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Polymorphisms of the human platelet antigen-1, -2, -3, -5, and -15 systems and acute cellular liver transplant rejection

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ABSTRACT

The human platelet antigen (HPA)-1, -2, -3, -5, and -15 systems are characterized as polymorphic alloantigens expressed on platelets and endothelial cells. In this retrospective study, we investigated, whether HPA-1, -2, -3, -5, and -15 incompatibilities are associated with acute cellular liver transplant rejection. A total of 96 Caucasian liver transplant recipients and corresponding donors were analyzed, 43 with biopsy proven acute cellular rejection (BPAR) and 53 without acute cellular rejection (No-BPAR). Polymorphisms of mentioned HPA systems were determined by polymerase chain reaction-sequence specific primers (PCR-SSP). Our data demonstrate that acute cellular rejection episodes were associated with HPA-3 incompatibility (58% HPA-3 incompatibility in BPAR group vs. 32% HPA-3 incompatibility in No-BPAR group, $p = 0.013$). Furthermore, the frequency of HPA-3bb genotype was significantly higher in BPAR recipients as compared to No-BPAR recipients (30% vs 6%, $p = 0.002$). On the other hand, there was no association between acute cellular rejection and the other tested HPA systems. We conclude that in the Caucasian population the HPA-3 system confers susceptibility to acute cellular rejection after liver transplantation.

1. Introduction

Human platelet antigen (HPA) systems are polymorphic glycoproteins mainly expressed on the surface of platelet membranes [1,2]. The HPA systems are numbered in their order of discovery. Currently, 35 different human platelet antigens have been specified in the Immuno Polymorphism Database (IPD-HPA) [3]. All except one (HPA-14) are defined by a single nucleotide polymorphism (SNP) in the gene encoding for the respective platelet glycoprotein resulting in an amino acid substitution in the corresponding protein [4]. Most HPA systems are based on SNPs, which are extremely rare (<0.1%). Only the HPA-1, -2, -3, -5, and -15 systems results from SNPs whose frequency is greater than 5% in Caucasian population [2,3].

The biallelic HPA-1, -2, -3, -4, -5, and -15 systems are inherited autosomally and codominantly; the more common antigen is designated "a" and the rarer antigen, "b" [2,5]. These HPA systems are localized on platelet glycoprotein receptors, namely, HPA-1 and HPA-4 on CD61 (GPIIIa) that is known as a part of vitronectin receptor, HPA-2 on CD42b (GPIbα) that functions as thrombin receptor, HPA-3 on CD41 (GPIIb) that is part of the fibrinogen, von Willebrand factor, fibronectin, vitronectin, and thrombospondin receptor, and HPA-5 on CD49b (GPIa) that is part of the collagen and laminin receptor [2,6]. The HPA-15 system is exceptional as it is carried by the glycosylphosphatidylinositol-linked protein CD109 that was found to be part of the transforming growth factor-β receptor system [7,8]. The HPA systems could act as alloantigens contributing to the induction of platelet-reactive antibodies. The development of those antibodies occurs most commonly in multi-transfused patients or during pregnancy [10].

Abbreviations: BPAR, biopsy proven acute cellular rejection; CD, cluster of differentiation; CI, confidence interval; CNI, calcineurin inhibitor; DNA, deoxyribonucleic acid; DSA, donor-specific human leukocyte antigen alloantibody; HPA, human platelet antigen; GP, glycoprotein; MMF, mycophenolate mofetil; mTORI, mTOR inhibitor; NAITP, neonatal alloimmune thrombocytopenia; PCR-SSP, polymerase chain reaction-sequence specific primers; PTP, post-transfusion purpura; SD, standard deviation; SNP, single nucleotide polymorphism; STER, corticosteroids.

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Alloimmunisation by platelet-reactive antibodies can result in clinical manifestations such as neonatal alloimmune thrombocytopenia (NAITP), post-transfusion purpura (PTP), transplantation-associated alloimmune thrombocytopenia, and platelet transfusion refractoriness [11–14]. There are hints that these antibodies are also involved in the development of antibody-mediated transplant rejection [15].

The HPA-1, -2, -4, -5, and -15 systems are not only expressed by platelets but also found on other cell types [2,9]. In context with acute cellular liver transplant rejection, it is important that at least the HPA-1, -4, and -5 systems are expressed on endothelial cells of portal vein as well as on monocytes [2,16]. It is generally believed that endothelial cells of portal vein are a main target of acute cellular rejection in liver transplantation [17]. If liver transplant donors and recipients are genetic different in the mentioned HPA systems, it is quite conceivable that the monocytes of the donor could contribute to the immunization and differentiation of cytotoxic CD8+ T-cells in liver transplant recipient. These cytotoxic CD8+ T-cells may recognize endothelial cells of portal vein as target causing an acute cellular liver transplant rejection. Thus, the aim of this study was to genotype the HPA-1, -2, -3, -5, and -15 systems in liver transplant recipients and their corresponding donors in order to analyze the impact of mentioned HPA systems on acute cellular liver transplant rejection.

2. Subjects and methods

2.1. Subjects

In this retrospective study, 96 recipients undergoing liver transplantations at the Department of Hepatobiliary and Transplant Surgery of the University Hospital Hamburg-Eppendorf between August 2009 and May 2014 and the corresponding donors were included. All recipients were over 18 years, most of them (>90%) Caucasians. The mean age at the date of transplantation, gender, immunosuppressive treatment, number of thrombotic events after transplantation, number of re-Transplantations, portion of donor-specific human leukocyte antigen alloantibody (DSA) in pretransplant serum, and primary indication for liver transplantation are summarized in Table 1. Based on primary clinical data liver transplant recipients were divided into two groups according to the presence or absence of acute cellular rejection. Recipients were considered as acute cellular rejectors if they had experienced one biopsy-proven acute cellular rejection episode (BPAR = 43) with a BANFF score $\geq$3/9, together with a rise in liver enzymes (aminotransferases, bilirubin) with no other identifiable cause and by a response to high dose corticosteroid treatment. Recipients who did not experience any acute cellular rejection episodes for at least one year after liver transplantation (date of liver transplantation plus at least the following year after liver transplantation) were considered as liver transplant recipients without acute cellular rejection (No-BPAR = 53).

The study design was approved by the Local Ethics Commission, and all involved patients gave their written informed consent.

2.2. Genotyping of HPA-1, -2, -3, -5, and -15 systems polymorphisms

The DNA was extracted from peripheral blood samples using the innuPREP Blood DNA mini Kit (Analytik Jena Biometra, Jena, Germany) according to the instruction manual.

The HPA-1, -2, -3, -5, and -15 genotypes were determined by PCR-SSP method described previously by Lyou et al. (HPA-1, -2, and -3) and Nie et al. (HPA-5 and -15) [18,19]. Fifteen percent of the samples have been regenotyped with the commercial available HPA Ready Gene Kit (inno-train, Kronberg, Germany) to check the accuracy of the results. The results of regenotyping showed a match of 99% with the beforehand observed data.

Table 1
Demographic and clinical characteristics of liver recipient's collectives.

	No-BPAR (n = 53)	BPAR (n = 43)
Gender (male/female)	28 (53%)/25 (47%)	28 (65%)/15 (35%)
Age (mean year ± SD) at the date of transplantation	55 ± 10	51 ± 11
Primary liver disease[*]		
Alcoholic cirrhosis	25 (32%)	18 (31%)
Alpha 1-antitrypsin deficiency	1 (1%)	3 (5%)
Budd-Chiari syndrome	2 (3%)	1 (2%)
Hepatocellular carcinoma	19 (24%)	9 (16%)
Hepatitis C cirrhosis	14 (18%)	9 (16%)
Hepatitis B cirrhosis	3 (4%)	6 (10%)
Hepatic failure through intoxication	2 (3%)	1 (2%)
Hemochromatosis	0 (0%)	1 (2%)
Primary biliary cirrhosis	2 (3%)	0 (0%)
Primary sclerosing cholangitis	2 (3%)	5 (9%)
Others	8 (10%)	5 (9%)
re-Transplantation	2 (4%)	3 (7%)
Thrombosis after transplantation	0 (0%)	2 (5%)
DSA in pretransplant serum		
DSA class I	4 (7%)	3 (7%)
DSA class II	3 (6%)	3 (7%)
DSA class I + class II	2 (4%)	1 (2%)
No DSA	13 (25%)	16 (37%)
No serum available	31 (58%)	20 (47%)
Immunosuppressive treatment at the time of discharge double regimen		
STER + CNI	10 (19%)	9 (21%)
mTORI + CNI	5 (9%)	4 (9%)
Others	2 (4%)	3 (7%)
Triple regimen		
STER + CNI + mTORI	24 (45%)	11 (26%)
STER + CNI + MMF	10 (19%)	13 (30%)
Others	2 (4%)	3 (7%)

[*] Multiple nominations are possible, STER: corticosteroids; mTORI: mTOR inhibitors; MMF: mycophenolate mofetil; CNI: calcineurin inhibitors.

2.3. Detection of DSAs

Recipients with available pretransplant serum were retrospectively tested for the presence of circulating class I (HLA-A, HLA-B, and HLA-C) DSAs and class II (HLA-DR, HLA-DQ, and HLA-DP) DSAs by using single antigen bead technology (One Lambda, Meerbusch, Germany). The test was performed as described in the instruction manual. A median fluorescence intensity $\geq$2500 was considered positive.

2.4. Statistical analysis

Differences in age between BPAR liver recipients and No-BPAR liver recipients were tested by Student's *t* test. Further statistical comparisons such as gender, immunosuppressive therapy, primary liver disease, DSAs, number of thrombotic events after transplantation, re-transplantation, genotype, and allele frequencies were calculated with Fisher's exact test. P values, OR, and 95% CI calculations were performed by R v2.12.1 (Foundation for Statistical Computing, Vienna, Austria). The significance of p values was finally controlled by the Benjamini-Hochberg method. Hardy-Weinberg equilibrium was tested using the HW calculator (Michael H. Court, Tufts University, Medford, USA). The power calculation was executed by G*Power 3.1 (Institute for Experimental Psychology, Heinrich Heine University, Düsseldorf, Germany).