Can Direct-Acting Antiviral Treatment Change the Immunologic Risk Profile in Patients Infected with Hepatitis C Virus Who Are on the Cadaveric Waiting List?

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ABSTRACT

Background. In patients with hepatitis C virus (HCV) infection, the activation of the immune system by the virus or viral proteins leads to the production of numerous autoantibodies and clinical manifestations. The objectives of this study were to investigate the relationship between HCV and anti-HLA antibodies, as well as the effect of viremia on the antibody response and of direct-acting antivirals (DAAs) on anti-HLA antibody persistence in patients on the waiting list for a cadaveric kidney transplant.

Methods. A total of 395 patients from the cadaveric renal transplant waiting list were included in the study. The patients were grouped according to the presence of HCV infection, and patients with HCV positivity were further divided into a spontaneous clearance group and a persistent group. Anti-HLA antibodies were examined before and after treatment of the patients in the persistent group. The One Lambda Luminex method (Thermo Fisher Scientific, Waltham, MA, United States) was used to assess both HLA class I and II alleles and the anti-HLA antibody profile.

Results. Anti-HLA class I and II antibodies were detected in 48.2% and 55.1%, respectively, of the patients infected with HCV and in 21.8% and 20.4%, respectively, of the patients who were not infected. The level of anti-HLA A3, A11, B72, B52, Cw6, Cw16, DR3, and DQ4 antibodies was significantly higher in the patients infected with HCV. There was no statistically significant difference in class I and II antibody titration between the HCV-infected spontaneous clearance group and the persistent group (class I mean fluorescence intensity [MFI] ± SD: 13,583 ± 6224, 13,450 ± 9540, $P = .808$; Class II MFI ± SD: 13,000 ± 8673, 8440 ± 8302, $P = .317$, respectively). There was no significant difference in the class I and class II anti-HLA antibody profile and titration in the persistent group after treatment with DAAs ($P > .05$).

Conclusions. The results of this study demonstrated that hepatitis C DAA treatment did not change the anti-HLA antibody profile and titration.

FREQUENT parenteral intervention increases the hepatitis C virus (HCV) exposure of patients undergoing hemodialysis [1]. The primary infection often leads to acute hepatitis, which progresses to a chronic infection in most cases, characterized by gradually advancing liver fibrosis, cirrhosis, and hepatocellular carcinoma [2]. About 30% of infected patients clear the virus spontaneously [3]. A major

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characteristic of HCV infection is the interindividual variability in terms of the ability to clear the virus either spontaneously or after treatment and the rate at which fibrosis develops [4]. The identification of markers to predict spontaneous resolution or chronic infection could have a meaningful clinical effect in determining whether therapy should be implemented or deferred [5].

HLAs are one of the most polymorphic genes in humans [6,7]. HLA molecules play an important role in the regulation of the host's immune response as signalers of self or foreign peptides or antigens to T-cell receptors. They are also involved in the initiation of a cytotoxic T-cell or helper T-cell response [8], as in HCV infection [9]. A variety of different HLA alleles could therefore influence viral persistence [4].

The activation of the immune system in response to the virus or viral proteins leads to the production of many autoantibodies and clinical manifestations in patients with HCV infection. These autoantibodies may be associated with underlying autoimmune disorders or liver inflammation due to the HCV infection [10]. In addition to the presence of circulating antibodies against HLA class I and class II molecules, HCV antibodies may cause an autoimmune response to HLA antigens and may play a pathogenic role in the induction of HCV-related chronic liver disease [11].

In the 1990s, treatment of chronic hepatitis C infection with interferon-alfa monotherapy achieved a sustained virologic response in only 10% of cases. Response rates increased up to 50% with the use of the combination of pegylated interferon alfa and ribavirin [12]. The advent of direct-acting antivirals (DAAs) has introduced a cure rate of > 90% [13].

The objective of this study was to assess a possible relationship between anti-HLA antibodies and HCV infection and to investigate the effectiveness of DAAs on anti-HLA antibody persistence in patients with end-stage renal disease (ESRD) who were on the cadaveric kidney transfer waiting list.

MATERIALS AND METHODS

Patient Characteristics

In all, 395 patients from the cadaveric renal donor waiting list were included in this study. In the group, 366 patients (92.6%) did not have HCV infection, whereas 29 (7.4%) patients tested positive for HCV infection. The HCV-infected group was further divided into a spontaneous clearance group (n = 21; positive HCV antibodies confirmed and negative HCV RNA without the patient ever having received anti-HCV therapy or HCV RNA detected only in the first 6 months after HCV seroconversion) and the persistent group (n = 8; detection of HCV RNA for more than 6 months [patients with chronic HCV infection]). Dasabuvir and combination ombitasvir, paritaprevir, and ritonavir (Viekira Pak) treatment was administered for 12 weeks to 8 patients with HCV viremia. The HCV infection resolved in all of the patients after treatment. It was confirmed that none of the patients carried the hepatitis B surface antigen or the HIV antibodies in order to exclude additional viral factors that could affect our results.

Anti-HCV Antibody and HCV RNA Assay

The HCV RNA level was assessed using the real-time polymerase chain reaction kit Artus HCV QS-RGQ QIAsymphony RGQ system; (Qiagen, Hilden, Germany). The measurement spectrum of the kit used was 35 IU/mL-17-700.000 IU/mL, where 1 IU/mL equals 1.21 copy/mL. A microenzyme-linked immunosorbent assay method was used to measure HCV antibodies.

HLA Tissue Group and Anti-HLA Antibody Typing

Molecular HLA typing revealed HLA-A, HLA-B, and HLA-DRB1 alleles in 395 patients, HLA-DQB1 alleles in 327 patients, and HLA-C alleles in 37 patients. HLA typing was performed using the One Lambda Luminex method (Thermo Fisher Scientific). Anti-HLA antibody screening using LabScreen PRA (Thermo Fisher Scientific) was performed for all of the patients on the waiting list 2 times per year. The threshold was a mean fluorescence intensity (MFI) > 1000. The MFI values were interpreted by the local histocompatibility expert. The change in anti-HLA antibody response after a mean of 12 months (range, 5-20 months) of HCV viremia treatment in 8 patients was assessed according to the MFI values.

Statistical Analysis

Data analysis was performed using the IBM SPSS Statistics for Windows, version 21.0 (IBM Corp, Armonk, NY, United States). The distribution of continuous variables was evaluated for normality. Demographic and clinical parameters were described as mean ± SD, and Student *t* test was used to determine the significance of the difference between the means of data sets displaying normal distribution. Nominal variables were evaluated with Fisher exact χ^2 test. Antigen and anti-HLA antibody frequencies for each locus were estimated using the direct-counting method. Differences between groups were evaluated using χ^2 analysis, odds ratio (OR) values and 95% confidence intervals (CIs). A value of $P < .05$ was accepted as significant.

RESULTS

The age and sex ratios included in this study of patients who were HCV-infected and non–HCV-infected were similar. The group with HCV infection had received more blood transfusions and transplants (Table 1).

No significant difference was detected in the HLA allele distribution when the HCV-infected and non–HCV-infected groups were compared. There was increased expression of the HLA-DRB1*07 allele in the HCV persistent group among those with the HCV infection (OR = 0.105; 95% CI, 0.01-0.78; $P = .033$). Anti-HLA class I and class II antibodies were detected in 48.2% and 55.1%, respectively, of the patients infected with HCV. Anti-HLA class I and class II antibodies were observed in 21.8% and 20.4%, respectively, of the patients in the non–HCV-infected group (Table 2). Of the patients with an anti-HLA class I antibody and infected with HCV (n = 14), 87% had received blood transfusions, 20% had been pregnant, and 80% had a history of previous transplantation. Of the patients with an anti-HLA class II antibody and infected with HCV (n = 13), 92% had received blood transfusions, 23% had been pregnant, and 85% had a history of previous transplantation. The analysis of antibodies revealed that the level of anti-HLA A3 (OR = 0.18; 95% CI, 0.05-0.6; $P = .0098$), anti-HLA A11 (OR = 0.23; 95% CI,

Table 1. Demographic Information

	All Patients (N = 395)	HCV-Infected Group (n = 29)	Non–HCV-Infected Group (n = 366)	*P* Value
Age, mean ± SD, y	51 ± 16	52 ± 10	51 ± 17	.642
Sex, female/male	198/197	12/17	186/180	.343
Mean no. blood transfusions ± SD	2.7 ± 4.4	4.6 ± 5	2.6 ± 4.4	.015*
Mean no. previous pregnancies ± SD	1.3 ± 2.3	0.9 ± 2	1.4 ± 2.4	.13
No. of patients who had undergone transplantation	73	17	56	.0001*
Donor type: live/cadaveric	44/29	13/4	31/25	.2
Dialysis duration, y (range)	8.05 (0-28)	16.34 (1-28)	7.39 (0-24)	< .0001*
Primary disease, no.				
Amyloidosis	6	0	6	1
Idiopathic	89	8	81	.49
Chronic glomerulonephritis	61	8	53	.1
HT	111	5	106	.2
CAKUT	47	7	40	.065
DM	41	0	41	.057
PKD	40	0	40	.058

CAKUT, congenital anomalies of the kidney and urinary tract; DM, diabetes mellitus; HCV, hepatitis C virus; HT, hypertension; PKD, polycystic kidney disease.
*Indicates statistical significance.

0.07-0.7; $P = .019$), anti-HLA B72 (OR = 0.21; 95% CI, 0.05-0.85; $P = .04$), anti-HLA B52 (OR = 0.31; 95% CI, 0.11-0.9; $P = .03$), anti-HLA Cw6 (OR = 0.03; 95% CI, 0.001-0.6; $P = .02$), anti-HLA Cw16 (OR = 0.03; 95% CI, 0.001-0.6; $P = .02$), anti-HLA DR3 (OR = 0.03; 95% CI, 0.001-0.6; $P = .02$), and anti-HLA DQ4 (OR = 0.3; 95% CI, 0.10-0.8; $P = .04$) was significantly greater in patients infected with HCV than in those patients not infected with HCV.

Anti-HLA class I antibodies were detected in 3 patients in the persistent group and 11 patients in the spontaneous clearance group ($P > .05$), whereas class II antibodies were detected in 4 patients in the persistent group and 9 patients in the spontaneous clearance group ($P > .05$). Among the patients infected with HCV, no statistically significant difference was detected in terms of class I and II antibody titration (MFI) between the spontaneous clearance group and the persistent group (Class I MFI: 13,583 ± 6224, 13,450 ± 9540, $P = .808$; Class II MFI: 13,000 ± 8673, 8440 ± 8302, $P = .317$, respectively). Similarly, no difference was found in terms of class I and II anti-HLA antibody profile and titrations in the persistent group after treatment (Table 2).

DISCUSSION

A significant feature of hepatitis C is the interindividual variability in the ability to clear the virus, either spontaneously or after treatment and the rate at which fibrosis develops [4]. Specific HLA alleles and their cognate epitopes have been associated with events such as drug hypersensitivity, spontaneous clearance, or viral persistence in HCV [14].

Numerous studies can be found in literature regarding predisposition to HCV infection, spontaneous clearance or persistency, and HLA alleles in different populations. Unfortunately, there is no consensus among these studies because of the polymorphic and ethnic distribution of HLA alleles.

HLA-B*35 and B*08 have been found to be associated with chronic HCV infection in the Russian and Kazakh populations, as has HLA-DRB1*03:01, DQB1*02:01, and DQA1*02:01 in most European and Caucasians, and DRB1*07 in the Brazilian population [15–17]. In a study performed on the Egyptian population, no significant difference was detected between patients who are anti–HCV negative and anti–HCV positive regarding A, B, and DR frequencies of HLA antigens, whereas an increased frequency of HLA-DRB1*07 allele was observed in HCV-positive family members in the same study population [18,19]. It has been reported that Turkish individuals who carry the B*38 antigen are predisposed to HCV infection [20]. In Chinese and Italian newborns, the DRB1*13 antigen was found to be protective against HCV infection,

Table 2. The Effect of HCV Viremia Treatment on Antibody Response

Patient Group No.	Non–HCV-Infected Group (n = 366) (92.7%)	HCV-Infected Group (n = 29) (7.3%)	*P* Value	Persistent Group		*P* Value
				Before Treatment (n = 8)	After Treatment (n = 8)	
PRA class I (-/+), n	286/80	15/14	.001*	5/3	4/4	.614
PRA class I max MFI, mean	8276	14,390	.002*	13,450	10,936	.361
Class II (-/+), n	291/75	16/13	.005*	4/4	5/3	.614
Class II max MFI, mean	10,602	12,392	.303	8440	9786	.572

MFI, mean fluorescence intensity; PRA, panel-reactive antibody.
*Indicates statistical significance.

although DRB1*07 is associated with a predisposition to the infection [16]. In addition, the HLA-DRB1*07:01 allele has been found to be associated with disease severity in the European population [16]. A meta-analysis study found that the alleles HLA-B*27 and A*03 were associated with spontaneous clearance in Ireland, the HLA-B*44 and Cw*12 in Spain; and in other HLA studies, the DQB1*02, DQB1*03, DRB1*04 and DRB1*11 alleles were related to spontaneous clearance [4,5,21]. In a study performed on a Turkish population of 189 patients with ESRD who were exposed to HCV infection, the HLA-Cw*06 and DRB1*10 alleles were frequent in the group that achieved viral clearance [20]. DRB1*07 was reported to be associated with viral persistence in a study from Pakistan [22]. In our study, there was no clear association between HLA antigens and HCV infection, though the increased expression of HLA-DRB1*07 is consistent with reports that it may be related to viral persistence.

Research investigating the effect of HLA antigens on HCV infection treatment has yielded varied results. In a study of 51 patients receiving interferon treatment, the HLA-A variants of HLA-A*03 and HLA-A*30 were associated with effective antiviral therapy. HLA-A*25 was correlated with undesirable treatment results [23]. The HLA-A*01 and HLA-A*02 alleles have been linked to a weak response to antiviral treatment and, therefore, undesirable results in the course of infection [23]. HLA-A*24 has demonstrated a somewhat positive response to interferon treatment, while HLA-A*25 is indicative of a weak treatment result [23]. In the Chinese population, DRB1*04 has been associated with unresponsiveness to interferon treatment in HCV infection [24]. However, no study has reported an association between the current DAA HCV drugs and HLA alleles [25]. The results of 8 patients who received DAA treatment and responded positively were evaluated in our study. No HLA allele was identified as conferring an effective response to treatment. Since the patient population is very limited in this study, further research is needed to support our results.

It has been proposed that amino acid variations in HLA alleles may affect antigen-binding sites and thus create distinct immune responses to viruses and different disease outcomes [16].

HCV has a single-stranded RNA genome and surface envelope protein (E2). The interaction of E2 and CD81 activates B lymphocytes and thereby induces the production of various immunoglobulins and autoantibodies [26]. The presence of antibodies against HLA class I and II molecules can represent an autoimmune response to HLA antigens and might play a role in the induction of chronic liver disease in patients with HCV [11].

Can et al [27] found a significant relationship between class I panel-reactive antibodies (PRAs) and anti-HCV positivity. Gentil et al [28] and Forman et al [29] compared patients who were anti–HCV positive and anti–HCV negative and demonstrated a statistically significant relationship between high PRA levels and HCV positivity. However, Özdemir et al [30] found that HCV infection had no effect on PRA sensitization. In our study, PRA class I and II positivity was higher in patients who were anti–HCV positive. Furthermore, the class I PRA antibody titer (MFI) was higher in patients considered anti–HCV positive. Anti-HLA antibody titers may increase as a result of overactivation and proliferation of B lymphocytes interacting with the surface protein of HCV. We investigated the effect of HCV treatment on anti-HLA antibody positivity and found that treatment did not substantially change the antibody profile of the patients. HCV treatment decreased the anti-HLA class I antibody titer a little, but it was not statistically significant. Treatment was not seen to have an effect on anti-HLA class II antibody titers. Previous influenza vaccine studies have shown that anti-HLA antibodies developed at 6 months after treatment [31]. To the best of our knowledge, there is no published research demonstrating the effect of DAA HCV treatment on the anti-HLA antibody profile. In this study, the anti-HLA antibody profile was examined at a mean of 12 months (range, 5-20 months) after treatment. Further studies with long-term PRA monitoring are needed to fully determine the effect of DAAs on antibody response.

The primary limitation of this study was the small number of patients and the high incidence of sensitization in the patients who were positive for HCV. Even if HCV infection appeared to trigger anti-HLA antibody production in our study, it should not be overlooked that a history of blood transfusions and transplantation are more common in patients with HCV infection.

This study demonstrated that DAA agents used to treat chronic HCV infection did not change the anti-HLA antibody profile or titration.

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