JOURNAL OF VIROLOGY, Feb. 2002, p. 1787–1789 Vol. 76, No. 4
0022-538X/02/$04.00+0 DOI: 10.1128/JVI.76.4.1787–1789.2002

Decreased Frequency of the *HLA-DRB1*11* Allele in Patients with Chronic Hepatitis C Virus Infection

Ayla Yenigün and Belma Durupinar*

Department of Microbiology and Clinical Microbiology, School of Medicine, Ondokuz Mayıs University, 55139 Samsun, Turkey

Received 25 April 2001/Accepted 13 November 2001

A genetically determined resistance or susceptibility to chronic hepatitis C virus (HCV) infection may make an important contribution to the course of liver disease and may be linked to the human major histocompatibility complex (MHC). The aim of this study was to investigate the HLA class II genotype profile in chronic hepatitis C and to determine the HLA-hepatitis C association. The experimental population was composed of 49 unrelated chronic HCV patients (31 females, 18 males; mean age, 54.4 ± 1.7 years; range, 34 to 73 years). The control population consisted of 43 ethnically matched healthy donors. *HLA-DR* and -DQ alleles were studied for patients and controls by a PCR–sequence-specific-primer low-resolution method. Anti-HCV was investigated with enzyme-linked immunosorbent assay II, and HCV RNA was investigated with reverse transcriptase nested PCR. The HLA class II allele, *DRB1*11*, was found at reduced frequency in 49 patients with chronic hepatitis C (anti-HCV and HCV RNA positive) compared to that for controls (22.4 versus 51.0%; $P < 0.01$, odds ratio = 0.3, confidence interval = 0.1 to 0.7). No further HLA associations with chronic HCV infection were observed, and there was no correlation between the stage of disease and HLA. *DRB1*11* was also found at reduced frequency in all HCV antibody-positive patients compared to controls (corrected P = not significant). *DRB1*11* was associated with chronic HCV infection, and it is possible that *HLA-DRB1*11* may have a protective feature in chronic HCV infection. In addition, *DRB1*11* was associated with protection from HCV infection. These findings suggest that host HLA class II genotype is an important factor determining the outcome of infection with HCV.

Hepatitis C virus (HCV) persists in the majority of infected individuals and is responsible for a wide spectrum of chronic liver lesions ranging from minimal inflammation to cirrhosis or hepatocellular carcinoma (8, 18, 19). The mechanisms whereby HCV establishes persistent infection and liver disease are still poorly understood. Both virus-related factors, such as viral heterogeneity and replicative activity (10, 14), and the host's determinants, such as lack of efficient immune responses (13, 15), are certainly involved in the pathogenesis of chronic hepatitis. Differences in the immunogenetic backgrounds of infected patients might in part account for the observed variation in the individual courses of disease. Indeed, polymorphisms of immune regulatory genes or HLA class I and II molecules are known to influence the host's ability to present or react to viral antigens (1, 7).

Several studies have aimed to identify major histocompatibility complex class II alleles associated with different outcomes of HCV infection, but the results have not been consistent.

In the present study, we have investigated the distribution of the HLA class II alleles in patients with chronic hepatitis C using the PCR–sequence-specific-primer low-resolution method. The aim of the present study was to investigate whether these alleles might be associated with protection from or susceptibility to chronic HCV infection.

(This work was presented in part at the 15th European Histocompatibility Conference, Granada, Spain, 27 to 30 March 2001 [abstract no. 236].)

* Corresponding author. Mailing address: Ondokuz Mayıs University School of Medicine, Department of Microbiology and Clinical Microbiology, 55139 Samsun, Turkey. Phone: 90-362-4576000/2539. Fax: 90-362-4576041. E-mail: belma.durupinar@worldnet.att.net.

MATERIALS AND METHODS

Study populations. A total of 58 patients with detectable HCV antibody were seen at the gastroenterology service at the Medical Faculty, Ondokuz Mayis University, between 1999 and 2000. Subjects with chronic hepatitis C infection were selected according to the following criteria: presence of HCV antibody and HCV RNA with abnormal liver function tests and/or biopsy evidence of HCV-related liver diseases. Forty-nine patients (31 females, 18 males; mean age, 54.4 ± 1.7 years; range, 34 to 73 years) who fulfilled these criteria were recruited into this study.

The control groups included 43 unrelated healthy donors (25 females, 18 males; mean age, 39.0 ± 1.0 years) without HCV infection and living in the same geographic area.

Virological testing. The presence of HCV antibodies was determined with a commercially available third-generation enzyme-linked immunosorbent assay (Abbott Imx; Abbott Diagnostics, Maidenhead, United Kingdom), and a line immunoassay detecting antibodies to several HCV regions (INNO-LIA HCV AbIII; Immunogenetics) was used as a confirmatory test. The presence or absence of HCV RNA was determined by an in-house nested PCR (G. Maertens, Innogenetics, Ghent, Belgium).

HLA genotyping. Genomic DNA was prepared from whole blood by denaturation-precipitation with trimethylammonium bromide salts for PCR amplification. HLA genotyping was performed for a total of 15 *HLA-DRB* and 5 *HLA-DQB* alleles by PCR amplification with *DRB*, and *DQB* low-resolution typing was performed by the PCR–sequence-specific-primer method. The PCR mixtures contained 50 ng of genomic DNA/μl, 10× PCR buffer, deoxynucleoside triphosphate, *Taq* polymerase (Promega, Madison, Wis.), and specific primers. PCR amplifications were carried out in a Gene Amp PCR System 9700 (Perkin-Elmer Cetus, Norwalk, Conn.). PCR mixtures were loaded in a 3-mm-wide slot in 2% agarose gels. Gels were examined under UV illumination, and results were documented by photography.

Statistics. Statistical analysis was performed by the chi-square test. The Fisher exact test was used when appropriate. In this study, our hypothesis was that the *HLA-DRB1*11* allele was associated with chronic HCV infection, based on preliminary work. The level of significance was set at 0.05: the P values were

TABLE 1. Frequency of *HLA-DR* and *-DQ* alleles in patient groups and controls

MHC[a] class II allele	% Healthy controls (n = 43)	% All HCV (n = 58)	% Chronic HCV (n = 49)	P	
				All vs controls	Chronic vs controls
DRB1					
*DRB1*01*	4.9	10.2	10.2	NS	NS
*DRB1*15*	20.9	6.9	8.2	0.05 (pc = NS)	NS
*DRB1*16*	9.3	8.2	8.2	NS	NS
*DRB1*03*	20.9	20.4	20.4	NS	NS
*DRB1*04*	23.4	34.7	34.7	NS	NS
*DRB1*11*	51.2	25.9	22.4	0.01 (pc = NS)	<0.008[b]
*DRB1*12*	0	0	0	NS	NS
*DRB1*13*	14.0	32.8	32.7	0.05 (pc = NS)	NS
*DRB1*14*	11.7	22.4	22.4	NS	NS
*DRB1*07*	21.0	22.4	22.4	NS	NS
*DRB1*08*	2.32	2.04	2.04	NS	NS
*DRB1*10*	0	2.04	2.04	NS	NS
*DRB3**	79.1	74.0	74.0	NS	NS
*DRB4**	39.5	53.1	53.1	NS	NS
*DRB5**	32.6	14.3	14.3	NS	NS
DQB1					
*DQB1*02*	37.2	36.7	36.7	NS	NS
*DQB1*03*	67.4	51.0	51.0	NS	NS
*DQB1*04*	2.3	2.0	2.0	NS	NS
*DQB1*05*	24.6	38.8	38.8	NS	NS
*DQB1*06*	23.5	36.7	36.7	NS	NS

[a] MHC, major histocompatibility complex.
[b] OR (95% confidence interval) = 0.276 (0.112–0.679).

corrected for multiple testing (pc), applying a correction factor of 20 (i.e., the total number of *HLA-DRB1* and *-DQB1* alleles defined) for new associations, and corrections for multiple comparisons were not necessary for the allele *HLA-DRB1*11*, which we have previously identified as being reduced in chronic HCV infection (unpublished data). Odds ratios (OR) with 95% confidence intervals were calculated.

RESULTS

Comparison of *HLA-DRB1* and *-DQB1* (Table 1) allele distribution at the phenotypic level was carried out between all HCV antibody-positive patients and controls and between chronic HCV patients and controls.

Comparison of chronic HCV patients with controls revealed a strong association with chronic infection. The allele *DRB1*11* (P = 0.008, OR [95% confidence interval] = 0.276 [0.112–0.679]) was found at a significantly lower frequency in the chronic HCV group than in the controls (22.4 versus 51.2%).

Comparison of all HCV antibody-positive cases with the control group confirmed the negative association with *DRB1*11* (25.9 versus 51.2% of controls; $P < 0.01$). In addition, there were a reduced frequency of *DRB1*15* in all HCV antibody-positive patients compared with that in controls (6.8 versus 20.9% of controls; pc = not significant [NS]) and a higher frequency of *DRB1*13* in all HCV antibody-positive patients compared with that in controls (32.8 versus 14.0% of controls; pc = NS).

No further HLA associations with chronic HCV infection were observed, and there was no correlation between stages of disease.

DISCUSSION

HCV is now recognized as one of the major causes of chronic liver diseases worldwide. One of the striking features of HCV infection is the very high rate of development of chronicity. Approximately 20% of infected patients successfully eliminate the virus, whereas the great majority of patients develop chronic infection with a wide spectrum of disease. Some will remain asymptomatic, whereas others may have a more severe course leading to cirrhosis and hepatocellular carcinoma (2, 8, 19, 20). There is circumstantial evidence that immune mechanisms make an important contribution to control of HCV infection (3, 6).

In a host immune reaction against viral infection, HLA class II may play a crucial role because it is a key protein in antigen presentation to T-helper (Th) cells by antigen-presenting cells. As Th cells recognize peptides presented by HLA class II molecules, it is logical to investigate HLA class II gene polymorphism in HCV infection (3–5).

This study was specifically designed to address the influence of host HLA class II genotype profile in chronic hepatitis C and HLA-hepatitis C association. We determined HLA class II alleles in patients with chronic hepatitis C and compared the frequencies of HLA alleles between the chronic hepatitis C patients and noninfected, healthy subjects. Consequently, we found that the HLA class II allele *DRB1*11* was significantly less frequent in patients than in controls, suggesting that *DRB1*11* may be associated with protection from chronic infection. The present study also found the *DQB1*03* allele to be present at a reduced frequency in chronic infection compared to that in self-limiting infection (HCV-infected patients who were HCV RNA negative and had normal levels of aminotransferases during a follow-up period of at least 2 years [unpublished data]). The association found between *DQB1*03* alleles and spontaneous recovery from HCV infection confirmed other investigators' previous results (1, 4, 22).

To date, data on possible associations between genes in the human major histocompatibility complex and the outcome of hepatitis C are quite divergent. A higher prevalence of *DRB1*04*, *DQA1*03*, *DQB1*0301*, or *DRB1*13* was found to be associated with resistance to HCV infections in two studies from the United Kingdom (1, 16, 17). In a French study, higher frequencies of *DQB1*0301* and *DRB1*1101* were reported for patients with transient hepatitis than for those with chronic hepatitis (1), and an Italian study found the haplotype *DRB1*1104*, *DQA1*0501*, *DQB1*03*1* to be protective against chronic HCV infection, whereas *DQA1*0201-DQB1*0201* predisposed patients to chronic hepatitis (22). Furthermore, other HLA associations were found in a non-Caucasian population (9). The discrepancy between these studies may be due to sample size or selection bias or may be genuine. Different studies use different definitions, e.g., symptom free, HCV RNA negative, etc. (4, 5). In addition, differences in the frequency of class II alleles in the background population and a geographical variation in the distribution of HCV genotypes could also be considered reasons for these differences.

In our study, we have carefully defined patient groups according to HCV RNA status. Patients who were HCV antibody positive and HCV RNA positive for at least 6 months after the likely exposure to HCV were considered chronic HCV pa-

tients; patients who were HCV antibody positive and HCV RNA negative and had normal levels of aminotransferases during a follow-up period of at least 2 years were considered patients with self-limiting HCV infection. With these criteria, our data suggest that *HLA-DRB1*11* constitutes an important genetic factor for resistance to chronic HCV infection. In addition, the present study also found that the *DRB1*11* allele was associated with protection from HCV infection.

In summary, the fact that the majority of patients do go on to develop chronic infection, despite the presence of the protective alleles, suggests that genetic factors ultimately determine the outcome of chronic HCV infection. Now that associations have been established between HLA class II and viral clearance and persistence, the mechanisms underlying these associations can be analyzed, and biological pathways can be identified for therapeutic intervention.

REFERENCES

1. **Alric, L., M. Fort, J. Izopet, J. P. Vinel, J. P. Charlet, J. Selves, J. Puel, J. P. Pascal, M. Duffaut, and M. Abbal.** 1997. Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. Gastroenterology **113:**1675–1681.
2. **Alter, M. J., H. S. Margolis, K. Krawezynski, F. N. Judson, A. Mares, W. J. Alexander, P. Y. Hu, J. K. Miller, M. A. Gerber, R. Sampliner, et al.** 1992. The natural history of community-acquired hepatitis C in the United States. N. Engl. J. Med. **327:**1899–1905.
3. **Botarelli, P., M. R. Brunetto, M. A. Minutello, P. Calvo, D. Unutmaz, A. J. Weiner, Q. L. Choo, J. R. Shuster, G. Kuo, F. Bonino, et al.** 1993. T-lymphocyte response to hepatitis C virus in different clinical courses of infection. Gastroenterology **104:**580–587.
4. **Cramp, M. E., P. Carucci, J. Underhill, N. V. Naoumov, R. Williams, and P. T. Donaldson.** 1998. Association between HLA class II genotype and spontaneous clearance of HCV infection. J. Hepatol. **29:**207–213.
5. **Donaldson, P. T.** 1999. The interrelationship between hepatitis C virus and HLA. Eur. J. Clin. Investig. **29:**280–283.
6. **Ferrari, C., A. Valli, L. Galati, A. Penna, P. Scacaglia, T. Giuberti, C. Schianchi, G. Missale, M. G. Marin, and F. Fiaccodori.** 1994. T cell response to structural and nonstructural hepatitis C virus antigen in persistent and self-limited hepatitis C virus infections. Hepatology **19:**286–295.
7. **Germain, R. N.** 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell **76:**287–299.
8. **Kiyosawa, K., T. Sodemaya, E. Tanaka, et al.** 1990. Interrelationship of blood transfusion, non-A, non-B hepatitis C and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology **14:**381–388.
9. **Kuzushita, N., N. Hayashi, T. Moribe, K. Katayama, T. Kanto, S. Nakatani, T. Kaneshige, T. Tatsumi, A. Ito, K. Machizuki, Y. Sasaki, A. Kasahara, and M. Hori.** 1998. Influence of HLA haplotypes on the clinical courses of individuals infected with hepatitis C virus. Hepatology **27:**240–244.
10. **Lau, J. Y., G. L. Davis, J. Kniffen, K. P. Qian, M. S. Ureda, C. S. Chan, M. Mizokami, P. O. Neuwald, and J. C. Wilber.** 1993. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet **341:**1501–1504.
11. **Lechmann, M., H. G. Ihlenfeldt, I. Braunschweiger, G. Giers, G. Jung, B. Matz, R. Kaiser, T. Sauerbruch, and U. Spengler.** 1996. T- and B-cell responses to different hepatitis C antigens in patients with chronic hepatitis C infection and in healthy anti-hepatitis C virus-positive blood donors without viremia. Hepatology **24:**790–795.
12. **Lechmann, M., E. M. Schneider, G. Giers, R. Kaiser, F. L. Dumoulin, T. Sauerbruch, and U. Spengler.** 1999. Increased frequency of the HLA-DR15 (B1*15011) allele in German patients with self-limited hepatitis C virus infection. Eur. J. Clin. Investig. **29:**337–343.
13. **Mondelli, M.** 1996. Is there a role for immune responses in the pathogenesis of hepatitis C? J. Hepatol. **25:**232–238.
14. **Silini, E., F. Bono, A. Cividini, A. Cerino, S. Bruno, S. Rossi, G. Belloni, B. Brugnetti, E. Civardi, L. Salvaneschi, et al.** 1995. Differential distribution of hepatitis C virus genotypes in patients with and without liver function abnormalities. Hepatology **21:**285–290.
15. **Spengler, U., M. Lechmann, B. Irrang, F. L. Dumoulin, and T. Sauerbruch.** 1996. Immune responses in the hepatitis C virus infection. J. Hepatol. **34**(Suppl. 2)**:**20–25.
16. **Thursz, M., R. Yallop, C. Trepo, R. Goldin, and H. C. Thomas.** 1999. Influence of MHC class II genotype on the outcome of hepatitis C virus infection. Lancet **354:**2119–2124.
17. **Tibbs, C., P. Donaldson, J. Underhill, L. Thomson, K. Manabe, and R. Williams.** 1996. Evidence that the HLA DQA1*03 allele confers protection from chronic HCV infection in Northern European Caucasoids. Hepatology **24:**1342–1345.
18. **Tremolada, F., C. Casarin, A. Alberti, C. Drago, A. Tagger, M. L. Ribero, and G. Realdi.** 1992. Long-term follow-up of non-A, non-B (type C) post-transfusion hepatitis. J. Hepatol. **16:**273–281.
19. **Tsukuma, H., T. Hiyama, S. Tanaka, et al.** 1993. Risk factors for hepatocellular carcinoma among patients with chronic liver diseases. N. Engl. J. Med. **328:**1797–1801.
20. **Van der Poel, C. L., H. T. Cuypers, and H. W. Reesink.** 1994. Hepatitis C virus six years on. Lancet **344:**1475–1479.
21. **Yoshimichi, H., M. Takashi, Y. Reiko, K. Tsutomu, F. Hiroaki, and K. Kiyoshi.** 2000. Human leukocyte antigen DRB1 1302 protects against bile duct damage and portal lymphocyte infiltration in patient with chronic hepatitis C. J. Hepatol. **32:**837–842.
22. **Zavaglia, C., M. Martinetti, E. Silini, R. Bottelli, C. Daielli, M. Asti, A. Airoldi, L. Salvaneschi, M. U. Mondelli, and G. Ideo.** 1998. Association between HLA class II alleles and protection from or susceptibility to chronic hepatitis C. J. Hepatol. **28:**1–7.