Low frequency of HLA-DRB1*11 in hepatitis C virus induced end stage liver disease

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Abstract

Hepatitis C virus (HCV) infection becomes chronic in more than 70% of patients, leading to end stage liver disease in about 20–30% of these patients. Apart from the virus itself, host factors that modulate the immune response are likely to be involved in determining the outcome of HCV infection. Studies on the association of human leucocyte antigens (HLAs) and HCV infection have shown inconsistent results. Selection of patient subgroups may be crucial. However, any association relevant to HCV disease progression will become evident, especially in those patients with end stage liver disease. Therefore, we analysed the phenotype frequencies of HLA antigens in two groups of 69 and 39 patients with HCV induced liver cirrhosis who had received a transplant or were awaiting liver transplantation. The first group was typed serologically and compared with 331 blood and liver donors. The second group, prospectively HLA typed by a polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) procedure for HLA-DRB and DQB alleles, was compared with another 170 PCR-SSO typed and randomly selected blood donors. Decreased frequencies for HLA-DR5 and HLA-DQ3 were found in one group of patients with HCV induced liver cirrhosis compared with the control groups. In the second analysis comparing 39 patients with end stage liver cirrhosis with blood donors, we confirmed the significant decrease in HLA-DRB1*11 and HLA-DQB1*03, which corresponded to serological HLA-DR5 and HLA-DQ3 antigens, respectively. Our results show that the presence of HLA-DRB1*11 and HLA-DQB1*03 alleles is associated with a reduced risk for the development of HCV induced end stage liver disease.

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Keywords: hepatitis C; HLA; liver cirrhosis; immunogenetics; hepatocellular carcinoma; genotype

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Hepatitis C virus (HCV) infection leads to the development of chronic hepatitis in about 70–80% of cases,[1–4] of whom 20–30% progress to liver cirrhosis or hepatocellular carcinoma (HCC).[5] The liver damage in HCV infected patients is probably mediated by both direct cytopathic effects and immune mediated mechanisms.[6 7] HCV genotype and viral load[8] are likely to modulate HCV induced liver disease. The importance of genetic factors for HCV infection arises from the observation of different courses of infection despite the same source of infection.[9] Crucial genetic factors influencing the immune reaction are the human leucocyte antigens (HLAs) encoded by the major histocompatibility complex (MHC). Recent studies have indicated an important role for the MHC class II antigen HLA-DR13 in viral clearance of hepatitis B,[10 11] and there is evidence of disease modulation in human immunodeficiency virus (HIV) infection.[12 13] However, associations between HLA determinants and susceptibility to HCV remain controversial. While some studies did not reveal any significant associations,[14–17] others highlighted the relevance of the serologically determined HLA-DR5 antigen or the corresponding DNA determined HLA-DRB1*11 alleles in chronic hepatitis C[18–21] and some found other antigens such as DRB1*09,[22 23] HLA-DR13,[24] DRB1*04, DQB1*03[25] or DRB1*1301, and DQA1*0103[26] associated with viral clearance of HCV infection.

HLA antigen frequency in patients with HCV induced end stage liver disease with or without HCC has not been addressed previously in European patients. These patients with HCV infection clearly have a poor outcome. Any HLA association relevant for chronicity or disease progression should become evident in these patients unless the mechanisms involved in viral persistence are contrary to those involved in promoting hepatocellular damage. Therefore, these patients are especially appropriate for studying the relevance of HLA antigens in HCV dependent disease progression.

Thus we analysed the phenotype frequencies of class I and class II HLA antigens in two such patient groups. Additionally, we studied the relation of HCV genotype and presence of HCC to HLA antigens.

Patients and methods

PATIENTS

The first part of the study involved 69 Caucasians who had undergone liver transplantation for HCV induced end stage liver cirrhosis at Hannover Medical School between January 1984 and June 1996. In patients transplanted

Abbreviations used in this paper: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; HLA, human leucocyte antigen; HIV, human immunodeficiency virus; MHC, major histocompatibility complex; PCR, polymerase chain reaction; RR, relative risk; SSO, sequence specific oligonucleotide.

since 1992 HCV serology was tested prospectively by antibodies and polymerase chain reaction (PCR) while the HCV status of patients transplanted before 1992 was determined retrospectively from sera stored at −80°C.[27] Patients with evidence of another cause of liver cirrhosis other than HCV infection were excluded from the study, especially those with viral coinfection with hepatitis B virus (HBV) and known alcohol consumption. There were 26 females and 43 males. The frequencies of the different HLA phenotypes were compared with 331 unrelated blood and organ donors registered at our blood bank or whose livers were transplanted between 1984 and 1995.

To confirm the significance of our results, we analysed a second group of 39 patients transplanted for or suffering from HCV induced end stage liver cirrhosis requiring liver transplantation compared with 170 unrelated blood donors (unrelated and different from control group 1) registered at our blood bank who were repeated blood donors with normal transaminases and no markers for HCV or HBV infection. Patients with end stage liver disease had signs of decompensation, severe impairment of liver function, and advanced cirrhosis. There were 17 females and 22 males in this group.

In 79 of 108 patients the histological diagnosis of HCC or its absence was available for evaluation because the explanted liver was examined. Twenty two patients had evidence of HCC while 57 had no evidence of HCC in explanted livers. HCV genotype was determined in 73 of 108 cases while the genotype was not available in the other patients because an appropriate serum sample was not available. HCV genotypes were determined as described previously.[28 29] Thus the minimal breakdown of HCV genotypes was 1a (n=8), 1b (n=53) and 2a, and 2b and 3a (n=12). As most patients showed genotype 1a or 1b, we compared three groups according to genotype 1a, 1b, or neither of these. Furthermore, we compared all patients with genotype 1 versus those with other genotypes and all patients with genotype 1b versus all others.

VIROLOGICAL PARAMETERS

Serological markers for HBV, HCV, and hepatitis D virus infection were determined using commercial assays (Abbott Laboratories, Illinois, USA). HCV-RNA and HBV-DNA were detected as described previously.[30 31]

HLA TYPING

HLA antigens of both class I and class II were serologically determined by standard microlymphocytotoxicity assay in our laboratory for tissue typing using both commercial available antiserum and our own antiserum. Additionally, in patients and controls from the second comparison, DNA based HLA class II typing for DRB1 and DQB1 was performed using our modified PCR-sequence specific oligonucleotide (PCR-SSO) procedure.[32 33]

STATISTICAL ANALYSIS

All data were analysed using the computer programme Excel for windows (v 5.0). Calculating phenotype frequencies and statistical data processing for the χ^2 test were performed using the computer program SPSS/PC (v 6.0.1), and Fisher's exact test was used where appropriate. p values provided by the χ^2 or Fisher's exact test were corrected by multiplying by the number of comparisons made (pc) according to the recommendations by Svejgaard and Ryder.[34] This is necessary if one group is tested because p values less than 0.05 will occur by chance without a true association when multiple comparisons are made. Thus in the first part of our study we multiplied by 57 and in the second part of the study we multiplied by 62. However, the multiplication process can be circumvented when a second group of patients is analysed and p values from the initial analysis are confirmed in the second group.[10]

Results

HLA FREQUENCY: FIRST PATIENT GROUP VERSUS FIRST CONTROL GROUP

Comparing phenotype frequencies of HLA class I (table 1) and class II (table 2) antigens in 69 patients with HCV induced liver cirrhosis with 331 blood and organ donors (control group 1), we found decreased frequencies of HLA-DR5 (12.3% *v* 31.8%; p=0.001, relative risk (RR)=0.3) and DQ3 (39.5% *v* 59.6%; p<0.029, RR=0.44) in patients with HCV induced liver cirrhosis. Increased frequencies were found for A19 (30.4% *v* 18.1 %; p=0.032, RR=1.98), B35 (24.6% *v* 14.2%; p=0.049, RR=1.98), B37 (7.2% *v* 1.8%; p=0.035, RR=4.23), Cw4 (32.8% *v* 19.8%; p=0.035, RR=1.98), and DR3 (30.8% *v* 18.5%; p=0.038, RR=1.96). However, there were no statistically significant differences in phenotype frequency after statistical correction by multiplying p values by the number of HLA antigens (n=57) tested.

HLA FREQUENCY: SECOND PATIENT GROUP VERSUS SECOND CONTROL GROUP

In a second comparison we analysed data for HLA typing by DNA analysis in a second group of 39 patients with HCV induced liver cirrhosis and 170 blood donors (control group 2) (tables 3, 4). In this analysis we found decreased frequencies of HLA-DRB1*11 (7.7% *v* 22.4%; p=0.04, RR=0.29) and HLA-DQB1*03 (38.5% *v* 58.8%; p=0.033, RR=0.53), and an increased frequency for HLA-B8 (40.6% *v* 20.6%; p=0.027, RR=2.64) in patients with HCV induced liver cirrhosis. If we subdivided HLA-DQB1*03 into HLA-DQB1*0301 and DQB1*0302, none of these alleles was significantly different (table 5). Likewise, the associated haplotypes HLA-DR4-DQB1*0301 (2.5% *v* 6.4%; p=0.573, RR=0.39) and HLA-DR4-DQB1*0302 (10.2% *v* 16.7%; p=0.375, RR=0.58) were not significantly different. No statistical difference in this analysis was detected after correction. Thus HLA-DR5/DRB1*11 and HLA-DQ3 / DQB1*03 were the only antigens/alleles found to be significantly lower in both studies, indicating an important role in disease protection. As with the first part of the study, no class

Table 1 HLA class I frequencies of serologically typed patients with hepatitis C virus (HCV) induced end stage liver disease versus blood and organ donors (control group 1)

	Blood and organ donors		HCV induced end stage liver cirrhosis		
	n	%	n	%	p Value
A1	82	24.8	13	18.8	NS
A2	176	53.2	32	46.4	NS
A3	99	29.9	14	20.3	NS
A9	66	19.9	20	29.0	NS
A10	42	12.7	6	8.7	NS
A11	31	9.4	10	14.5	NS
A19	*60*	*18.1*	*21*	*30.4*	*0.032*
A28	34	10.3	8	11.6	NS
	331		69		
B5	48	14.5	7	10.1	NS
B7	88	26.6	16	23.2	NS
B8	51	15.4	16	23.2	NS
B12	96	29.0	24	34.8	NS
B13	22	6.6	7	10.1	NS
B14	16	4.8	3	4.3	NS
B15	48	14.5	6	8.7	NS
B16	34	10.3	2	2.9	NS
B17	27	8.2	5	7.2	NS
B18	33	10.0	4	5.8	NS
B21	17	5.1	4	5.8	NS
B22	20	6.0	3	4.3	NS
B27	22	6.6	5	7.2	NS
B35	47	14.2	17	24.6	0.049
B37	6	1.8	5	7.2	0.035
B40	48	14.5	7	10.1	NS
B41	4	1.2		0.0	NS
B42	3	0.9		0.0	NS
B47		0.0		0.0	NS
B53		0.0		0.0	NS
B70	2	0.6		0.0	
	331	100.0	69		
Cw1	26	8.7	3	4.7	NS
Cw2	27	9.1	4	6.3	NS
Cw3	97	32.6	14	21.9	NS
Cw4	59	19.8	21	32.8	0.035
Cw5	46	15.4	11	17.2	NS
Cw6	54	18.1	18	28.1	NS
Cw7	140	47.0	27	42.2	NS
Cw8	11	3.7	1	1.6	NS
	298		64		

Table 2 HLA class II frequencies of serologically typed patients with hepatitis C virus (HCV) induced end stage liver disease versus blood and organ donors (control group 1)

	Blood and organ donors		HCV induced end stage liver cirrhosis		
	n	%	n	%	p Value
DR1	49	14.8	12	18.5	NS
DR2	109	33.0	17	26.2	NS
DR3	61	18.5	20	30.8	0.038
DR4	86	26.1	14	21.5	NS
DR5	*105*	*31.8*	*8*	*12.3*	*0.001*
DR6	99	30.0	21	32.3	NS
DR7	87	26.4	24	36.9	NS
DR8	18	5.5	1	1.5	NS
DR9	1	0.3		0.0	NS
DR10	3	0.9	2	3.1	NS
	330		65		
DQ1	179	65.1	27	71.1	NS
DQ2	99	36.0	21	55.3	NS
DQ3	*164*	*59.6*	*15*	*39.5*	*0.029*
DQ4	9	3.3			NS
	275		38		

I allele was found to be significantly different between patients and controls.

We also performed an analysis to investigate secondary association and linkage associations such as HLA-DR4-DQB1*0302. However, none of the analyses showed a significant association (data not shown); HLA-DR11 was just significantly lower after exclusion of HLA-DQB1*0302 (p=0.049).

Table 3 HLA class I frequencies of patients with hepatitis C virus (HCV) induced end stage liver disease (n=32) versus blood donors (n=170, control group 2)

	Blood donors		HCV induced end stage liver cirrhosis		
	n	%	n	%	p Value
A1	61	35.9	13	40.6	NS
A2	81	47.6	13	40.6	NS
A3	57	33.5	12	37.5	NS
A9	28	16.5	7	21.9	NS
A10	23	13.5	2	6.3	NS
A11	11	6.5	3	9.4	NS
A19	42	24.7	4	12.5	NS
A28	17	10.0	2	6.3	NS
	170		32		
B5	11	6.5	5	15.6	NS
B7	52	30.6	9	28.1	NS
B8	35	20.6	13	40.6	0.027
B12	39	22.9	4	12.5	NS
B13	10	5.9	2	6.3	NS
B14	7	4.1	2	6.3	NS
B15	24	14.1	1	3.1	NS
B16	9	5.3	1	3.1	NS
B17	20	11.8	2	6.3	NS
B18	16	9.4	1	3.1	NS
B21	5	2.9	1	3.1	NS
B22	11	6.5	2	6.3	NS
B27	22	12.9	2	6.3	NS
B35	25	14.7	9	28.1	NS
B37	4	2.4	1	3.1	NS
B40	24	14.1	5	15.6	NS
B41	4	2.4	0	0.0	NS
B47	3	1.8	0	0.0	NS
B48	1	0.6	0	0.0	NS
	170		32		
Cw1	13	7.6	1	3.2	NS
Cw2	25	14.7	2	6.5	NS
Cw3	47	27.7	8	25.8	NS
Cw4	27	15.9	10	32.3	NS
Cw5	26	15.3	2	6.5	NS
Cw6	34	20.0	4	12.9	NS
Cw7	91	53.5	18	58.1	NS
Cw8	6	3.5	2	6.5	NS
	170		31		

Table 4 HLA class II frequencies of patients with hepatitis C virus (HCV) induced end stage liver disease (n=39) versus blood donors (n=170, control group 2) who were typed by a polymerase chain reaction-sequence specific oligonucleotide procedure

	Blood donors		HCV induced end stage liver cirrhosis		
	n	%	n	%	p Value
DRB1*1	31	17.6	5	12.8	NS
DRB1*2	68	40.0	12	30.8	NS
DRB1*3	41	24.1	12	30.8	NS
DRB1*4	42	24.7	5	12.8	NS
DRB1*7	41	24.1	13	33.3	NS
DRB1*8	11	6.5	1	2.6	NS
DRB1*9	4	2.4	2	5.1	NS
DRB1*10		0.0	1	2.6	NS
*DRB1*11*	*38*	*22.4*	*3*	*7.7*	*0.04*
DRB1*12	9	5.3	2	5.1	NS
DRB1*13	21	12.4	10	25.6	NS
DRB1*14	11	6.5	4	10.3	NS
	170		39		
DQB1*02	64	37.6	21	53.8	NS.
*DQB1*03*	*100*	*58.8*	*15*	*38.5*	*0.033*
DQB1*04	11	6.5	3	7.7	NS
DQB1*05	49	28.8	9	23.1	NS
DQB1*06	79	46.5	19	48.7	NS
	170		39		

HLA FREQUENCY IN PATIENTS WITH OR WITHOUT HCC

In patients with HCC (n=22) in the explanted liver we found an increase in HLA-B37 (22.2% *v* 1.8%; p=0.02, RR=12.44) prior to compulsory multiplication and HLA-DR1 was found

*Table 5 DQB1*03 allele frequencies of patients with hepatitis C virus (HCV) induced end stage liver disease versus blood donors, who were typed by a polymerase chain reaction-sequence specific oligonucleotide procedure*

	Blood donors		HCV induced end stage liver cirrhosis		
	n	%	*n*	%	*p Value*
DQB1*0301	56	32.9	8	20.5	NS
DQB1*0302	31	18.2	4	10.3	NS
DQB1*3032	20	11.8	5	12.8	NS
DQB1*0304	1	0.6	0		NS
	100		15		

less frequently (4.3% *v* 30.8%; p=0.05, RR=0.082). None remained significant after essential multiplication.

HLA FREQUENCY IN RELATION TO HCV GENOTYPES

HLA patterns did not differ remarkably among patient groups divided on the basis of different HCV genotypes. The only difference was for patients with genotype 1a who had increased frequencies of HLA-B12 (5/8 (62.5%)) compared with those with genotype 1b (12/53 (22.6%); p=0.032, RR=5.694) and all others (16/65 (24%); p=0.039, RR=5.104), and HLA-B16 (3/8 (37.5%) *v* 0/53 (0%; p=0.002, RR=na) and 0/65, ⩽0.001, RR=na). However, no significance difference was detected after correction.

HLA FREQUENCY IN RELATION TO PATIENT SEX

HLA patterns did not differ in male and female patients.

Discussion

Based on earlier results from European studies,[16 18 20 21] we assumed that the phenotype frequency of HLA-DR5 antigen and the corresponding allele DRB1*11 would be significantly decreased in patients with HCV induced liver cirrhosis compared with controls. As expected, in two independent comparisons we found that the HLA-DR5 antigen or the corresponding HLA-DRB1*11 allele was significantly less frequent in patients with HCV induced end stage liver cirrhosis. In addition, HLA-DQB1*03 was found less frequently in patients with HCV induced end stage liver disease. In common with HLA-DRB1*11, HLA-DQB1*03 has been reported to be less frequent in patients with viral clearance.[20 21]

Whether HLA-DRB1*11 or DQB1*03 is the more relevant factor is difficult to determine as HLA-DRB1*11 is in strong linkage disequilibrium with DQB1*0301. Neither HLA-DRB1*11 nor HLA-DRB1*03 remained significantly different after exclusion of the other. Some studies found DQB1*03 to be relevant without identifying DRB1*11 as a relevant cofactor,[25] while those identifying HLA-DRB1*11 as a relevant factor also found DQB1*03 to be relevant.[20 21] This would argue for a more important role of DQB1*03 versus DR1*11. Although HLA-DRB1*11 and DQB1*03 have most frequently been associated with resolving HCV infection,[20 21 35–37] other alleles have also been identified in individual studies.[24 38]

However, these studies were performed in patients selected because of their known HCV status. In two studies performed in women selected by their rhesus status and infected with a uniform source of HCV, neither HLA-DRB1*11 nor DQB1*03 was associated with viral persistence. Only HLA-DRB1*01 was found to be associated with viral clearance.[39 40]

We have recently been involved in a large European multicentre study demonstrating that HLA-DR1*11 and HLA-DQB1*0301 are the most prominent factors in viral clearance of HCV.[41] Comparing all of our patients with those with persistent hepatitis C from the European study[41] supports the relevance of HLA-DR5 for lower disease progression. We found 13 of 104 patients (12.5%) to be HLA-DR5 positive in the study reported here, only among patients with very late stage HCV induced liver disease, compared with 39 of 170 (22.9%) in the European study (p=0.032 uncorrected χ^2 test). This shows that HLA-DR5 is even less frequent in patients with the most advanced form of chronic hepatitis C compared with those selected only on the basis of positive HCV RNA status for more than six months. This would indicate that HLA-DRB1*11 and DQB1*03 alleles are associated with a favourable outcome of chronic hepatitis C instead of viral clearance. Perhaps the low frequency of HLA-DRB1*11 and DQB1*03 in many studies analysing patients with replicative hepatitis C compared with patients with self limited HCV infection may be due to over representation of patients with more progressive disease compared with those with none or minimal disease. This view is supported by studies in European patients with different stages of HCV disease.[18 42]

The relevance of the immune system in ameliorating HCV infection is also supported by the more rapid disease progression in HIV infected[43] and agammaglobulinaemic patients.[44 45] Both diseases show a disturbance in CD4+ T cell competence or antibody production. In agreement with this hypothesis, viral clearance was found to be associated with either a vigorous CD4+ T cell reaction[46] or high antibody titres.[47] However, reactivation of hepatitis can occur after discontinuation of immunosuppressive treatment.[48] Thus while the CD4+ T cell response may contribute to amelioration of the disease,[49] CD8+ cytotoxic T cells may contribute to hepatocellular damage. This is in agreement with data showing a significant correlation of CD8+ T cell numbers and alanine aminotransferase levels.[50]

Apart from direct involvement in the pathogenesis of HCV infection, HLA DRB1*11 may be in linkage disequilibrium with an as yet unidentified disease predisposing gene.[51] However, no association was found between chronic hepatitis C and the TAP and LMP2 polymorphic alleles,[52] which are located close to the HLA-DQ locus.

Most studies published so far only included HLA class II typing. Although we included HLA class I antigens in this analysis, no antigen was significantly associated with HCV induced

end stage liver disease. Similarly, analysis of HLA pattern in relation to HCV genotype or the presence or absence of HCC did not reveal any major influences.

In conclusion, our results show that the presence of HLA-DR5 antigen/HLA-DRB1*11 and HLA-DQ3/HLA-DQB1*03, respectively, is associated with a reduced risk of developing end stage liver disease induced by HCV infection in Caucasians, independent of HCV genotype.

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