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Hepatitis C virus-associated oral lichen planus: is the geographical heterogeneity related to HLA-DR6?

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BACKGROUND: The association between hepatitis C virus (HCV) and oral lichen planus (OLP) is more common in the Mediterranean area and Japan, possibly because of immunogenetic factors.

METHODS: Intermediate-resolution HLA-DRB typing by hybridization with oligonucleotide probes was performed in 31 Italian OLP patients with HCV infection, in 45 Italian OLP and in 48 British OLP patients without HCV infection. As healthy controls we included data from 145 unrelated Italian and 101 unrelated British bone marrow donors.

RESULTS: Italian HCV+ve OLP patients possessed the HLA-DR6 allele more frequently than Italian and British OLP patients without HCV infection (51.6% vs. 17.7% vs. 16.7%; *P* corrected = 0.028 and 0.017, respectively). There was no difference in the frequency of the HLA-DR6 allele between Italian and British control subjects.

CONCLUSIONS: The present data suggest that HLA-DR6 may be responsible for the peculiar geographic heterogeneity of the association between HCV and OLP.
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Introduction

Lichen planus (LP) is a relatively common disorder of stratified squamous epithelia, frequently exclusively involving the oral cavity (1). Given its immunological nature, oral lichen planus (OLP) is unlikely to be caused by a single antigen, as studies of T-cell receptor variable region genes from lesional T cells did not reveal the use of a restricted number of different variable region gene (2). OLP is probably the common result of the influence of a limited range of extrinsic antigens, altered self-antigens or super antigens. Precipitating factors have been identified in a minority of patients, and these include dental materials, drugs, stress, trauma and infections (1). In some LP patients there appears to be a genetic predisposition as evidenced by the frequent association of cutaneous idiopathic LP with the HLA-DR1 allele (3, 4), particularly the DRB1*0101 allele (5), whereas patients with idiopathic OLP do not have the same immunogenetic profile (6–9). The frequent association of LP with chronic liver disease (CLD) is well known (10) and controlled studies from Italy (11, 12), Spain (13, 14), USA (15–17), Japan (18) have confirmed that hepatitis C virus (HCV) is the main correlate, especially in OLP, this being one of the most frequent extrahepatic manifestation of HCV infection (19). Moreover, HCV viral sequences have been recently found in samples of affected oral tissue, suggesting that HCV may be involved in the development of the oral lesions (20–22). However, in OLP patients from England, the Netherlands and Northern France, a low prevalence of HCV infection (0–4%) has been found (23–25).

These conflicting data may not be completely explained by geographical differences in HCV infection, in fact similar geographic variability has been demonstrated for other extrahepatic abnormalities linked to HCV infection, such as serum autoantibodies, porphyria cutanea tarda (PCT) and lymphoma (26–28).

Several studies have suggested that immunogenetic, rather than viral factors, may influence the natural history and clinical presentation HCV-infected patients. Specific major histocompatibility complex (MHC) class II alleles may influence susceptibility (HLA-DRB1*0701, HLA-DRB4*0101) or resistance (HLA-DRB1*1101, HLA-DQB1*0301) to persistent HCV infection (29). Some histopathological changes in HCV-infected patients may be influenced by the host's immune reaction regulated via HLA class II alleles (30).

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In addition an immunogenetic influence on the development of extrahepatic manifestations of HCV infection is strongly suggested by reports showing that the HLA-DR11 phenotype confers susceptibility, that DR7 appears to protect against HCV-related mixed cryoglobulinemia (31) and that HCV-related OLP may be associated with HLA-DR6 (32).

Evidence is also growing to suggest that OLP is a reaction with different causes including more than one genetic or pathogenic entity (1). Nevertheless, there are no studies jointly examining the HLA aspect in patients with OLP from different countries.

The aim of this study was to assess whether MHC class II alleles influence the geographic heterogeneity of the association between OLP and HCV infection.

Patients and methods

Patients

The study included 76 Italian and 48 British patients, all affected by histologically proven OLP, without signs of dysplasia, without skin involvement. All the OLP patients were Caucasian and without serological evidence of hepatitis B virus (HBV) infection. Patients suspected to have drug or restoration related lichenoid lesions were excluded. The Italian group was formed by 31 consecutive OLP patients with HCV infection (ItaOLP-HCV + ve) and 45 sex and age matched OLP patients without HCV infection (ItaOLP-HCV−ve). All the Italian patients were recruited at the Oral Medicine Section of the Department of Biomedical Science and Human Oncology of the University of Turin. The British group consisted of 48 consecutive OLP patients without HCV infection (UKOLP-HCV−ve) recruited at the Department of Oral Medicine of the Eastman Dental Institute, UCL, University of London. The healthy control group included 145 Italian unrelated bone marrow donors without history of LP or evidence of liver disease and HCV infection. Information about the distribution of HLA-DR alleles of 101 British unrelated bone marrow donors, typed using similar methods, were obtained from previously published data (33). Informed consent and local ethical committee approvals were obtained.

Virologic assessments

Serum anti-HCV antibodies (HCVAb) were assayed by second or third generation enzyme-linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Raritan, NJ, USA) and results confirmed with second or third generation RIBA (Chiron Corp, Emeryville, CA, USA) in all OLP patients and controls, according to the manufacturers' instructions. Sera were considered positive when ELISA results were confirmed by RIBA.

Serum HCV-RNA was detected with reverse transcription polymerase chain reaction (RT- PCR) (Amplicor, Roche Diagnostic Systems, Branchburg, NY, USA). Genotyping was performed by reverse hybridization with the line probe assay (InnoLIPA^{TM} HCV, Innogenetics, Ghent, Belgium). Genotypes were expressed according to a consensus nomenclature (34).

HLA-DRB typing

A micro-salting out method modified for small amounts (1–2 ml) of ethylenediaminetetraacetic acid (EDTA)-peripheral blood was used to prepare genomic DNA (35). For intermediate-resolution DRB typing, locus-specific amplification was performed using primer pairs designed to amplify the complete polymorphic HLA-DRB1 exon 2. PCR products were blotted onto nylon membranes and hybridized with digoxigenin-11–2′-deoxyuridine-5′-triphosphate (DIG-11-dUTP)-labelled sequence specific oligonucleotide probes. Annealed probes were visualized using anti-digoxigenin-alkaline-phosphatase labelled antibody followed by disodium 3-(4-methoxyspirol 1,2-dioxetane-3,2′(5′-chloro)tricyclo-[3.3.1.1(3,7)]decan-4-yl)phenyl (CSPD) visualization according to the manufacturer's recommendations. The protocols, the sequences of the primers used and of the oligonucleotides selected for intermediate-resolution DRB typing are described in the XII Workshop technical manual (36). All the reagents were purchased from Boehringer Mannhein GmbH, Germany.

Statistical analysis

Chi-square analysis was used to compare dichotomous variables. Unpaired Student's *t*-tests was used to compare continuous variables. All the tests were two-tailed. Corresponding *P*-values were considered significant at values < 0.05.

For HLA statistical analysis, patients were grouped according to geographic origin and to the presence or absence of HCV infection (ItaOLP-HCV + ve, ItaOLP-HCV−ve, UKOLP-HCV−ve). HLA allele frequencies were compared using a standard Woolf-Haldane analysis (37). Probability values obtained were corrected (*P* corrected) for the number of comparisons. A corrected *P*-value < 0.05 was considered statistically significant.

Results

Clinical characteristics of the patients

The characteristics of the OLP patients are summarized in Table 1. There were no significant differences between the two Italian and the British groups regarding age, gender, presence of erosive oral lesions, diabetes and hypertension (Table 1). Most (74.2%) of the ItaOLP-HCV + ve patients were infected by genotype 1b, the most common HCV subtype in Italy. Details about the liver enzymes and histological lesion in the liver have been published elsewhere (32).

HLA DRB typing

The HLA-DR allele frequencies of the patients and controls are shown in Table 2. There was no significant difference between the OLP subjects taken together (ItaOLP-HCV + ve, ItaOLP-HCV−ve, UKOLP-HCV−ve) and healthy controls. Similarly, no significant difference was found comparing HCV seronegative OLP (ItaOLP-HCV−ve and UKOLP-HCV−ve) or HCV seropositive OLP (ItaOLP-HCV + ve) with healthy controls.

Allele DR5 was increased in ItaOLP-HCV−ve group compared with UKOLP-HCV−ve (46.6% vs. 18.7%,

Table 1 Clinical characteristics of the OLP patients

	Italian		
	HCV+ve (n = 31)	*HCV−ve (n = 45)*	*British (HCV−ve) (n = 48)*
Age (years) (range)[a,b]	62.3 ± 11.49 (31–79)	58.18 ± 12.26 (27–80)	57.62 ± 14.77 (26–94)
Female (%)[c]	19 (61)	29 (64)	22 (46)
Patients with erosive lesions (%)[d]	10 (32)	12 (27)	17 (35)
Patients with diabetes (%)[e]	3 (10)	5 (11)	4 (8)
Patients with hypertension (%)[f]	6 (19)	5 (11)	4 (8)

[a]Mean ± SD.
[b]Italian HCV+ve vs. Italian and British HCV−ve, $P = 0.144$ and $P = 0.139$, respectively.
[c]Italian HCV+ve vs. Italian vs. British HCV−ve, $P = 0.159$.
[d]Italian HCV+ve vs. Italian vs. British HCV−ve, $P = 0.65$.
[e]Italian HCV+ve vs. Italian vs. British HCV−ve, $P = 0.90$.
[f]Italian HCV+ve vs. Italian vs. British HCV−ve, $P = 0.139$.

Table 2 Frequencies of HLA-DR alleles in patients affected by OLP and healthy controls from Italy and England

	Italian			*British*	
HLA-DRB gene	*OLP-HCV+ve [n = 31 (%)]*	*OLP-HCV−ve [n = 45 (%)]*	*Controls [n = 145 (%)]*	*OLP-HCV−ve [n = 48 (%)]*	*Controls [n = 101 (%)]*
DR01	7 (22.6)	10 (22.2)	19 (13.1)	11 (22.9)	20 (19.8)
DR02	5 (16.1)	7 (15.5)	34 (23.5)	11 (22.9)	20 (19.8)
DR03	2 (6.4)	7 (15.5)	32 (22.1)	12 (25)	23 (22.8)
DR04	6 (19.3)	8 (17.8)	24 (16.5)	18 (37.5)	26 (25.7)
DR05	10 (32.2)	21 (46.6)[b]	65 (44.8)	9 (18.7)[b]	21 (20.8)
*11[a]	9 (29)	20 (44.4)	63 (43.4)[c]	8 (16.7)	13 (12.9)[c]
*12	1 (3.2)	1 (2.2)	2 (1.4)	1 (2)	8 (7.9)
DR06	16 (51.6)[d,e]	8 (17.7)[d]	49 (33.8)	8 (16.7)[e]	25 (24.7)
*13[a]	10 (32.3)	6 (13.3)	36 (24.8)	8 (16.7)	19 (18.8)
*14	6 (19.3)	2 (4.4)	13 (9)	0 (0)	6 (5.9)
DR07	10 (32.2)	15 (33.3)	31 (21.3)	16 (33.3)	38 (37.6)
DR08	1 (3.2)	4 (8.8)	8 (5.5)	0 (0)	7 (6.9)
DR09	2 (6.4)	0 (0)	2 (1.3)	2 (4.2)	4 (4)
DR10	1 (3.2)	4 (8.8)	6 (4.1)	1 (2)	3 (3)

[a]*Indicates the splits of the broad antigen.
[b]Italian HCV−ve vs. British HCV−ve: *P*-value = 0.0048, corrected *P*-value (pc) = 0.060.
[c]British healthy controls vs. Italian healthy controls: pc < 0.001, relative risk (rr) = 0.184, (95% confidence interval, 0.094–0.362).
[d]Italian OLP-HCV+ve vs. Italian OLP-HCV−ve: pc = 0.028, rr = 4.93, (95% confidence interval, 1.74–13.95).
[e]Italian OLP-HCV+ve vs. British OLP-HCV−ve: pc = 0.017, rr = 5.33, (95% confidence interval, 1.89–15.02).

respectively, $\chi^2 = 7.96$, $P = 0.0048$) but the differences only approached the statistical significance after correction for multiple testing (pc = 0.06). The allele DRB1*11 in particular was increased among ItaOLP-HCV−ve as compared with UKOLP-HCV−ve (44.4% vs. 16.7%, respectively, $\chi^2 = 8.14$, $P = 0.0043$, pc = 0.055) but this allele was significantly less frequent in the British healthy controls, than in the Italian healthy controls [12.9% vs. 43.4%, respectively: pc < 0.001, relative risk (rr) = 0.184, (95% confidence interval, 0.094–0.362)] independently by the presence of OLP.

Conversely, the frequency of HLA-DR6 was higher in ItaOLP-HCV+ve than ItaOLP-HCV−ve (51.6% vs. 17.7%, pc = 0.028; rr = 4.93) and UKOLP-HCV−ve (51.6% vs. 16.7%, pc = 0.017; and rr = 5.33), but there was no significant difference between healthy Italian and British controls regarding this allele (Table 2). The prevalence of DRB1*14 seemed increased in ItaOLP-HCV+ve as compared with Italian and British patients without HCV infection (19.3% vs. 4.4% vs. 0%), although the corrected probability approached, but failed to reach, statistical significance (pc = 0.09).

Discussion

The prevalence of HCV infection in LP appears to be higher in persons with oral lesions from Southern Europe (mainly Italy and Spain), Japan and USA than among patients from Northern Europe (19). These geographical differences appear unrelated to the particular HCV genotypes (38) or to any co-infection with hepatitis G virus or transfusion transmitted virus (TTV) (39) or to molecular mimicry (40), and seem only partially related to the level of the HCV endemicity in the general population. Indeed, the negative results reported from Britain and Holland (24, 25) possibly reflect the low incidence of HCV infections in those populations, whereas the very high prevalence of HCV infection among Japanese LP patients (38–62%), mainly

those coming from southern regions (Kyushu) (18, 41) was probably influenced by the high frequency of HCV in the general population (up to 7.9% in the sixth decade is HCV-infected) (42). However, a small-scale study from Egypt (43), which has the highest reported prevalence of HCV infection in the world (44), did not report a significant association between LP and HCV.

Interestingly, geographic heterogeneity in the prevalence of HCV infection similar to that observed in OLP patients is also reported in patients with other extrahepatic abnormalities linked to HCV infection, such as the presence of some serum autoantibodies, PCT and lymphoma (26–28) indirectly suggesting the existence of possible genetic differences among different populations. Indeed, it has been reported that PCT susceptibility is different in British and Italian patients, being correlated with a mutation in the HLA-linked haemochromatosis gene C 282Y in the British and to the H63D gene and HCV in the Italian groups (27). Most idiopathic cutaneous LP worldwide is related to the HLA-DR1 (DRB1*0101) allele, whereas OLP is not (5).

The present study suggests that the peculiar epidemiology of the association between OLP and HCV could be related to the HLA-DR6 allele which, mainly the DRB1*14 allele, is significantly increased in Italian OLP patients with HCV infection whereas its frequency is almost the same in clinically comparable Italian and British OLP patients without HCV infection. Notably, we did not find any difference in HLA-DR6 allele frequencies between Italian and British healthy patients.

HLA-DR6 has been associated with self-elimination of HBV in African, European and Asian populations (45), and with less severe hepatic disease in Japanese HCV-infected patients (30), possibly through a more efficient peptide presentation to CD4 positive T cells causing a vigorous T-cell response (46). Interestingly, CD4-mediated damage of basal keratinocytes is believed to be the crucial pathogenetic mechanism responsible for OLP lesions and very recently HCV-specific T cells has been found in the oral mucosa of patients with chronic hepatitis C and OLP (47) whereas both *in situ* hybridization and extractive RT-PCR techniques revealed the presence of replicative intermediate HCV-RNA in OLP specimens (20–22).

The aforementioned data suggest that the selective presentation of certain HCV-encoded peptides by HLA-DR6 molecules on the surface of monocytes to CD4 T cells may provoke a lichenoid attack upon the oral mucosa. Alternatively, similarly efficient antigen presentation could be because of a linked polymorphism in a neighbouring immunoregulatory gene (48).

Notably, increased HLA-DR6 frequency has been reported in pemphigus vulgaris (49), chronic active autoimmune hepatitis in children (50), sarcoidosis (51), and in human immunodeficiency virus (HIV)-infected patients with slow disease progression (52).

In conclusion, the results of the present study indicate that the HLA class II allele HLA-DR6 may be responsible for the peculiar geographic heterogeneity of the association between HCV and OLP and strongly supports the notion that this allele possibly influences host immune responses in the pathogenesis of HCV-related OLP.

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