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Immunology

Major histocompatibility complex haplotypes and class II genes in non-Jewish patients with pemphigus vulgaris

(HLA/extended haplotypes/autoimmunity)

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ABSTRACT **Previous studies demonstrated that HLA-DR4 was markedly increased among Ashkenazi Jewish patients with pemphigus vulgaris (PV), almost entirely as the common Jewish extended haplotype [HLA-B38, SC21, DR4, DQw8] or as the haplotype HLA-B35, SC31, DR4, DQw8, and that HLA-DR4, DQw8 was distributed among patients in a manner consistent with dominant expression of a class II (D-region or D-region-linked) susceptibility gene. In the present study of major histocompatibility complex (MHC) haplotypes in 25 non-Jewish PV patients, DR4, DQw8 was found in 12 of the patients and DRw6, DQw5 was found in 15. Only 3 patients had neither. Only 1 of the DR4, DQw8 haplotypes was [HLA-B38, SC21, DR4, DQw8] and 2 were HLA-B35, SC31, DR4, DQw8; most were the presumed fragments (SC31, DR4, DQw8) or (SC21, DR4, DQw8) or DR4, DQw8 with some other complotype. Of the patients with DRw6, DQw5, all were DRw14, DQw5, and 6 had a rare Caucasian haplotype, HLA-Bw55, SB45, DRw14, DQw5. Four of 6 of these were found in patients of Italian extraction, as was the 1 normal example. The non-Jewish patients were of more Southern European extraction than our controls. This suggests that there are two major MHC susceptibility alleles in American patients with PV. The more ancient apparently arose on a haplotype in the Jews, HLA-B38(35), SC21(SC31), DR4, DQw8, and spread to other populations largely as D-region segments. The other arose in or near Italy on the haplotype HLA-Bw55, SB45, DRw14, DQw5 and has also partially fragmented so that many patients carry only DRw14, DQw5. The available data do not permit the specific localization of either the DR4, DQw8- or the DRw14, DQw5-linked susceptibility genes.**

Pemphigus vulgaris (PV) is a blistering autoimmune disease that affects the skin and mucous membranes. There is deposition *in vivo* of an antibody to intercellular cement substance (ICS) of the skin, and most patients have anti-ICS in serum (1). The antibody appears to be pathogenetic since newborns of mothers with PV have serum anti-ICS and transient blisters (2); intraperitoneal injection of anti-ICS into newborn mice results in clinical disease (3).

Previous studies have demonstrated an increase in specific HLA-D region (HLA-DR, DQ, and DP) alleles in patients compared with controls. Genes of this region are called class II major histocompatibility complex (MHC) genes and encode molecules involved in the immune response to soluble protein antigens (4, 5). Between class II and class I MHC genes (HLA-A, -B, and -C) lie four genes encoding three complement proteins, factor B, C2, and C4, which are inherited as a single genetic unit called a complotype, designated (in arbitrary order) by its BF, C2, C4A, and C4B alleles (6). Some specific HLA-B, complotype, HLA-DR allelic sets occur on randomly ascertained normal Caucasian haplotypes at frequencies significantly greater than those predicted by the frequencies of their component alleles and exhibit positive linkage disequilibrium. These are frozen or "extended haplotypes" and considerable evidence suggests that, at least for the HLA-B/DR interval, the intervening DNA is fixed and the same, or nearly the same, on such haplotypes in unrelated individuals (7). In many diseases exhibiting MHC associations, it is these extended haplotypes or their fragments that provide the individual allele markers noted in patients, as observed in insulin-dependent diabetes mellitus (8, 9) and celiac disease (10).

Greek patients with PV have been reported to have an increased frequency of HLA-B22 (w54, w55, or w56) (11). We found a striking increase in the frequency of the complotype SB45 among non-Jewish PV patients (12). Ashkenazi Jewish PV patients have increased frequencies of HLA-A26, B38, and DR4 (13–15), whereas HLA-DRw6 is increased in non-Ashkenazi Jews and in Austrian non-Jews with PV (16). We recently found a marked increase in the frequency of two haplotypes in Jewish patients with PV: [HLA-B38, SC21, DR4] and HLA-B35, SC31, DR4. Virtually all patients had one or the other of these haplotypes or their HLA-D region fragments (SC31, DR4, DQw8), (SC21, DR4, DQw8), or, rarely, DR4, DQw8 alone (17). The distribution of HLA-DR4 among Jewish patients with PV was consistent with dominant inheritance.

The present study reports MHC haplotypes in non-Jewish patients with PV. The frequencies of individual MHC alleles and extended haplotypes in patients are compared with family controls and overall normal Caucasian MHC haplotypes. We also report studies of subtypes of HLA-D region genes defined by restriction fragment length polymorphisms (RFLPs) in these non-Jewish patients.

METHODS

Patients and Samples. Blood samples were obtained from 25 randomly ascertained unrelated non-Jewish Caucasian patients with PV and their immediate family members. Complotypes were reported earlier in 23 of these 25 patients (12). Control homozygous cells for subtypes of DQw1 (DQw5 and DQw6), DQw3 (DQw7 and DQw8), and DRw6 (DRw13 and DRw14) were those of the Tenth International Histocompatibility Workshop (18) and included 9059 (U.S. Caucasian), 9063 (Italian), and 9062 (Dutch) for HLA-DR13, DQw6 as well as 9025 (Dutch) for HLA-DR4, DQw7 and 9026 (Ashkenazi Jewish), 9029 (Italian), and 9032 (Dutch) for DR4,

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Abbreviations: MHC, major histocompatibility complex; PV, pemphigus vulgaris; RFLP, restriction fragment length polymorphism.

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DQw8. Additional control cells for subtyping by RFLP were from healthy normal individuals of known ethnicity who carried serologically determined HLA-DRw6 and DQw1, HLA-DR4, and DQw3. Haplotype assignments were made from family studies, including the RFLP variants.

Clinical and Laboratory Criteria for Diagnoses. All patients had typical pemphigus lesions of skin or mucosa or both. The diagnosis was confirmed by direct and indirect immunofluorescence (19). Patients were questioned about the ethnicity and European country of origin of their grandparents. Only those with non-Jewish ancestry were included in this study as non-Jews except that one patient (1689) reported that one of her four grandparents was Jewish.

MHC Marker Studies. Assays for C4, BF, and C2 typing were done by methods previously described (20–22). HLA-A, -B, and -C antigens were detected by the standard N.I.H. lymphocyte microcytotoxicity assay (23), and HLA-DR and DQ typing was done according to the Oxford (7th International Histocompatibility) Workshop (24).

Haplotype Analysis. The haplotypes occurring in the propositi constituted the patient chromosomes. Haplotypes not occurring in patients but found in other family members constituted family control haplotypes (9). Randomly selected independent MHC haplotypes from normal European Caucasians were used as overall normal controls.

Extended MHC haplotypes were defined previously as HLA-B, complotype, HLA-DR allelic combinations exhibiting statistically significant linkage disequilibrium (6). There are about a dozen common extended haplotypes on normal Caucasian chromosomes.

DQB and DRB Subtypes. The cDNA probes used to study RFLP in DQB and DRB genes by agarose gel electrophoresis and Southern blotting were provided by the Tenth International Histocompatibility Workshop (25). The distinctions between HLA-DRw13, DQw5; DRw13, DQw6; DRw14, DQw5; and DRw14, DQw6 were made by *Taq* I digestion and probing with DRB and DQB. DRw14, DQw5 was detected by the presence of an 11.4-kilobase (kb) fragment using the DRB probe and by 5.3- and 2.0-kb *Taq* I fragments and a 5.8-kb *Bam*HI fragment using the DQB probe (26, 27). Assignments of DQw7 and DQw8 were made on the basis of the presence of the following fragments for DQw7: *Taq* I, 4.6 kb; *Bam*HI, 3.6 kb; *Kpn* I, 5.3 and 1.2 kb; and *Pvu* II, 3.2 kb; and for DQw8: *Taq* I, 1.9 and 2.6 kb; *Bam*HI, 10.3 kb; and *Kpn* I, 19.1 kb.

Analysis for Mode of Inheritance. To test the fit of the distribution of homozygotes and heterozygotes for individual markers to that predicted by a simpe dominant model of inheritance, calculations were made by methods recently described (17).

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 or Fisher's exact test. Nominal P values for comparisons of multiple variables were corrected by multiplication by the number of comparisons.

RESULTS

Allele Frequencies. MHC alleles and complotypes with frequencies in non-Jewish patients with PV significantly different from those in family or overall control chromosome populations or both are shown in Table 1. It is seen that there were increases in the frequencies with significant P values for HLA-Bw55, DRw6, and SB45 in patients compared with family or overall Caucasian controls (P = 0.04, 0.01, 0.02, 0.001—0.04, and 0.002, respectively). Although DQw1 would be expected to be significantly increased because of its strong linkage disequilibrium with HLA-DRw6, no significant increase in this allele was observed in comparison with either control population of chromosomes. There were no statistically significant increases in alleles increased among Ashkenazi Jewish patients with PV such as HLA-A26 or B38. HLA-DR4, on the other hand, was significantly increased, as it had been in Jewish patients, with P = 0.02 when compared with family control haplotypes. Family control haplotypes were significantly deficient in DR4 (and had an excess of DR5) compared with overall control haplotypes, suggesting ethnic differences between the study families and the overall controls.

Haplotype Associations with HLA-DR4. Among the non-Jewish patients, 13 of 50 patient chromosomes (26%) carried HLA-DR4, compared with 3 of 64 (5%) among non-Jewish family control chromosomes and 17.9% of overall normal Caucasian chromosomes. Table 2 shows the MHC alleles on each chromosome of the 25 patients. Of the 13 DR4-bearing chromosomes in patients, 3 were on haplotypes common in Ashkenazi Jews, particularly patients with PV, HLA-B35, SC31, DR4 (two examples), and [HLA-B38, SC21, DR4] (single example) and the rest had a variety of complotypes and HLA-B specificities. It is striking that, of 25 non-Jewish patients, all but 3 carried either HLA-DR4 or DRw6 or both. Similarly, all but 2 of 25 patients were positive for HLA-DQw1 or DQw3 or both.

Haplotype Associations with HLA-DRw6. Of the 50 non-Jewish patient haplotypes, 17 (34%) bore HLA-DRw6, DQw1 compared with 13 (26%) with HLA-DR4 and 20 (40%) with some other HLA-DR type. An unusual Caucasian haplotype, HLA-Bw55, SB45, DRw6, was found on 6 patient chromosomes of the 17 that bore HLA-DRw6, DQw1. The frequency of this haplotype among the patients was highly significantly increased ($P = 10^{-8}$) compared to the controls. Only 1 of 1999 normal Caucasian chromosomes carried this haplotype and the bearer was of Italian ethnicity.

Table 1. MHC allele and haplotype frequencies in non-Jewish patients with PV

Allele	Pt*		FC†		OC‡		P value§		
	No.	Freq.	No.	Freq.	No.	Freq.	Pt vs. FC	Pt vs. OC	FC vs. OC
Bw55	4	0.080	1	0.017	19	0.010	NS	0.002 (0.04)	NS
DR4	13	0.260	3	0.050	358	0.179	0.002 (0.02)	NS	<0.004 (0.03)
DR5	5	0.100	18	0.300	305	0.153	0.009 (NS)	NS	0.002 (0.02)
DRw6	17	0.340	8	0.133	198	0.099	0.010 (NS)	0.001 (0.01)	NS
*C4A*4*	9	0.180	1	0.017	141	0.070	0.003 (0.02)	0.003 (0.02)	NS
*C4B*5*	6	0.120	0	0.000	11	0.006	0.007 (0.04)	0.0001 (0.001)	NS
SB45	6	0.120	0	0.000	7	0.004	0.007 (NS)	0.0001 (0.002)	NS
Bw55, SB45, DRw6	6	0.120	0	0.000	1	0.0005	0.007 (NS)	10^{-9} (10^{-8})	NS

Pt, patient; FC, family control; OC, overall control; NS, not significant.
*n = 50.
†n = 60.
‡n = 1999.
§Nominal P values are given with corrected P values in parentheses.

Table 2. MHC haplotypes in non-Jewish patients with PV

Family no.	HLA A	C	B	Comp	DR	DQ	HLA A	C	B	Comp	DR	DQ
2014	2		w55	SB45	w6	w1	28		51	FC30	w6	w-
2001	11	w3	w55	SB45	w6	w1	11		7	SC31	w6	w1
1696	30	w3	w55	SB45	w6	w1	11	w3	w58	FC31	1	w1
1770	1		w55	SB45	w6	w1	26		w63	SC01	5	w3
1944	2		w55	SB45	w6	w1	3	w4	35	SC31	4	w3
1689	11	w3	w55	SB45	w6	w1	26		38	SC21	4	w3
2180	3		7	SC31	w6	w1	3		51	SC32	4	w3
2016	26		38	SC21	w6	w1	3	w4	35	SB42	5	w3
1698	11	w3	51	SC31	w6	w1	1		7	SC31	2	w1
1774	24		49	SC01	w6	w1	29	w6	13	SC31	7	w2
1683	26		38	FC31	w6	w1	1	w6	w57	SC61	4	w3
1739	1		8	FC31	w6	w1	25		18	SO42	2	w1
1945	24	w1	51	SC43	w6	w1	11	w4	35	SC30	1	w1
1695	2	w5	44	FC31	w6	w1	29		w58	FC31	w8	w-
1740	2	w5	44	SC30	w6	w1	1	w4	35	SC31	4	w3
1719	23	w6	45	SC2(1,17)	4	w3	2		7	SC31	2	w1
2007	2		7	SC10	4	w3	3		7	SC31	2	w1
1699	24	w6	w50	SC31	4	w3	2	w5	44	SC30	4	w3
1685	3		44	SC32	4	w3	31		51	SC31	5	w3
1688	3		7	SC31	4	w3	1		8	SC01	3	w2
1727	1		w41	SC31	4	w3	2	w6	37	FC31	1	w1
1750	1		51	SC32	4	w3	2	w4	35	SC31	5	w3
1738	2	w2	w61	SC31	5	w-	11	w4	35	SC30	1	w1
1759	29		44	FC31	7	w2	3		44	FC31	7	w2
1702	29		44	FC31	7	w2	3	w4	35	FC(3,2)0	2	w1

The haplotypes are arranged so that the HLA-DRw6 haplotypes and DR4 haplotypes are shown on the left, if they are present in heterozygotes. Alleles and possible segments of HLA-Bw55, SB45, DRw14, DQw5, [HLA-B38, SC21, DR4, DQw8], and HLA-B35, SC31, DR4, DQw8 are underlined. All underlined DRw6 were DRw14, all underlined DQw1 were DQw5, and all underlined DQw3 were DQw8 except for the second haplotype of subject 1699, which was DQw7 by RFLP analysis (see text and Tables 3 and 4). The presumed origin of the second haplotype in subject 1689 was a known Jewish grandfather. Comp, complotype.

HLA-DQ and DR Subtypes of Patient DRw6 and DR4 Haplotypes. Genomic DNA from the 25 non-Jewish patients was analyzed for RFLPs that distinguish DQw7 and DQw8 (subtypes of DQw3), DQw5 and DQw6 (subtypes of DQw1), as shown in Fig. 1, and DRw13 from DRw14 (subtypes of DRw6), as shown in Fig. 2. From these analyses (Table 3), it was clear that all instances of DRw6 (including all examples of the haplotype HLA-Bw55, SB45, DRw6) were DRw14, DQw5 (11/11). On the other hand, of 18 non-Jewish control DRw6 haplotypes, only 5 were DRw14, DQw5 (Table 3).

As shown in Table 4, 12 of 12 non-Jewish patient DR4 haplotypes carried DQw8. The single exceptional patient with HLA-DR4, DQw7 had DR4, DQw8 on the other chromosome. In contrast, 8/22 or 36% of non-Jewish control DR4 haplotypes carried DQw7 rather than DQw8 specificities.

Ethnicity of Patient and Control Haplotypes. Of 50 non-Jewish patient haplotypes, 27 were Southern European or Middle Eastern (17 Italian, 4 Portuguese, 3 Armenian, 4 Lebanese) and 23 were Northern European (German, English, Irish, Polish, Austrian, Scottish, Russian, Swedish, Dutch, and French). This ethnic distribution was significantly different ($P = 0.004$) from that of 241 non-Jewish control haplotypes for which ethnicity was known (78 Southern European, 163 Northern European). Most (4 of 6) instances of the DRw6-bearing haplotype HLA-Bw55, SB45, DRw6 were in patients of Italian ancestry. There were, however, no ethnic differences between overall DR4 and DRw6 haplotypes.

Other D-region RFLPs. We did not detect the following bands in our patients or controls using the DQB probe: *Bam*HI, 8.3 and 2.5 kb; *Pvu* II, 6.9 kb; and *Pst* I, 12.0 and 5.3 kb reported to be associated with HLA-DRw6 in studies of patients with PV (16).

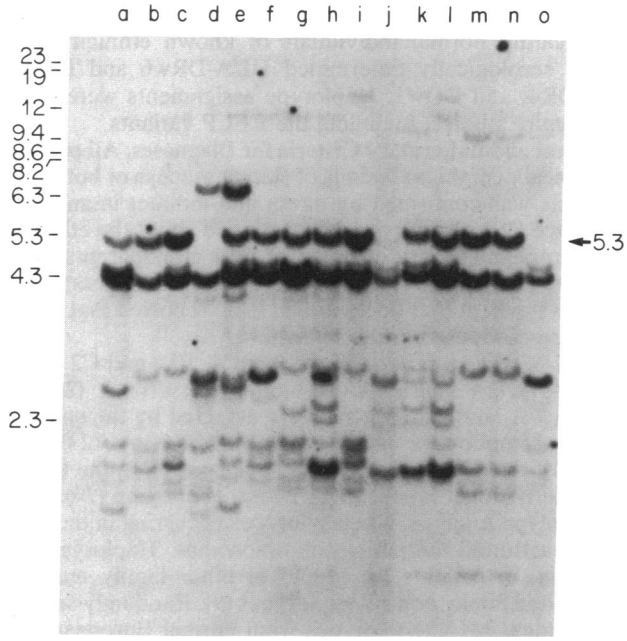

FIG. 1. Autoradiograph of RFLP patterns in non-Jewish patients with PV and DRw6, DQw1. DNA was digested with *Taq* I and the membrane was probed with a DQB cDNA probe. Note the 5.3-kb band characteristic of DQw5. Lanes a–c and e–i, patients; lanes d and j–l, DRw6, DQw1 normal non-Jewish controls; lanes m–o, consanguineous homozygous normal controls. Samples are as follows: DRw14, DQw5/DRw14, DQw- (lane a); DRw14, DQw5; DR1, DQw5 (lanes b and c); DR14, DQw6/DRw15, DQw6 (lane d); heterozygotes for DRw14, DQw5 and DR7, DQw2 (lanes e and f); DRw16, DQw5 (lane g); DR5, DQw3 (lane h); DR4, DQw8, DRw15, DQw6 (lane i); and DR4, DQw8 (lane l); DRw13, DQw6/DR4, DQw8 (lane j); DRw13, DQw5/DR4, DQw8 (lane k); homozygous DRw13, DQw5 (lanes m and n); and homozygous DRw13, DQw6 (lane o). Sizes are given in kb.

Analyses for Mode of Inheritance. Analysis of DR4, DRw6, and DR4 + DRw6 distributions among the non-Jewish pem-

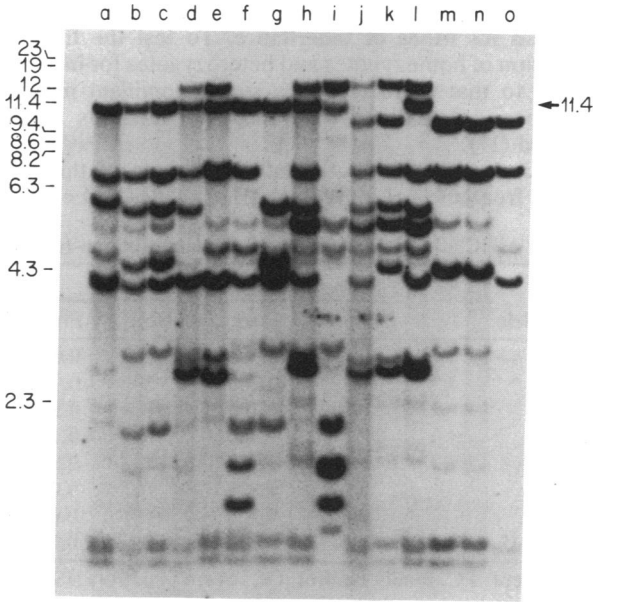

FIG. 2. Lanes and samples as in Fig. 1, except that the probe was DRB. Note the 11.4-kb band characteristic of DRw14, DQw5. Sizes are given in kb.

Table 3. HLA-DR, DQ subtypes on haplotypes carrying HLA-DRw6, DQw1

Haplotype	Non-Jewish patient				Non-Jewish control			
	DRw14, DQw5	DRw14, DQw6	DRw13, DQw5	DRw13, DQw6	DRw14, DQw5	DRw14, DQw6	DRw13, DQw5	DRw13, DQw6
HLA-Bw55, SB45, DRw6	4	0	0	0	0	0	0	0
Other DRw6	7	0	0	0	5	4	6	3
All DRw6	11	0	0	0	5	4	6	3

phigus patients revealed that neither recessive nor dominant inheritance could be excluded and that the data were consistent with either.

DISCUSSION

Our previous studies of Jewish patients with PV revealed that MHC-mediated susceptibility was almost invariably in one of two closely related, dominantly expressed DR4, DQw8-containing extended, [HLA-B38, SC21, DR4], or presumably extended, HLA-B35, SC31, DR4, haplotypes. In the present study of non-Jewish patients with PV, what may be the D-region fragments of these same haplotypes and rarely the complete haplotypes accounted for 26% of patient chromosomes. Evidence for similarity and possibly common origin of these DR4 haplotypes in Jewish and non-Jewish patients was obtained by the finding that 12 of 12 non-Jewish patient DR4 haplotypes carried DQw8. The possible exception was a patient who was homozygous for HLA-DR4 but heterozygous for DQw7 and DQw8 so that there was in fact no exception. An additional 34% of haplotypes in non-Jewish patients carried the HLA-DRw14 subtype of DRw6, all with DQw5. In all, 22 of 25 non-Jewish patients carried DRw14, DQw5 or DR4, DQw8 or both. Of the 17 HLA-DRw14-bearing haplotypes, 6 were a possible extended haplotype, HLA-Bw55, SB45, DRw14. Since the non-Jewish patients were of a more Southern European origin than our control non-Jewish haplotypes and 4 of the 6 patient instances and the one normal instance of HLA-Bw55, SB45, DRw14 were of Italian origin, it is likely that the DRw6-related susceptibility gene arose in or near Italy. It is of interest that the frequency of DRw6 among our normal Italian control haplotypes was higher than our overall control haplotypes ($P_{nominal} = 0.03$) (unpublished observations) but considerably lower than in our non-Jewish pemphigus patients. A common origin for the DRw14 among patients is supported by the fact that all 11 such haplotypes among non-Jewish patients (and the 2 found among Jewish patients previously) carried DQw5, as did all instances of HLA-Bw55, SB45, DRw14.

These data suggest that there are two major susceptibility genes for PV in European Caucasians, one associated with the DR4, DQw8 and the other associated with the DRw14, DQw5 portions of the respective extended or presumably extended haplotypes. The first may be the more ancient since it has spread considerably to non-Jews and may have arisen on a precursor of [HLA-B38, SC21, DR4] and HLA-B35, SC31, DR4. The other, more recent gene, appears to have arisen on HLA-Bw55, SB45, DRw14 in Southern Europe.

An analysis of the distribution of homozygotes and heterozygotes for DR4, DRw6, or both did not allow a definitive indication of the mode of inheritance of the MHC susceptibility gene for pemphigus among non-Jewish pemphigus patients. This was undoubtedly related to the fact that there were two major susceptibility alleles in this population of patients rather than one as among the Jewish patients. It is most likely that inheritance is dominant as it is in the Jews, although demonstration of this may be difficult.

In addition to the two common susceptibility genes associated with DR4 and DRw14, it is clear that there is a rarer susceptibility allele on the extended haplotype [HLA-B44, FC31, DR7, DQw2] since one of the non-Jewish patients was a homozygote and another was a heterozygote for this haplotype (with DR2, DQw1 on the other chromosome). Furthermore, the one Jewish patient who had neither DR4 nor DRw6 had DR7 (and DR2).

Our prior finding that the complotype SB45 was increased in non-Jewish patients (12) is now entirely explainable. It is a marker for HLA-Bw55, SB45, DRw14, DQw5.

The previously noted (13–15) associations of HLA-DR4 and DRw6 with PV are here put into perspective, particularly with respect to ethnic specificity and relationship to known or presumed extended haplotypes. We could not confirm the presence of unique pemphigus-related DQB restriction fragments even though we used the enzymes reported to detect such fragments, *Pvu* II, *Pst* I, and *Bam*HI, associated with DRw6, as reported by Szafer and co-workers (16). Moreover, a 6.1-kb *Eco*RV fragment found by these authors in none of 16 patients was found by us in 10 of 25 patients. These discrepancies may relate to differences in the cDNA probes used or to a relative rarity of DRw14, DQw5 and HLA-Bw55, SB45, DRw14 among Jewish patients and controls or to ethnic differences between Israeli and American Ashkenazi Jews. Particularly puzzling is the high frequency of DRw6 among the Israeli patients in contrast to our low frequency.

The finding (28) that there are identical DRBI sequences in 90% of DR4-positive pemphigus patients of unspecified but presumably Jewish ethnicity is entirely consistent with the identity of much, if not all, of the HLA-D region on the complete or partial extended haplotypes with HLA-DR4 that we observed in our previous study. We expect that most, if

Table 4. HLA-DQ subtypes on haplotypes carrying HLA-DR4, DQw3

Haplotype or fragment	Non-Jewish patient		Non-Jewish control		Jewish patient		Jewish control	
	DQw7	DQw8	DQw7	DQw8	DQw7	DQw8	DQw7	DQw8
[HLA-B38, SC21, DR4]	0	1	0	2	0	10	0	15
(SC21, DR4)	0	1	0	2	0	2	2	1
HLA-B35, SC31, DR4	0	1	0	0	0	1	4	1
(SC31, DR4)	0	6	3	1	0	2	0	2
DR4	0	2	5	9	0	1	1	6
[HLA-B44, SC30, DR4]	1	0	7	0	0	0	4	0
[HLA-Bw62, SC33, DR4]	0	0	0	7	0	0	0	0
Total	1	11	15	21	0	16	11	25

Data from the Jewish patients are those from a previous study (17).

not all, D-region sequences in DR4, DQw8-positive patients will be identical to those of the same extended haplotypes in normal Jews. Finding DQB hypervariable region identity does not at all mean that DQB is "the" susceptibility gene. The finding that the DRw6-associated DRB sequences are different from those associated with DR4 is not surprising. However, the fact that the susceptibility DRw6 allele was strongly associated with *DQB1.3* (or DQw5, as we have confirmed in the present study) is not directly related to susceptibility since DQw5 may also be associated with other DR specificities not increased in pemphigus patients, such as DR1 and DR2. Similarly, DQw8 is not in itself a susceptibility allele since extended haplotypes with DQw8, such as [HLA-Bw62, SC33, DR4], are not increased among patients with PV. The findings of Sinha and colleagues of a DRw6 pemphigus-associated DQB allele (29) by nucleotide sequencing in the hypervariable regions can be interpreted similarly. One would expect this finding to correlate with the DRw14, DQw5 of patients and of the rare normal controls with the HLA-Bw55, SB45, DRw14, DQw5 haplotype. Since it is likely that the entire HLA-B to DQ interval is fixed on the HLA-Bw55, SB45, DRw14, DQw5 haplotype or its class II region in the case of segments, it is also likely that the same or similar hypervariable regions as occur on these haplotypes were described by Scharf and co-workers (28) and Sinha and colleagues (29). Because of this fixity it is not possible to determine which class II or class II-like gene is the susceptibility allele.

Our observations suggest that neither we nor any other workers have identified a pemphigus MHC susceptibility allele. Our results provide suggestive evidence that there are two common ethnically restricted, class II genes or genes closely linked to class II genes (because D-region segments of susceptibility haplotypes are found in excess in patients) that mediate susceptibility in a dominant fashion. Because extended haplotypes have fixed alleles, it is not possible at this point to determine which gene(s) in the HLA-DR, DQ region is responsible.

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