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Association of DQw7 (DQB1*0301) with ocular cicatricial pemphigoid

(autoimmune susceptibility gene/haplotype analysis)

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ABSTRACT **Ocular cicatricial pemphigoid (OCP) is an autoimmune blistering disease that affects the conjunctiva and multiple mucous membranes. Class I and II and complement genetic markers of the major histocompatibility complex were studied in 20 Caucasian OCP patients and members of their families. Frequencies of individual alleles and common fixed or extended haplotypes in the patients were compared with those in normal family control haplotypes and with overall normal Caucasian haplotypes. The most striking increase compared with overall controls was noted in HLA-DQw3 ($P = 0.006$), unassociated with any extended haplotype. All but 1 of the 20 patients carried DQw3 in linkage with HLA-DR4 (increased significantly with $P = 0.042$ compared with overall normal genotype controls) or DR5. The DQw3, on analysis by restriction fragment length polymorphism in genomic DNA, was, in every instance, DQw7 (3.1, DQB1*0301). The frequency of DQB1*0301 in patient haplotypes compared with overall normal DR4 and DR5 DQw3-bearing haplotypes was statistically significantly increased ($P < 0.003$, relative risk = 9.6). The distribution of homozygotes and heterozygotes for DQB1*0301 among the patients was consistent with dominant but not recessive inheritance of DQB1*0301 or a gene, probably a class II allele, in linkage disequilibrium with it as the major histocompatibility complex susceptibility gene for OCP.**

Ocular cicatricial pemphigoid (OCP) is an autoimmune blistering disease that affects multiple mucous membranes (1–3). If not treated or treated inappropriately when it affects the eyes, it can cause blindness. The pathologic processes of chronic cicatrizing conjunctivitis and progressive conjunctival subepithelial fibrosis that characterize this disease result in severe xerosis of the eye and ocular keratinization.

The deposition of immunoglobulins and complement components at the basement membrane zone (BMZ) (4–6) of the involved mucosa appears to be pathogenetic. Inflammatory mediators in the preocular tear film contribute to the final pathologic changes (3). Circulating antibodies to BMZ have been demonstrated in the serum of OCP patients, using skin and buccal mucosa as substrate (7, 8).

There have only been a few studies of HLA antigen frequencies in patients with OCP. The initial reports (6, 9) of an increased frequency of HLA-B12 (HLA-B44 or B45) were not confirmed in later studies (3, 10). Recently (11), we reported an increase in the frequency of the HLA-DR4 allele in OCP patients. In patients with pemphigus vulgaris, the increased frequency of DR4 is ascribable to the increased frequency of two haplotypes, [HLA-B38, SC21, DR4, DQw8] and [HLA-B35, SC31, DR4, DQw8], particularly in Ashkenazi Jewish patients (12). The first of these is a known extended haplotype (13) (a haplotype with fixed DNA over at least the HLA-B/DR interval) in this ethnic group. In a number of major histocompatibility complex (MHC) allele-associated diseases, the increase in specific alleles is secondary to the increase in one or more extended haplotypes that carry susceptibility alleles. Thus, in celiac disease, an increase in HLA-DQw2 results from increases in the frequency of [HLA-B8, SC01, DR3, DQw2] and [HLA-B44, FC31, DR7, DQw2] (14). Similarly, in type 1 diabetes mellitus and rheumatoid arthritis, the increased frequencies of individual alleles are associated with increases in specific extended haplotypes that bear them (15, 16).

To determine if the increased frequency of DR4 in OCP was associated with an increase in a specific DR4-bearing extended haplotype and, in particular, if the DR4 was DQw7- or DQw8-associated, patients with OCP and their families were studied for HLA-A, -B, -DR, and -DQ, the serum complement proteins BF, C2, C4A, and C4B, and restriction fragment length polymorphisms (RFLPs) in *DRB*, *DQB*, and *DPB*. Our findings suggest that HLA-DQB1*0301, rather than an extended MHC haplotype bearing this allele, is a marker for susceptibility to OCP. It is possible that HLA-DQB1*0301 or a class II MHC gene in linkage disequilibrium with it determines the disease by specifying production of an antibody to a BMZ protein in the conjunctiva and other mucous membranes.

MATERIALS AND METHODS

Patients and Samples. Blood samples were obtained from 20 randomly ascertained unrelated Caucasian patients with OCP and their immediate family members. Control homozygous cells for subtypes of DQw3 (DQw7 and DQw8) were obtained from the Tenth International Histocompatibility Workshop (17) and included 9025 for HLA-DR4, DQw7 and 9026, 9029, and 9032 for DR4, DQw8. Additional control cells for subtyping by RFLP were from healthy normal Caucasian individuals who carried serologically determined HLA-DR4, -DR5, and -DQw3. Haplotype assignments were made from family studies, including the RFLP variants and complotypes (18).

Clinical and Laboratory Criteria for Diagnosis. The diagnosis of OCP was made on the basis of clinical features, which included cicatrizing conjunctivitis with or without symblepharon formation. It was confirmed by routine histologic examination and by the demonstration of IgG and C3 deposition at the epithelial BMZ of the conjunctiva (10).

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Abbreviations: OCP, ocular cicatricial pemphigoid; MHC, major histocompatibility complex; RFLP, restriction fragment length polymorphism; BMZ, basement membrane zone.

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MHC Marker Studies. C4, BF, and C2 typings were done by techniques described earlier (17, 19–21). HLA-A, -B, and -C antigens were detected by the National Institutes of Health lymphocyte microcytotoxicity assay (22) and HLA-DR and -DQ typing was done using the technique of the Seventh International Histocompatibility Workshop (23).

Haplotype Analysis. The haplotypes occurring in the propositi constituted the patient chromosomes or haplotypes. Haplotypes not occurring in patients but found in other family members constituted normal family control haplotypes (15). Randomly selected independent normal MHC haplotypes from Caucasians of European ancestry were used as overall normal controls.

DQB and DRB Subtypes. Typing of the *DRB* and *DQB* region was done by RFLP analysis on DNA obtained from peripheral blood lymphocytes of 18 patients and immediate family members to assign haplotypes. The probes used to study the RFLP in *DQB*, *DQA*, and *DRB* genes were cDNA probes provided by the Tenth International Histocompatibility Workshop (24). Assignments of DQw7 and DQw8 were made on the basis of the presence of the following fragments. For DQw7: *Taq* I 4.6 kilobases (kb), *Bam*HI, 3.6 kb; *Kpn* I, 5.3 and 1.2 kb; and *Pvu* II, 3.2 kb. For DQw8: *Taq* I, 1.9 and 2.6 kb; *Bam*HI, 10.3 kb; and *Kpn* I, 19.1 kb (25). Since there was no statistically significant increased frequency of DQw2 and DQw1 in patients, subtyping of these alleles by RFLP was not done. The frequencies of DQw7 and DQw8 in the 23 patient DQw3 haplotypes were compared to their frequencies in 46 normal Caucasian DQw3-bearing haplotypes.

The assignment of DQA was based on the presence of a 4.6-kb fragment upon *Taq* I digestion using the DQA probe (26). In eight patients (nine haplotypes since one patient was homozygous for DR5), the subtyping of the DR5 haplotypes was done by digestion with *Taq* I and the DRB probe. DRw11 was characterized by the presence of three polymorphic fragments of 11.6, 6.1, and 4.0 kb (27).

DPw Subtyping. Typing of the DP region was done by RFLP analysis as described by the Tenth International Workshop (28) on DNA of 18 OCP patients and their family members and 50 normal Caucasian controls. The DNA was digested with *Msp* I (M), *Pst* I (P), and *Taq* I (T) endonucleases and the membrane was hybridized with a DPB probe provided by the Tenth International Workshop (28, 29); assignments of DPB alleles were made on the basis of the presence of the following *Msp* I (M), *Pst* I (P), or *Taq* I (T) fragment sizes: DPw1, 5.0 and 6.7 kb (M), 2.85 kb (P), and 1.7 kb (T); DPw2, 2.0 and 2.2 kb (M), 2.4 and 1.9 kb (P); DPw3, 3.1 kb (M), 2.85 kb (P), and 1.7 kb (T); DPw4, 2.0 and 1.1 kb (M); DPw5, 6.7 and 2.0 kb (M), 2.85 kb (P), and 1.7 kb (T).

Statistical Analyses. Statistical significance of the differences in frequency of individual MHC alleles and extended haplotypes in the patient and control populations was estimated by χ^2 analysis using (observed − expected)^2/expected, 2 × 2 contingency tables, or Fisher's exact test, as appropriate. Nominal P values for comparisons of multiple variables were corrected by multiplication by the number of comparisons. The number of homozygotes and heterozygotes for DQw7 was used to test for recessive or dominant inheritance (12, 30).

RESULTS

Allele Frequencies. MHC haplotypes, determined from family studies of 20 patients, are presented in Table 1. The frequencies of MHC alleles in these patients were compared with 29 family control haplotypes and 2186 overall normal Caucasian control haplotypes. After correction, there were no significant differences in frequency of any HLA-A, HLA-B, or complotype alleles, including HLA-B44 and SC30. The frequency of HLA-DR4 was 0.342 in patients, 0.111 in family controls, and 0.173 in overall controls. The increase in patient DR4 compared with family controls ($P_{nominal}$ = 0.03) was not significant after correction but compared with overall controls was significant ($P_{corrected}$ = 0.042). The frequency of DR5 in patients did not differ significantly from that in either control group. In contrast, although the frequency of HLA-DQw3 at 0.605 in patients was not significantly increased over family controls at 0.423, it was when compared with overall controls at 0.348 ($P_{corrected}$ = 0.006). Of the 20 patients, 19 carried DQw3.

Haplotype Associations and DQw3 Subtypes. As seen in Table 1, all DR4 and DR5-bearing patient haplotypes carried

Table 1. Haplotypes in OCP patients with HLA-A, -B, -DR, -DQ, and -DP alleles and complotypes

Family no.	A	B	Comp.	DR	DQw*	DPw	A	B	Comp.	DR	DQw*	DPw
2627†	24	27	SC32	4/5	3	ND	2	w70	SC42	4/5	3	ND
2628	24	44	SC31	ND	3/7	4	26	w55	SC45	7	2	1
2629	2	44	SC30	4	3/7	4	32	w61	SC42	1	1	1
2487	3	44	SC42	1	1	ND	2	35	FC(3,2)0	7	2	ND
2637	2	w50	SC31	5‡	3/7	4	2	44	SC31	2	1	3
2641	11	w52	SC31	4	3/7	3	1	8	SC01	3	2	4
2635	26	35	FC31	4	3/7	4	2	14	SC2(1,2)	1	1	3
2640	11	35	ND	5‡	3/7	3	29	44	ND	5/1	1	4
2632	24	39	SC42	4	3/7	4	1	8	SC01	3	1	3
2636	32	35	SC31	4	3/7	3	1	w62	SC31	4	3/8	4
2631	2	44	SC30	4	3/7	4	32	44	FC30	5	3/7	3
2638	2	44	SC31	5‡	3/7	4	2	w51	FC32	4	3/8	3
2630	2	w62	SC42	4	3/7	4	2	7	SC31	2	1	3
2639	2	35	SC30	5‡	3/7	4	33	14	FC31	5	3/7	4
2633	1	w57	SC61	4	3/7	4	2	w60	SC31	1	1	4
2634	2	7	SC31	5‡	3/7	4	1	8	SC01	3	2	1
2644	2	w61	SC31	4	3/7	2	3	7	SC31	2	1	1
2500	3	35	SC31	5‡	3/7	1	30	13	SC01	7	2	5
2643	2	44	ND	4	3/7	3	26	27	ND	1	1	2
2642	24	35	SC31	5‡	3/7	4	23	63	FC31	ND	3/8	3

Comp., complotype; ND, not done.
*DQw1, -2, and -3 typing was by microcytotoxicity assay; subtyping of DQw3 into DQw7 and DQw8 was by RFLP.
†2627 was not analyzed by RFLP.
‡HLA-DR5 was DRw11 (rather than DRw12) by RFLP.

Table 2. Distribution of homozygotes and heterozygotes for HLA-DQw7 and non-HLA-DQw7 in relation to mode of inheritance

HLA-DQw7	No. observed	No. expected for dominant	χ^2	P	No. expected for recessive	χ^2	P
Homozygotes	2	3.19	0.44		5.26	2.02	
Heterozygotes	16	13.62	0.42		9.47	4.50	
Non-HLA-DQw7	1	2.18	0.64		4.26	2.49	
Total			1.50	NS		9.01	<0.02

NS, not significant.

DQw3. No DR4 or DR5-bearing extended haplotype was increased in frequency in the patients. Of 9 DR5 haplotypes studied in 8 patients, 7 were DRw11. Both of the non-DRw11 were in patients who had either DR4 or DRw11 on their other haplotypes.

Of the 23 patient DQw3-bearing haplotypes tested, 20 (86.9%) were DQw7 (12 with DR4, 8 with DR5) and 3 were DQw8 (all with DR4) (Fig. 1). All 3 patients with DQw8 had DQw7 on the other haplotype. Thus, of 18 OCP patients with DQw3 tested, 17 (94.4%) had DQw7. Of 46 control haplotypes, 15/36 DR4 haplotypes and 8/10 DR5 haplotypes were DQw7, the rest being DQw8. This difference in distribution of DQw7 and DQw8 on DQw3 haplotypes was highly significant ($P < 0.003$).

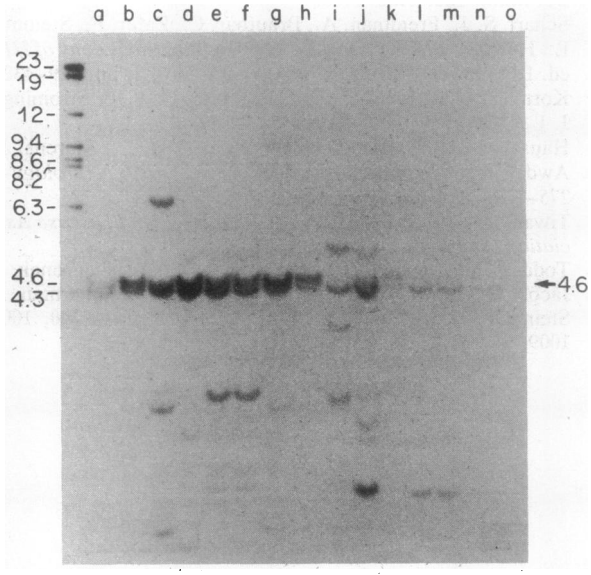

FIG. 1. Autoradiogram indicating RFLP pattern of DQw7 in OCP patients. DNA was digested with *Taq* I and probed with DQB. Note the 4.6-kb fragment. Lanes a–g, patient DNA; lanes h–j, normal control DNA; and lanes k–o, homozygous typing cell line DNA. The haplotypes are as follows (family no. in parentheses):

(2637)	a	A2	Bw50	SC31	DR5	DQw7	A2	B44	SC31	DR2	DQw1
(2641)	b	A11	Bw52	SC31	DR4	DQw7	A1	B8	SC01	DR3	DQw2
(2829)	c	A2	B44	SC30	DR4	DQw7	A32	Bw61	SC42	DR1	DQw1
(2639)	d	A2	B35	SC30	DR5	DQw7	A33	B14	FC31	DR5	DQw7
(2631)	e	A2	B44	SC30	DR4	DQw7	A32	B44	FC30	DR5	DQw7
(2630)	f	A2	Bw62	SC42	DR4	DQw7	A2	B7	SC31	DR2	DQw1
(2634)	g	A2	B7	SC31	DR5	DQw7	A1	B8	SC01	DR3	DQw2
	h	A2	B7	SC31	DR5	DQw7	A1	B8	SC01	DR3	DQw2
	i	A2	B38	SC21	DR4	DQw8	A2	Bw55	SC23	DRw6	DQw1
	j	A26	B35	SC31	DR4	DQw7	A26	B14	SC21	DR1	DQw1
	k	A2	B44	SC30	DR4	DQw7					
	l	A26	B38	SC21	DR4	DQw8					
	m	A10	B16	ND	DR4	DQw8					
	n	A23	Bw65	ND	DR4	DQw8					
	o	A26	B38	SC21	DR4	DQw8					

HLA-DQA and DP Polymorphism and Associations. The 4.6-kb *Taq* I DQA band was present in 9/15 DR4-DQw7 and all 7 DR5-DQw7 OCP patient haplotypes examined. The frequency of this fragment was not significantly increased in patient compared with control haplotypes.

The frequencies of DP subtypes as determined by RFLP in 36 patient haplotypes were not significantly different from those in 50 normal Caucasian control haplotypes.

Mode of Inheritance of DQw7. Table 2 gives the results of analysis of the distribution of DQw7 homozygotes and heterozygotes among OCP patients. Recessive inheritance can be rejected ($P < 0.02$), but the distribution is consistent with dominant inheritance.

DISCUSSION

OCP and pemphigus vulgaris are diseases associated with antibodies to components of the skin and mucous membranes. The antibody in pemphigus vulgaris is directed against a protein of the epidermal intercellular cement substance (31), whereas the antigen(s) of OCP have not been definitively characterized (32). Although both diseases are associated with HLA-DR4 (11, 33) in some patients, in other respects the MHC associations are quite different. In this report, we have shown that the HLA-DR4 increase in OCP appears to be secondary to the increase in DQw7 and not to a DR4-carrying extended haplotype.

In many diseases, fixed or extended haplotypes provide the individual MHC allele markers for MHC susceptibility. Extended haplotypes are conserved stretches of DNA involving at least the HLA-B-DR/DQ interval (13). If a disease susceptibility gene is on such a haplotype, it will occur on all or most examples of that haplotype in unrelated persons of the population who carry it. Extended haplotypes are the markers for gluten-sensitive enteropathy (14), type 1 diabetes mellitus (15), and pemphigus vulgaris (12, 30).

In contrast, there are diseases in which the susceptibility gene is an HLA allele and not an unidentified gene within any known extended haplotype. In northern European Caucasians with multiple sclerosis, DR2 appears to be a marker of the disease (34, 35). Similarly, in many ethnic groups, HLA-B27 is found in the majority of patients with ankylosing spondylitis (36). Because DR4-DQw7 and DR5-DQw7 are the markers for OCP in the present group of patients, it appears likely that the susceptibility allele is DQw7 or an allele in linkage disequilibrium with it on both susceptibility haplotypes.

The inheritance of the DQw7 DQB1*0301 susceptibility gene for OCP appears to be dominant. It has been postulated that aspartic acid at residue 57 of the DQB1 gene confers resistance to insulin-dependent diabetes mellitus (37) and DQB1*0301 is such a gene. In contrast, this same gene in OCP appears to mark disease susceptibility, perhaps as a dominantly expressed immune response gene controlling production of autoantibody to a basement membrane antigen.

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