches; AR: acute cellular rejection.

Table 10 Data on the relationship between killer immunoglobulin-like receptor gene phenotype (presence/absence of gene) in the recipient and the risk of acute cellular rejection after liver transplantation in the total patient population

Recipient *KIR* gene phenotype		Incidence of AR	*P* value
2DL1	Negative (*n* = 3)	0%	0.37
	Positive (*n* = 140)	29%	
2DL2	Negative (*n* = 79)	28%	0.92
	Positive (*n* = 64)	29%	
2DL3	Negative (*n* = 10)	20%	0.44
	Positive (*n* = 133)	28%	
2DL4	Negative (*n* = 0)	-	-
	Positive (*n* = 143)	29%	
2DL5	Negative (*n* = 79)	32%	0.27
	Positive (*n* = 64)	24%	
2DP1	Negative (*n* = 3)	0%	0.54
	Positive (*n* = 140)	31%	
2DS1	Negative (*n* = 91)	32%	0.24
	Positive (*n* = 52)	20%	
2DS2	Negative (*n* = 76)	27%	0.85
	Positive (*n* = 67)	29%	
2DS3	Negative (*n* = 108)	28%	0.79
	Positive (*n* = 35)	31%	
2DS4DEL[1]	Negative (*n* = 23)	9%	0.03
	Positive (*n* = 120)	31%	
2DS4WT[2]	Negative (*n* = 77)	27%	0.84
	Positive (*n* = 66)	29%	
2DS4TOTA[3]	Negative (*n* = 3)	0%	0.67
	Positive (*n* = 140)	29%	
2DS5	Negative (*n* = 104)	32%	0.47
	Positive (*n* = 39)	24%	
3DL1E4[4]	Negative (*n* = 3)	0%	0.61
	Positive (*n* = 140)	29%	
3DL1E9[5]	Negative (*n* = 3)	0%	0.62
	Positive (*n* = 140)	28%	
3DL2	Negative (*n* = 0)	-	-
	Positive (*n* = 143)	29%	
3DL3	Negative (*n* = 0)	-	-
	Positive (*n* = 143)	29%	
3DP1	Negative (*n* = 0)	-	-
	Positive (*n* = 143)	29%	
3DS1	Negative (*n* = 91)	31%	0.39
	Positive (*n* = 52)	25%	

[1]2DS4DEL refers to the 22-bp deletion variant of 2DS4; [2]2DS4WT refers to the full-length form of the gene; [3]2DS4TOT refers to the total number of 2DS4, *i.e.*, 2DS4DEL and 2DS4WT combined; [4,5]3DL1E4 and 3DL1E9 refers to exon 4 and 9 of the *3DL1* gene respectively. The various KIR genes genotyped are listed in the leftmost column. Genes with S in the name (*e.g.*, KIR2DS4) encode activating KIRs, genes with L in the name (*e.g.*, KIR3DL1) encode inhibiting KIRs and genes with a P in the name (*e.g.*, KIR3DP1) are pseudogenes. Uncorrected *P* value is calculated by the χ^2 test or the Fisher's exact test where appropriate. KIR: Killer immunoglobulin-like receptor; AR: Acute cellular rejection.

positive (Figure 1). Bile ducts were positive for CD56 in equal fractions in patients with PSC (30%), PBC (30%) and other liver diseases (30%). The numbers and type of lymphocytes observed in the AR biopsies obtained from patients with PSC, PBC and non-immunological liver diseases revealed no significant differences between the groups (Table 13). CD56 positive lymphocytes were only sporadically detected in the portal tracts. Scattered CD57-positive cells were seen in both the portal tracts, periportal and sinusoidal areas in a similar fraction of patients in all three groups, and there were no statistically significant differences between the numbers of CD57-positive cells per square millimeter of liver tissue. Double staining, CD3 with immunoperoxidase and diaminobenzidine, followed by CD57 with alkaline phosphatase, showed no CD57-positive cells, indicating that all or practically all CD57-positive cells in the liver biopsies were $CD3^+CD57^+$ NKT cells.

DISCUSSION

The potential importance of donor-recipient HLA matching in LTX is debated. At some centers, including our own, resources are still spent providing HLA data for liver allograft recipients even though the information is not accounted for in the organ allocation process. It has been consistently shown that recipients with PSC have an increased AR risk compared to recipients with other underlying liver diseases. PSC is strongly HLA-associated[34,43-45], and in the present analysis we demonstrate that this HLA background also associates with increased risk of AR in non-PSC recipients. These results provide an impetus to more thoroughly explore the role of recipient immunogenetic determinants on liver graft outcomes, rather than overly focusing on genetic matching between the donor and the recipient.

The strongest HLA risk factor in PSC is HLA-B*08[43,46]. This association is a representative of the so-called 8.1 ancestral haplotype (AH8.1), which is defined by a series

Table 11 Data on the relationship between killer immunoglobulin-like receptor gene phenotype in the recipient and the risk of acute cellular rejection after liver transplantation in patients with primary sclerosing cholangitis compared with patients without primary sclerosing cholangitis

Recipient *KIR* gene phenotype	Incidence of AR	*P* value
2DL1		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 4)	40%	
(other)		1.00
Negative (n = 3)	0%	
Positive (n = 96)	25%	
2DL2		
(PSC)		0.37
Negative (n = 23)	33%	
Positive (n =21)	48%	
(other)		0.71
Negative (n = 54)	26%	
Positive (n = 45)	22%	
2DL3		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		1.00
Negative (n = 10)	20%	
Positive (n = 89)	24%	
2DL4		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		-
Negative (n = 0)	-	
Positive (n = 99)	24%	
2DL5		
(PSC)		0.17
Negative (n = 28)	48%	
Positive (n = 16)	25%	
(other)		0.81
Negative (n = 51)	25%	
Positive (n = 48)	22%	
2DP1		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		1.00
Negative (n = 3)	0%	
Positive (n = 96)	25%	
2DS1		
(PSC)		0.14
Negative (n = 13)	26%	
Positive (n = 31)	20%	
(other)		0.51
Negative (n = 60)	26%	
Positive (n = 39)	20%	
2DS2		
(PSC)		0.37
Negative (n = 23)	33%	
Positive (n = 21)	48%	
(other)		0.48
Negative (n = 54)	26%	
Positive (n = 45)	20%	
2DS3		
(PSC)		0.78
Negative (n = 34)	41%	
Positive (n = 11)	36%	
(other)		0.57
Negative (n = 76)	22%	
Positive (n = 25)	28%	
2DS4DEL[1]		
(PSC)		0.11
Negative (n = 8)	38%	
Positive (n = 36)	43%	
(other)		0.10
Negative (n = 15)	7%	
Positive (n = 85)	27%	
2DS4WT[2]		
(PSC)		0.81
Negative (n = 21)	38%	
Positive (n = 23)	42%	
(other)		0.71
Negative (n = 56)	22%	
Positive (n = 43)	26%	
2DS4TOTA[3]		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		1.00
Negative (n = 3)	0%	
Positive (n = 96)	25%	
2DS5		
(PSC)		0.46
Negative (n = 34)	43%	
Positive (n = 10)	30%	
(other)		0.56
Negative (n = 69)	25%	
Positive (n = 30)	20%	
3DL1E4[4]		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		-
Negative (n = 0)	-	
Positive (n = 99)	25%	
3DL1E9[5]		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		1.00
Negative (n = 3)	0%	
Positive (n = 96)	25%	
3DL2		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		-
Negative (n = 0)	-	
Positive (n = 98)	24%	
3DL3		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		-
Negative (n = 0)	-	
Positive (n = 99)	24%	
3DP1		
(PSC)		-
Negative (n = 0)	-	
Positive (n = 44)	40%	
(other)		-
Negative (n = 0)	-	
Positive (n = 99)	24%	

3DS1		
(PSC)		0.42
Negative (*n* = 31)	44%	
Positive (*n* =13)	31%	
(other)		0.81
Negative (*n* = 61)	25%	
Positive (*n* = 40)	23%	

[1]2DS4DEL refers to the 22-bp deletion variant of 2DS4; [2]2DS4WT refers to the full-length form of the gene; [3]2DS4TOT refers to the total number of 2DS4, *e.g.*, 2DS4DEL and 2DS4WT combined; [4,5]3DL1E4 and 3DL1E9 refers to exon 4 and 9 of the *3DL1* gene respectively. The various KIR genes genotyped are listed in the leftmost column. Genes with S in the name (*e.g.*, KIR2DS4) encode activating KIRs, genes with L in the name (*e.g.*, KIR3DL1) encode inhibiting KIRs and genes with a P in the name (*e.g.*, KIR3DP1) are pseudogenes. Uncorrected *P* value was calculated by the χ^2 test or the Fisher's exact test where appropriate. KIR: Killer immunoglobulin-like receptor; AR: Acute cellular rejection; PSC: Primary sclerosing cholangitis.

Table 12 Killer immunoglobuline-like receptor gene copy number of the recipient and acute cellular rejection after liver transplantation

KIR gene	AR mean copy number (95% CI)	Non-AR mean copy number (95%CI)	*P* value
KIR2DL1	1.69 (1.54-1.84)	1.64 (1.53-1.76)	0.64
KIR2DL2	0.52 (0.34-0.71)	0.53 (0.40-0.65)	0.97
KIR 2DL3	1.50 (1.30-1.70)	1.45 (1.33-1.58)	0.69
KIR 2DL4	2.10 (2.00-2.19)	1.98 (1.91-2.05)	0.06
KIR 2DL5	0.50 (0.27-0.73)	0.63 (0.49-0.78)	0.32
KIR 2DP1	1.71 (1.57-1.86)	1.70 (1.59-1.81)	0.89
KIR 2DS1	0.26 (0.12-0.40)	0.43 (0.33-0.54)	0.08
KIR 2DS2	0.62 (0.30-0.43)	0.54 (0.41-0.66)	0.56
KIR 2DS3	0.29 (0.13-0.44)	0.32 (0.20-0.40)	0.77
KIR 2DS4DEL[1]	1.17 (1.01-1.32)	1.08 (0.94-1.21)	0.45
KIR 2DS4WT[2]	0.55 (0.36-0.73)	0.48 (0.37-0.59)	0.53
KIR 2DS4TOT[3]	1.74 (1.60-1.88)	1.56 (1.45-1.67)	0.06
KIR 2DS5	0.21 (0.08-0.35)	0.32 (0.22-0.42)	0.25
KIR 3DL1E4[4]	1.74 (1.60-1.88)	1.57 (1.46-1.67)	0.08
KIR 3DL1E9[5]	1.74 (1.60-1.88)	1.56 (1.46-1.67)	0.06
KIR 3DL2	-	-	-
KIR 3DL3	-	-	-
KIR 3DP1	-	-	-
KIR 3DS1	0.33 (0.30-0.51)	0.40 (0.30-0.51)	0.47

The various *KIR* genes genotyped are listed in the leftmost column. Genes with S in the name (*e.g.*, KIR2DS4) encode activating KIRs, genes with L in the name (*e.g.*, KIR3DL1) encode inhibiting KIRs and genes with a P in its name (*e.g.*, KIR3DP1) are pseudogenes. Statistical comparison was performed using the independent sample *t* test. [1]2DS4DEL refers to the 22-bp deletion variant of 2DS4; [2]2DS4WT refers to the full-length form of the gene; [3]2DS4TOT refers to the total number of 2DS4, *i.e.*, 2DS4DEL and 2DS4WT combined; [4,5]3DL1E4 and 3DL1E9 refers to exon 4 and 9 of the 3DL1 gene respectively. KIR: Killer immunoglobulin-like receptor; AR: Acute cellular rejection.

of correlated genetic variants within the HLA, including also the HLA-C*07 and DRB1*03 alleles[44,45,47]. The AH8.1 has profound influence on immune function, as also underscored by previously reported associations with a wide range of immune-mediated diseases[44,45]. Results in the present study account for both HLA-B*08 and HLA-C*07 and suggest that the underlying effect represented by these associations localizes in or near these neighboring genes. The dosage effect observed for the PSC associated alleles is also highly supportive of a true effect, *i.e.*, with an AR frequency of 56% and 57%, respectively, in homozygous individuals, as compared with 21% and 18%, respectively, in individuals without HLA-B*08 and HLA-C*07. Importantly, the effect of AH8.1 on AR risk was not restricted to patients with PSC, suggesting that the immunological abnormalities in AH8.1 carriers[45] is of general importance for the pathophysiology in AR. The present results reproduce a previously reported association reported between AH8.1 and AR in liver transplantation[12,48]. There have also been notions of AH8.1 in determining graft survival in kidney transplantation[49]. It is thus timely to explore the immunological basis of this association and the relationship between AR and autoimmunity associations related to the AH8.1 haplotype.

Further support of recipient-related immunogenetic determinants for AR holds true for the DRB1*04 associations, which protect against PSC as well as AR in patients with and without PSC. The AR frequency of individuals without DRB1*04 was 35% (comparable to the overall AR frequency of 28%), while dropping to 12% and 0% for the presence of one and two DRB1*04 alleles, respectively, suggesting a dosage effect of the protective mechanisms represented by the DRB1*04 haplotype. A similar relationship has been noted for type 1 diabetes in kidney transplantation[50]. While DRB1*04 is a strong risk factor for type 1 diabetes[51], type 1 diabetic recipients of diverse ethnicities who carry DRB1*04 experience improved graft survival[50]. A similar shared involvement has also been reported for non-HLA risk loci in autoimmune risk loci, *e.g.*, for the cytotoxic T-lymphocyte antigen 4 (*CTLA4*) gene[52-54]. Most likely the ongoing characterization of shared genetic risk factors between different autoimmune diseases should incorporate recipient factors for allograft-targeted immune reactions[55], for instance by means of screening large recipient populations with and without AR using the Immunochip[43,56].

The precision and homogeneity of the diagnosis of AR is critical for further studies of the genetics of AR along suggested lines. In the present study, great care was taken to include only biopsy proven cases of AR, and the resulting recipient group with AR thus consisted of Banff RAI 3-6 cases with only two exceptions (who showed RAI > 6 in their biopsies). Cases with different etiologies with potentially similar immune manifestations (*e.g.*, ischemia/reperfusion related injuries, biliary pathologies and infections, including early HCV recurrence) were systematically excluded. A multitude of immunological mechanisms are at play in the allograft during the first weeks after liver transplantation, and lack of precision in diagnosis of AR will diminish power considerably in genetic association studies[57,58]. Ideally, data on confounders and rejection characteristics should be prospectively collected, not retrospectively as in the present analysis. Lack of statistical power and phenotypic heterogeneity probably underlies the conflicting results of AR associations for many non-HLA loci investigated [*e.g.*, interleukin 10 (*IL-10*)[59-63],

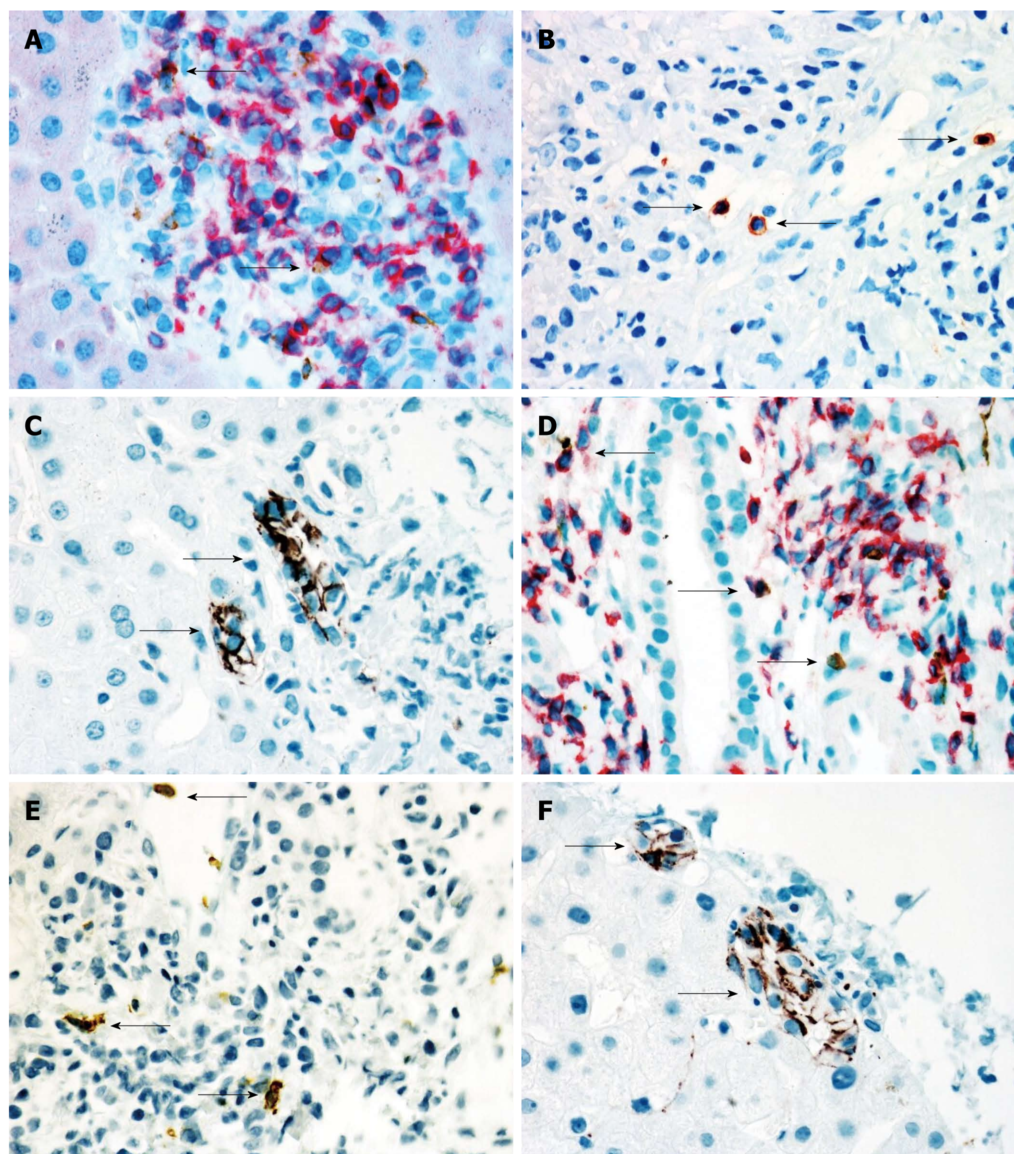

Figure 1 Immunoenzyme stainings in a liver biopsy from a transplanted primary sclerosing cholangitis patient with acute cellular rejection scored according to the Banff criteria as RAI 5 (panels A-C), and from a transplanted control patient (secondary liver metastases) with the same grade of cellular rejection (panels D-F). Panels A and D show double immunoenzyme staining for CD3 (red) and CD56 (brown, arrows) in a portal area. Panels B and E show immunoperoxidase staining for CD57 (brown, arrows) in a portal area. Panels C and F show CD56-staining of bile ducts (arrows). Only scattered CD56- and CD57 positive leucocytes can been seen in both patients (Original magnification × 400).

IL-4 and IL-4 receptor[61,64], *IL-6*[65,66], chemokine receptor 2 (*CCR2*)[67], *CCR5*[67-69], and intercellular adhesion molecule-1 (*ICAM1*)[70,71]]. The present study is also underpowered to detect non-HLA associations for a complex trait like AR[72], but of the same size as those first reporting on what has later shown to be consistent HLA associations in other diseases[46,73]. Similarly, it cannot be excluded that lack of AR association for rare HLA alleles in the present analysis may be due to type Ⅱ statistical errors.

Some studies have suggested a beneficial effect of HLA matching in LTX[12,48], others have found HLA matching to be detrimental or even exerting a dualistic effect[74]; being beneficial regarding AR, but detrimental to graft/patient survival. In most studies, however, HLA compatibility has not proven to have significant impact on outcome after LTX, and HLA genotype is presently not taken into account in the donor selection protocols. Data in the present population supports the latter conclusion, but also raises the possibility that recipient HLA type may be of consequence for individualized adjustment of the immunosuppressive regimens if findings are confirmed in future studies. Donor-recipient HLA mismatching has previously been reported to be of greater importance in non-autoimmune liver diseases where primary HLA associations do not exist[12]. A more thorough dissection of the HLA-related risk for AR on the side of the recipient only, may thus help clarify HLA determinants on the donor side of relevance to donor-recipient matching.

NK cells have been suggested to engage in the development of liver allograft tolerance[75-77], and animal experiments have suggested that NK cells infiltrate the liver

Table 13 Data showing scarce, but similar numbers of natural killer cells and natural killer T cells in biopsies with equal grade of AR in patients with various primary liver diseases

MNC cell subset[1]	PSC (n = 10)	PBC (n = 10)	Non-imm (n = 10)	P value
CD56 (NK/NKT)	1.6 ± 0.3	1.8 ± 0.4	1.8 ± 0.3	0.9
CD57 (NK/NKT)	5.0 ± 0.9	4.0 ± 0.4	4.6 ± 0.6	0.1
CD3-CD56$^+$ (NK)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.7
CD3-CD57$^+$ (NK)	0	0	0	-

[1]Mean absolute number of NK/NKT cells/mm^2. Statistics were performed comparing mean value between groups using one-way ANOVA. MNC: Mononuclear cells; PSC: Primary sclerosing cholangitis; PBC: Primary biliary cirrhosis; Non-imm: Non-immunological disease; NKT: Natural killer T cell; NK: Natural killer.

graft before T lymphocytes[78]. Inspired by work in autoimmune diseases[16], a series of studies have assessed the impact of donor and recipient genetic variation relevant to NK cell activation on risk of AR. While some studies suggest that this interaction may influence the risk of AR after LTX[22,23,41], other studies have failed to support this[19,20]. A major problem in designing the statistical analysis of HLA-KIR interactions, is that biological correlates are poorly defined and the number of potentially relevant interactions is thus high. According to conservative Bonferroni-thresholds for statistical significance, none of the reported associations remain significant. This is also the case for the present dataset. In an analysis accounting for the known biological differences between the HLA-C1 (weak inhibition *via* KIR) and HLA-C2 (strong inhibition *via* KIR)[16], we were unable to detect an influence of the HLA-C2 variant on AR risk[20,21]. Based on previous studies reporting an increased frequency of NK cells in the portal infiltrate of patients with PSC when compared with other liver diseases[79,80], we wanted to examine if similar findings were present in rejection infiltrates in liver transplants. Since the use of NK cell specific monoclonal antibodies was unsuccessful in formalin-fixed material, we used antibodies to CD56 and CD57 to detect putative NK- and NKT cells. The extremely low number of CD56 positive cells in biopsies from patients with AR does not exclude the possibility that these cells contribute to immune an immune reaction primarily driven by the infiltration of T cells. However, the sum of evidence suggests that a genetically determined NK cell hyperreactivity is unlikely to play a major pathogenetic role in AR in LTX.

In conclusion, we detected a significant impact from PSC-associated HLA variants on risk of AR. The findings are similar in PSC and non-PSC recipients and provide important confirmatory support to previous studies in LTX and kidney transplantation, together serving as a basis for further studies of the underlying mechanisms. In addition, we propose that there is a need to query genetic risk of AR along the model of other immune mediated diseases. This with spescial focus on recipients, and aiming for comprehensive genetic coverage by means of genome-wide association studies or targeted genotyping arrays (*e.g.*, the Immunochip) in adequately sized study populations.

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COMMENTS

Background

Donor-recipient matching for human leukocyte antigen (HLA) in liver transplantation is not part of routine clinical practice. Also for other genetic loci, *e.g.*, the killer-immunoglobuline-like receptors (KIR), the clinical importance of donor-recipient matching is unclear. Patients with primary sclerosing cholangitis (PSC) have consistently been reported to exhibit an increased risk of acute cellular rejections (AR) as compared with other indications for liver transplantation. The role of genetic risk factors for PSC in determining risk of AR has not previously been investigated.

Research frontiers

Recent genome-wide association studies have determined multiple robust genetic risk factors for PSC, out of which the strongest localize to the HLA complex on chromosome 6. Genetic risk factors for AR have traditionally been performed along the logic of donor-recipient mismatching, but no consistent findings have so far been made. In particular, recent articles suggest that particular HLA and KIR combinations may be important, but conflicting data also exist.

Innovations and breakthroughs

This is the first study to demonstrate a clear role for HLA variants associated with PSC susceptibility in determining risk of AR. Importantly, findings also apply to patients with other underlying liver diseases. Authors found no significant impact of donor-recipient matching on the risk of AR, neither for HLA nor HLA and KIR combinations.

Applications

Further studies are needed to establish the biological basis for the observed associations. Importantly, the findings suggest that studies querying the role of recipient genetics in AR may provide useful insights into AR pathophysiology. Rather than overly focusing on donor-recipient genetic matching, further studies (*e.g.*, genome-wide association studies) on the recipient side are warranted.

Peer review

The authors offer a valuable contribution to the still debated issue of HLA variants in acute liver rejection development. Further, they have investigated HLA-C and KIR genotypes to demonstrate a lack of association with AR.

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