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Common major histocompatibility complex class II markers in clinical variants of cicatricial pemphigoid

(PCR/HLA alleles and haplotypes/autoimmunity/eye diseases)

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ABSTRACT **Cicatricial pemphigoid (CP) is a chronic autoimmune blistering disease affecting multiple mucous membranes derived from stratified squamous epithelium and occasionally the skin. CP has a wide spectrum of disease manifestations. Patients with oral pemphigoid (OP) have a benign self-limited disease in which pathological changes are restricted to the oral mucosa. On the other hand, patients with ocular cicatricial pemphigoid (OCP), a chronic condition marked with relapses and remissions, have ocular involvement and also perhaps involvement of other mucous membranes. All clinical subsets are characterized by the presence of a similar antibasement zone autoantibody. The factors that determine the development of one form of CP or the other are not known. In a previous study, we described the association between OCP and the *DQB1*0301* allele ($P = 0.006$). In this study, we have analyzed 22 Caucasian patients with OP and their family members for major histocompatibility complex *DRB* generic, *DQA1*, and *DQB1* allele associations by PCR-sequence-specific oligonucleotide probe hybridization. The results were compared to those obtained from 17 Caucasian patients with OCP and to control Caucasian alleles and haplotypes. The *DQB1*0301* allele frequency was 38.6% in OP, 52.9% in OCP, and 17.8% in controls. Statistically significant associations were detected between the *DQB1*0301* allele and both OP ($P = 0.0047$) and OCP ($P < 0.0001$). In addition, *DRB1*04* showed a statistically significant association ($P = 0.005$) with OCP when compared to controls. Analysis of major histocompatibility complex class II haplotypes showed significant statistical associations between both OCP and OP and the HLA-DRB1*04, DRB4*0101, DQA1*03, DQB1*0301 haplotype ($P < 0.0001$ and $P = 0.0012$, respectively). Our results indicate that *DQB1*0301* is a marker of both oral and ocular forms of CP. The analysis of the amino acid sequence of the *DQB1* alleles present in both OP and OCP suggested that amino acid residues at position 57 and positions 71–77 may also be markers of CP.**

Cicatricial pemphigoid (CP) is an autoimmune blistering disease that involves multiple mucous membranes and occasionally the skin (1). The pathogenesis is attributed to the deposition *in vivo* of an anti-basement membrane antibody and complement leading to blister formation and subsequent progressive subepithelial fibrosis (2). CP has various clinical manifestations. When only the oral mucosa (oral pemphigoid, OP) is involved, the condition is usually benign and self-limited. In contrast, ocular CP (OCP) frequently involves other mucous membranes and has severe clinical manifestations and a chronic course requiring the use of high doses of corticosteroids and immunosuppressive agents to prevent blindness.

In previous studies, we showed (3) an association between the *DQB1*0301* allele and OCP ($P < 0.003$; relative risk = 9.6) when compared to a large population of healthy normal Caucasians. In that study, 19 out of 20 OCP patients carried DQ7 (*DQB1*0301*) (3). In addition, the presence of HLA DQ7 was not due to the presence of any HLA-DR4, DQ7-extended haplotypes, suggesting that the susceptibility to OCP was related to the presence of the *DQB1*0301* allele itself or another gene in linkage disequilibrium with it.

In this report, 22 Caucasian patients and their families (19 families) with OP were analyzed for major histocompatibility complex (MHC) class II *DRB* (generic), *DQA1*, and *DQB1* alleles after amplification of genomic DNA by the polymerase chain reaction (PCR) and sequence-specific oligonucleotide probe hybridization. The results were compared to those obtained from 17 OCP patients and their family controls and those of 42 control Caucasian families studied for bone marrow transplantation. Our results indicate that *HLA-DQB1*0301* is a marker of susceptibility for both the oral and ocular forms of CP. In addition, our results suggest that amino acid residues at positions 57 and 71–77 of the DQB1 molecule may be markers of CP.

MATERIALS AND METHODS

Twenty-two patients with OP were studied for MHC class II alleles and haplotype associations. Segregation analysis within families and definitive assignment of alleles to haplotypes were carried out in 19 of the 22 families. In addition, 17 patients with OCP (12 patients were reported previously) and their family members (15 families) were also used in our analysis. Their results were compared to those obtained after analysis of 42 families (129 normal haplotypes) studied for bone marrow transplantation. The diagnosis of OP was based on the presence of bullous or erosive lesions in the oral cavity. In each patient, routine histopathologic examination indicated a subepithelial split with a mixed cellular dermal infiltrate. Direct immunofluorescence examination of perilesional tissue demonstrated the presence of IgG and complement at the basement zone. Patients with OCP had the presence of immunoreactants at the conjunctival basement zone. Histologic and immunopathologic characterization was done in every patient studied.

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Abbreviations: CP, cicatricial pemphigoid; OP, oral pemphigoid; OCP, ocular CP; MHC, major histocompatibility complex.

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Table 1. MHC class II haplotypes in patients with OP

	First haplotype						Second haplotype					
ID	DRB1	DRB3	DRB4	DRB5	DQA1	DQB1	DRB1	DRB3	DRB4	DRB5	DQA1	DQB1
AL	11	02	—	—	0501	**0301**	08	—	—	—	0401	0402
LOR	11	02	—	—	0501	**0301**	07	—	0101	—	0201	0201
BIS	11	02	—	—	0501	**0301**	04	—	0101	—	03	**0301**
CL	11	02	—	—	0501	**0301**	1001	—	—	—	0101	0501
NAD	11	02	—	—	0501	**0301**	0101	—	—	—	0101	0501
SI	11	02	—	—	0501	**0301**	0102	—	—	—	0101	0501
GE	11	02	—	—	0501	**0301**	0101	—	—	—	0101	0501
CAP	11	02	—	—	0501	**0301**	12	02	—	—	0501	**0301**
KO	11	02	—	—	0501	**0301**	08	—	—	—	0401	0402
BA	04	—	0101	—	03	**0301**	0101	—	—	—	0101	0501
BE	04	—	0101	—	03	**0301**	0301	0101	—	—	0501	0201
QU	04	—	0101	—	03	**0301**	0101	—	—	—	0101	0501
MO	04	—	0101	—	03	**0301**	1501	—	—	0101	0102	0602
CO	04	—	0101	—	03	**0301**	0102	—	—	—	0101	0501
SM	1303	0101	—	—	0501	**0301**	0301	0101	—	—	0501	0201
BSH	1501	—	—	0101	0102	0602	09	—	0101	—	03	0303
WA	1501	—	—	0101	0102	0602	0101	—	—	—	0101	0501
TE	1501	—	—	0101	0102	0602	16	—	—	02	0102	0502
HE	1501	—	—	0101	0102	0602	0402	—	0101	—	03	0302
MU	07	—	0101	—	0201	0303	04	—	0101	—	03	0302
CNN	0102	—	—	—	0101	0501	0102	—	—	—	0101	0501
SAM	0301	02	—	—	0501	0201	0102	—	—	—	0101	0501

Boldface type, DQB1*0301; underlined, DQB1 Asp-57; ID, patient identification code.

DNA Isolation. DNA was isolated with the Stratagene DNA isolation kit or by the salting out method with minor modifications (4).

PCR Amplifications. DNA samples were amplified by PCR for *DRB* generic, *DQA1*, and *DQB1* loci in a 100-μl reaction mixture containing 50 pmol of each primer, 50 mM KCl, 10 mM Tris·HCl (pH 8.3), all four dNTPs (each at 200 mM), 2.5 units of *Taq* polymerase [AmpliTaq DNA polymerase (Perkin–Elmer/Cetus) or *Taq* polymerase (Promega)], and 1.5–2 mM $MgCl_2$. The primers and conditions used in this study have been published elsewhere (5–9). Several negative controls (no DNA) were always included to detect any contamination.

PCR-amplified products (5 μl) were separated by electrophoresis in TBE/2% NuSieve/1% SeaKem Agarose (FMC) gel at 150 V for 1 h (1× TBE = 0.9 mM Tris–borate/0.002 M EDTA), followed by photography of the ethidium bromide-stained DNA PCR products. Dot-blot, prehybridization, and hybridization procedures were carried out as described (8, 9).

DQA1 and *DQB1* alleles were determined in the locus-specific PCR-amplified products by sequence-specific oligonucleotide probe hybridization as described (6, 7). For *DRB*, a panel of 36 sequence-specific oligonucleotide probes were used. The majority of these PCR sequence-specific oligonucleotide probes have been described (9). A few sequence-specific oligonucleotide probes, used in this study and not described before, were synthesized based on published *DRB1* second exon sequences (10) and have been tested against an extensive panel of both homozygous (11) and heterozygous samples in our laboratory.

Statistical Analysis. Independent haplotypes and alleles were determined by direct counting based on segregation analysis within families. Statistical significance of the differences in frequency of individual MHC alleles and haplotypes

Table 2. MHC class II haplotypes in patients with OCP

	First haplotype						Second haplotype					
ID	DRB1	DRB3	DRB4	DRB5	DQA1	DQB1	DRB1	DRB3	DRB4	DRB5	DQA1	DQB1
BO	04	—	0101	—	03	**0301**	04	—	0101	—	03	**0301**
HO	04	—	0101	—	03	**0301**	0103	—	—	—	0101	0501
LAY	04	—	0101	—	03	**0301**	1501	—	—	0101	0102	0602
HU	04	—	0101	—	03	**0301**	12	02	—	—	0501	**0301**
ON	04	—	0101	—	03	**0301**	01	—	—	—	0101	0501
DAV	04	—	0101	—	03	**0301**	0101	—	—	—	0101	0501
DON	04	—	0101	—	03	**0301**	04	—	0101	—	03	**0301**
DA	04	—	0101	—	03	**0301**	0102	—	—	—	0101	0501
LEO	11	02	—	—	0501	**0301**	11	02	—	—	0501	**0301**
GUT	11	02	—	—	0501	**0301**	0301	0101	—	—	0501	0201
KAT	11	02	—	—	0501	**0301**	0101	—	—	—	0501	0504
ANT	12	0101	—	—	0501	**0301**	1401	02	—	—	0101	0503
LOM	0301	0101	—	—	0501	**0301**	0301	0101	—	—	0501	0201
LOP	0901	—	0101	—	03	0303	0101	—	—	—	0101	0501
DY	1501	—	—	0101	0102	0602	0103	—	—	—	0101	0501
STO	04	—	0101	—	03	0302	13	0301	—	—	0102	0604
HAR	0301	0101	—	—	0501	0201	0301	0101	—	—	0501	0201

Boldface type, DQB1*0301; underlined, DQB1 Asp-57; ID, patient identification code.

in the patients and control population was estimated by χ^2 analysis or Fisher's exact test, as appropriate, with the aid of the INSTAT software (GraphPad, San Diego).

RESULTS

The MHC class II alleles and haplotypes of patients with OP and OCP are shown in Tables 1 and 2. The phenotype frequency of *HLA-DQB1*0301* among 22 patients with OP and 17 OCP patients was 68.18% (15/22) and 76.47% (13/17), respectively. Among OP patients, 2 were homozygotes for *DQB1*0301*, 13 were heterozygotes, and 7 did not carry *DQB1*0301*. Among OCP patients, 4 patients were homozygotes for *DQB1*0301*, 4 were negative for *DQB1*0301*, and 9 were heterozygotes for *DQB1*0301*. The allele frequencies and statistical comparisons for *DRB1*, *DQA1*, and *DQB1* are shown in Table 3.

Table 3. Statistical comparison of MHC class II *DRB1*, *DQA1*, and *DQB1* alleles in OP and OCP

	OP (44)		OCP (34)		Normal (129)		Significance		
Allele	No.	%	No.	%	No.	%	OP vs. normal	OP vs. OCP	OCP vs. normal
DQA1									
0101/0104	12	27.2	6	17.64	22	17.05	NS	NS	NS
0102	5	11.3	3	8.82	18	13.95	NS	NS	NS
0103	—	—	—	—	11	8.52	—	—	—
0201	2	4.5	—	—	21	16.27	NS	—	—
03	9	20.4	12	35.29	18	13.95	NS	NS	0.01
0401	2	4.5	—	—	5	3.87	NS	—	—
0501	14	31.8	13	38.23	34	26.35	NS	NS	NS
DQB1									
0501	11	24.9	5	14.7	17	13.17	NS	NS	NS
0502	1	2.3	—	—	4	3.1	NS	—	—
0503	—	—	1	2.94	5	3.87	—	NS	NS
0504	—	—	1	2.94	—	—	—	—	NS
0602	5	11.3	2	5.88	10	7.75	NS	—	NS
0603	—	—	—	—	10	7.75	—	NS	—
0604	—	—	1	2.94	3	2.32	—	—	NS
0605	—	—	—	—	1	0.77	—	—	—
0201	4	9	4	11.76	32	24.8	0.03	NS	NS
0301	17	38.6	18	52.94	23	17.82	0.007	NS	0.001
0302	2	4.5	1	2.94	15	11.62	NS	NS	NS
0303	2	4.5	1	2.94	5	3.87	NS	NS	NS
0402	2	4.5	—	—	4	3.1	NS	—	—
DRB1									
0101	5	11.3	4	11.76	7	5.42	NS	NS	NS
0102	5	11.3	1	2.94	6	4.65	NS	NS	NS
0103	—	—	2	5.88	1	0.77	—	—	NS
01	—	—	—	—	1	0.77	—	—	—
15	5	11.3	2	5.88	13	10.07	NS	NS	NS
16	1	2.3	—	—	2	1.55	NS	—	—
03	3	7	5	14.7	14	10.85	NS	NS	NS
04	7	16	11	32.35	13	10.07	NS	NS	0.005
0402	1	2.3	—	—	5	3.87	NS	—	—
11	9	20.4	4	11.76	16	12.4	NS	NS	NS
12	1	2.3	2	5.88	3	2.32	NS	NS	NS
1301/1302	1	2.3	1	2.94	15	11.6	NS	NS	NS
1304	—	—	—	—	1	0.77	—	—	—
1401	—	—	1	2.94	4	3.1	—	—	NS
1404	—	—	—	—	1	0.77	—	—	—
07	2	4.5	—	—	20	15.5	NS	—	—
08	2	4.5	—	—	5	3.87	NS	—	—
0901	1	2.3	1	2.94	1	0.77	NS	NS	NS
1001	1	2.3	—	—	1	0.77	NS	—	—

No., the number of independent instances; NS, not significant. Numbers in parentheses are *n*.

The *DQB1*0301* allele frequency was 38.6% in OP and 52.94% in OCP. When class II allele frequencies in patients with OP were compared with those present in control individuals, only *DQB1*0301* showed a statistically significant association ($P = 0.007$). In addition, and as expected, the *DQB1*0301* allele showed a strong statistical association with OCP ($P < 0.0001$), as described before (3).

For the *DRB1* locus, *DRB1*04* showed a statistically significant association with OCP when compared to control individuals ($P = 0.005$). However, no association was detected between the *DRB1*04* allele and OP (Table 3).

Family studies were carried out to determine haplotype frequencies (shown in Table 4) in all but three patients with OP and three patients with OCP. The haplotypes of these patients were then deduced based on known associations between MHC class II alleles.

A strong statistical association between OCP and the HLA-DRB1*04, DRB4*0101, DQA1*03, DQB1*0301 haplotype was detected ($P < 0.0001$). A similar association was also found for OP ($P = 0.0012$). These findings contrasted with the lack of association of two other DQB1*0301-carrying haplotypes (DRB1*11, DRB3*02, DQA1*0501, DQB1*0301 and DRB1*12, DRB3*02, DQA1*0501, DQB1*0301).

DISCUSSION

We have reported (3) a strong association between OCP and the *DQB1*0301* allele by restriction fragment length polymorphism analysis. In that report, the *DQB1*0301* allele was present in 19 of 20 OCP patients analyzed. In the present study, we analyze MHC class II markers and haplotypes in two subsets of CP. In this study, 17 OCP patients, 12 from the original series and 5 additional patients, were analyzed by PCR sequence-specific oligonucleotide hybridization methods. The *DQB1*0301* allele was found in 13 of 17 patients (76.47%). The allele frequency for *DQB1*0301* was 52.94% and was found to be statistically significantly increased compared with that in normal individuals ($P < 0.0001$). Twenty-two patients with OP were studied for MHC class II allele and haplotype associations. Family studies were performed in 19 of 22 patients. The same allele, *DQB1*0301*, was present in 15 of 22 patients (68.18%) and was significantly associated with this subset of CP compared to normal controls ($P = 0.007$). The frequencies of *DRB1*04*, *DRB1*11*, or *DRB1*12* alone were not statistically significantly increased in OP when compared to normal individuals. These findings suggested that in both OCP and OP subsets of CP, the primary MHC associations were with *DQB1*0301*.

As in OCP, a significant association between OP and the HLA-DRB1*04, DRB4*0101, DQA1*03, DQB1*0301 haplotype was detected ($P < 0.0001$ and $P = 0.0012$, respectively). Other DQB1*0301-carrying haplotypes did not appear to be significantly increased in OP or OCP.

A number of autoimmune diseases are associated with *DQB1* alleles. The *DQB1*0201* allele was found to be associated with gluten-sensitive enteropathy (celiac disease) (12), while *DQB1*0302* was found to be associated with insulin-dependent diabetes mellitus (13). The amino acid at position 57 (absence of aspartic acid) has been associated with increased risk for insulin-dependent diabetes mellitus. In addition, Arg-52 of the DQA1 chain has also been suggested to have a role in susceptibility to insulin-dependent diabetes mellitus (14) with non-Asp/Arg residues associated with maximal susceptibility. About 30% of patients with either OP or OCP did not carry *DQB1*0301* (many had *DQB1*0602* or *-0303*). The analysis of the amino acid residues present in the OP and OCP patients showed that 35 of 39 (90%) CP patients carried Asp in position 57 compared to 56 of the 129 (43%) control haplotypes ($P < 0.0001$). Thus, it is possible that this residue is involved in susceptibility to CP.

Table 4. Comparisons of MHC class II haplotypes in patients with OP and OCP and normal individuals

Haplotype						Normal (129)		OP (44)		OCP (34)		Significance		
DRB1	DRB3	DRB4	DRB5	DQB1	DQA1	No.	%	No.	%	No.	%	OP vs. normal	OCP vs. normal	OP vs. OCP
16			0102	0502	0102	1	0.77					—	—	—
16			02	0502	0102	1	0.77	1	2.3			NS	—	—
15			0102	0201	0103	1	0.77					—	—	—
15			0101	0602	0102	10	7.75	5	11	2	5.88	NS	NS	NS
15			02	0502	0102	1	0.77							
15			0101	0502	0102	1	0.77							
1404	02			0503	0101	1	0.77							
1401	02			0503	0101	4	3.1			1	2.94		NS	
1304	0101			0301	0501	1	0.77							
1303	0101			0301	0501			1	2.3					
1301/02	0101			0603	0103	5	3.87							
1301/02	02			0603	0103	3	2.32							
1301/02	02			0604	0102	1	0.77							
1301/02	0301			0603	0103	2	1.55							
1301/02	0301			0604	0102	2	1.55			1	2.94		NS	
1301/02	0301			0501	0101	1	0.77							
1301/02	0301			0605	0102	1	0.77							
12	02			0301	0501	3	2.32	1	2.3	2	5.88	NS	NS	NS
11	02			0301	0501	16	12.4	9	20	4	11.76	NS	NS	NS
1001				0501	0101	1	0.77	1	2.3			NS	—	—
0901		0101		0303	0301	1	0.77	1	2.3	1	2.94	NS	NS	NS
08				0402	0401	4	3.1	2	4.5			NS		
08				0301	0401	1	0.77							
07		0101		0201	0201	16	12.4	1	2.3			NS		
07		0101		0303	0201	4	3.1	1	2.3			NS		
0404		0101		0302	0301	1	0.77							
0402		0101		0302	0301	4	3.1	1	2.3			NS		
0402		0101		0301	0301	1	0.77							
04		0101		0302	0301	10	7.75	1	2.3	1	2.94	NS	NS	NS
04		0101		0201	0201	1	0.77							
04		0101		0301	0301	1	0.77	0	14	10	29.41	0.0012	<0.0001	NS
03	0101			0201	0501	12	9.3	2	4.5	4	11.76	NS	NS	NS
03	02			0201	0501	2	1.55	1	2.3			NS		
0103				0501	0101	1	0.77			1	2.94		NS	
0103				0301	0501					1	2.94			
0102				0501	0101	6	4.6	5	11	1	2.94	NS	NS	NS
0101				0501	0101	7	5.42	3	6.8	4	8.82	NS	NS	NS
0101				0504	0501					1	2.94			
01				0501	0101	1	0.77	2	4.5			NS		
0101				0301	0501					1	2.94		NS	

Total number of haplotypes is in parentheses. No. refers to the number of independent instances for each haplotype.

There have been reports of association of the *DQB1*0302, -0303, -0602*, and *DQB1*0301* alleles and the presence of anti-phospholipid antibodies in certain clinical conditions associated with systemic lupus erythematosus, such as spontaneous intravascular thrombosis, livedo reticularis, Libman–Sachs endocarditis, thrombocytopenia, and recurrent abortions (15). These alleles share identical amino acid residues at positions 71–77 in the second exon. Of interest, 36 of 39 CP patients carried these alleles (92.3%) ($P < 0.0001$), indicating that these residues may also be involved in susceptibility to CP.

Our results indicate that similar MHC class II markers are present in two clinical subsets of CP, suggesting that they are part of a spectrum of a single disorder. The association of *DQB1*0301* allele (about 70% of patients) and the presence of similar amino acid residues in positions 57 and 71–77 in about 90% of the patients (*DQB1*0301, -0302, 0303*, and *-0602* alleles) suggest a common susceptibility to develop CP.

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