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Immunology

Major histocompatibility complex haplotype studies in Ashkenazi Jewish patients with pemphigus vulgaris

(extended haplotypes/ethnicity/autoimmune susceptibility gene)

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ABSTRACT **Of 26 Ashkenazi Jewish patients with pemphigus vulgaris, 24 (92.3%) carried the major histocompatibility complex (MHC) class II alleles HLA-DR4, DQw3, of which all were of the subtype DR4, DQw8. From studies of the patients and their families, haplotypes were defined. It was found that, of the patients who carried HLA-DR4, DQw8, 75% carried one or the other (and in one case, both) of two haplotypes [HLA-B38, SC21, DR4] or HLA-B35, SC31, DR4. The former is a known extended haplotype among normal Jews, with a frequency of 0.102, and the latter may also be an extended haplotype in this ethnic group, with a frequency of 0.017 among normal haplotypes from Jews. Of the remaining DR4-positive patients, all but one had a presumed D-region segment (defined as SC21, DR4, DQw8 or SC31, DR4, DQw8 with variable HLA-B) of these haplotypes. Only one patient had DR4, DQw8 without any other markers of the extended haplotypes. The number of homozygotes and heterozygotes for DR4, DQw8 was consistent with dominant but not recessive ($P < 0.01$) inheritance of a class II or a class II-linked susceptibility gene for the disease. Since the disease is entirely attributable to the presence of an antibody to an intraepidermal intercellular cement substance, it is likely that the class II susceptibility gene (on [HLA-B38, SC21, DR4, DQw8], HLA-B35, SC31, DR4, DQw8, or their segments, in Jewish patients) controls the production of the antibody as a dominantly expressed immune response gene.**

Pemphigus vulgaris is a rare blistering autoimmune disease that affects the skin and mucous membranes. Patients have circulating antibody to an intercellular cement substance, and deposition *in vivo* of this antibody is a hallmark of the disease (1). The antibody appears to be pathogenetic, since newborn infants of mothers with pemphigus may have blisters (2), and newborn mice injected with the antibody from patients have clinical pemphigus (3). The disease is reported to have a particularly high incidence among Jews.

A number of autoimmune diseases, including pemphigus vulgaris, are associated with HLA and other major histocompatibility complex (MHC) alleles. Specific alleles have higher frequencies (presumed susceptibility alleles or markers for these alleles), whereas others have lower frequencies (presumed protective alleles or markers) compared with frequencies in ethnically matched control populations. In general, the reasons for these associations are unknown.

Previous studies have focused on class II or HLA-D region (HLA-DR, -DQ, and -DP) (4–6) alleles as possible susceptibility genes. Genes of this region encode transmembrane glycoproteins involved in the immune response to soluble protein antigens that exhibit marked genetic polymorphism differing strikingly among ethnic groups, even within the major races. There are four polymorphic genetic loci, *C2*, *BF*, *C4A*, and *C4B*, that control the synthesis of three complement proteins, C2, factor B, and C4. The four complement genes are inherited and occur in populations as single genetic units called complotypes, designated in arbitrary order by their *BF*, *C2*, *C4A*, *C4B* alleles. At least a third of MHC haplotypes in Caucasians consist of specific HLA-B, complotype, HLA-DR allelic sets in frequencies significantly higher than those predicted by the frequencies of their individual alleles, and their components are thus said to show positive linkage disequilibrium. There are a dozen or more of these frozen or "extended haplotypes," and considerable evidence suggests that, at least for the chromosomal region between HLA-B and HLA-DR, intervening DNA is similarly fixed on all instances of such haplotypes in unrelated persons (7, 8).

It has been presumed that most MHC allele–autoimmune disease associations result from certain class II molecules somehow specifying an abnormal immune response which in turn leads to the disease. In this view, the associations of class I or complement alleles with a disease is explained by their known linkage disequilibrium with class II alleles (9). Our view is different. Since there is fixity of alleles controlling the synthesis of specific proteins (10–12) and functions (13) on extended haplotypes, we conclude that there is also fixity of disease-susceptibility alleles. From family studies of several diseases, we have found that the increase in frequency of a number of specific alleles is secondary to increases in extended haplotypes (14–16).

Because of the fixity of alleles on extended haplotypes, it is not possible to know which specific genes are involved in disease susceptibility, and all markers of associated extended haplotypes are possible candidates. On the other hand, information on the nature of susceptibility genes can be obtained by taking into account which derivative segments of extended haplotypes (class I–complotype, complotype–class II, or individual alleles alone) are increased among patients.

In studies of unrelated Jewish patients with pemphigus vulgaris, significant increases were found in the frequencies of HLA-A26 (17), HLA-B38 (17–19), HLA-DR4 (17, 18, 20, 21), and the complotype SC21 (22). These findings suggested that the extended haplotype [HLA-(A26), B38, SC21, DR4] might be the marker for pemphigus vulgaris in Jews. The present study was designed to provide extended MHC haplotype analysis in Ashkenazi patients with pemphigus vulgaris.

METHODS

Blood samples from 26 random unrelated Ashkenazi Jewish patients with pemphigus vulgaris and their families were

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

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collected into 7-ml Vacutainer tubes (Becton Dickinson) containing 10.5 mg of potassium EDTA and into a heparinized syringe containing 500–1000 units of sodium heparin and diluted with an equal volume of RPMI 1640 medium.

The diagnosis of pemphigus vulgaris was established by the presence of mucocutaneous blisters and the deposition of IgG antibody in the intraepidermal intercellular cement substance in biopsy samples of perilesional skin (23). Studies of the MHC in the patients presented in this paper have not been previously reported except for the complotypes (22) in 25 of 26.

Typing for HLA-A, -B, -C, -DR, and -DQ was by the microlymphocytotoxicity assay (24, 25). *BF* (26), *C4* (27) and *C2* (28) genetic typings were performed by previously described methods. Complotypes are given in arbitrary order as *BF*, *C2*, *C4A*, and *C4B* alleles in abbreviated form. Thus, "SC01" denotes *BF*S*, *C2*C*, *C4A*Q0*, *C4B*1* (29).

Control homozygous cells for subtypes of DQw3 (DQw7 and DQw8) were those of the Tenth International Histocompatibility Workshop (30) and included cell lines 9025 (Dutch) for HLA-DR4, DQw7 and 9026 (Ashkenazi Jewish), 9029 (Italian), and 9032 (Dutch) for HLA-DR4, DQw8. Cell line 9026 and two of our own cell lines were from Ashkenazi Jewish persons homozygous for [HLA-B38, SC21, DR4, DQw8]. Additional control haplotypes for subtyping of DR4 by restriction fragment length polymorphism (RFLP) were obtained from healthy normal individuals of known ethnicity who carried DR4. Haplotype assignments were made from family studies, including the RFLP variants.

A cDNA probe provided by the Tenth International Histocompatibility Workshop (31) was used to study RFLP in DQB genes. 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; and for DQw8: *Taq* I 1.9 and 2.6 kb, *Bam*HI 10.3 kb, and *Kpn* I 19.1 kb (32).

Control haplotypes for statistical analysis were those that occurred in normal families (no MHC-associated diseases) and in healthy individuals from family studies if the haplotypes did not occur in patients (14). Patients and controls were from families where all four grandparents were Jewish and claimed Eastern or Central European ancestry. There were 118 control and 52 patient haplotypes.

To test the fit of the distribution of homozygotes and heterozygotes for individual markers to that predicted by a simple dominant model of inheritance, the following calculations were made. The observed frequency among patients of any allele A is equal to ½ the sum of the "true disease frequency" (TDF_A) + the frequency in matched normal controls (NF_A), allowing one to solve for TDF_A. The predicted frequency of homozygotes for A equals $TDF_A \times NF_A$, whereas the frequency of homozygotes for all alleles except A equals $[(1 - TDF_A) \times (1 - NF_A)]$. The frequency of heterozygotes for A and all other alleles equals $[TDF_A \times (1 - NF_A) + (1 - TDF_A) \times NF_A]$. For recessive inheritance of an MHC allele A as a marker for disease susceptibility, the frequency of homozygotes is expected to be equal to a (the allele frequency of A) squared, the frequency of homozygotes for all alleles other than A equals $(1 - a)^2$, and the frequency of heterozygotes is expected to equal $2a(1 - a)$. Some individuals gave single DR and DQ typing responses and did not receive the allele from a heterozygous parent or pass it on to a heterozygous child. It was therefore not possible to be certain in such cases whether these individuals were homozygotes for the specificity or heterozygotes for the specificity and a specificity not detected ("blank"). All such cases were considered homozygous for the detected specificity because this was the most likely assignment and because such an assignment was most conservative with respect to favoring recessive and rejecting dominant inheritance. Such assignments are indicated by parentheses in Table 2.

Significance of the differences between groups and deviation of observed homozygote/heterozygote distributions from those predicted by recessive or dominant inheritance was determined by χ^2 analysis or Fisher's exact test.

RESULTS

The frequencies of HLA-A26, B38, and DR4 were significantly increased among patients compared with those of a population of normal haplotypes from Jews, as shown in Table 1. In addition, as previously reported for most of these patients, there was a significant increase in the complotype SC21 and a decrease in SC01. Of other DR alleles, DRw6 showed a mild but insignificant increase and DR2 and DR7 had frequencies similar to those of normal Jews. The remaining major HLA-DR alleles, DR1, DR3, and DR5, showed marked reductions in frequency compared to Jewish controls.

Table 2 shows the haplotypes in Jewish patients with pemphigus vulgaris, arranged so that one can judge the presence of the extended haplotype [HLA-B38, SC21, DR4], the possible extended haplotype HLA-B35, SC31, DR4, or their segments. It is clear that the majority of patients carry one or the other of these haplotypes and a few carry the complotype-DR markers as a presumed D-region segment of the extended haplotypes, but only one patient had DR4, DQw3 alone. No instances of the general DR4-carrying Caucasian extended haplotypes [HLA-B44, SC30, DR4] or [HLA-Bw62, SC33, DR4] were found among the patients. Although extended haplotypes are defined by HLA-B, complotype, and DR alleles, HLA-A26 occurs so frequently on both haplotypes in patients that it is clearly part of their fixed regions.

It can be seen from the list of patient haplotypes given in Table 2 that the alleles showing significantly increased frequencies in Table 1 were often all found together on the extended haplotype [HLA-B38, SC21, DR4], occurring on 14 of 52 patient haplotypes. A second haplotype, HLA-B35, SC31, DR4, not previously identified by analysis of three-point linkage disequilibrium as an extended haplotype in any ethnic group, perhaps related to its relatively low normal

Table 1. Common MHC allele frequencies in patients and controls

Allele	Frequency: In patients (haplotype n = 52)	Frequency: In controls (haplotype n = 118)	χ^2	P
A1	0.118	0.144		n.s.
A2	0.137	0.212		n.s.
A26	0.392	0.144	12.8	0.0001
B35	0.231	0.161		n.s.
B38	0.327	0.136	8.4	0.004
DR1	0.039	0.144		n.s.
DR2	0.077	0.102		n.s.
DR3	0.019	0.068		n.s.
DR4	0.519	0.212	16.1	0.0001
DR5	0.135	0.254		n.s.
DRw6	0.077	0.034		n.s.
DR7	0.135	0.136		n.s.
SC31	0.365	0.390		n.s.
SC21	0.404	0.229	5.5	0.02
SC01	0.000	0.068		0.05*

Only HLA-A and B alleles with frequencies in excess of 0.10 and selected complotypes in excess of 0.02 in either patient or control populations are shown. The frequencies of DR1–7 are all shown. n.s., Not significant.

*Estimated by Fisher's exact test.

Table 2. Haplotypes in Jewish patients with pemphigus vulgaris, highlighting the alleles of [HLA-A26, B38, SC21, DR4] and HLA-A26, B35, SC31, DR4

Family no.	HLA A	HLA B	Complo-type	DR	DQ	HLA A	HLA B	Complo-type	DR	DQ
1799	1	58	SC21	4	w3	11	52	SC(3,2)0	2	w1
1716	2	27	SC21	4	w3	3	18	SB41	w6	w1
1772	30	38	SC21	4	w3	32	44	SC42	5	w3
1789	26	38	SC21	4	w3	1	8	SC31	2	w1
1725	26	38	SC21	4	w3	30	13	SC31	7	w2
1778	26	38	SC21	4	w3	24	14	SC2(1,2)	1	w1
2005	26	38	SC21	4	w3	26	27	SC31	5	w3
1724	26	38	SC21	4	w3	28	14	SC2(1,2)	1	w1
1703	26	38	SC21	4	w3	30	13	SC31	7	w2
1793	26	38	SC21	4	w3	26	38	SC21	w6	w1
2370	26	38	SC21	4	w3	24	38	SC21	w6	w1
1906	26	38	SC21	4	(w3)	1	35	SC31	5	(w3)
2002	26	38	SC21	4	w3	3	41	FC31	4	w3
1807	26	38	SC21	4	(w3)	26	38	SC31	(4)	(w3)
1797	26	38	SC21	(4)	(w3)	26	35	SC31	(4)	(w3)
1773	26	35	SC31	4	w3	2	51	SC31	2	w1
1749	26	35	SC31	4	w3	1	35	SC31	5	w3
1946	26	35	SC31	4	w3	2	50	S1C2(1,17)	7	w2
1757	26	35	SC31	4	w3	24	35	SC31	5	(w3)
1767	1	35	SC31	4	(w3)	2	44	SC32	5	(w3)
1766	1	44	SC31	4	w3	28	14	SC2(1,2)	7	w2
1796	2	44	SC31	4	w3	24	35	FC21	3	w2
1769		41	SC31	4	w3	9	35	SC31	5	w3
1763	3	14	SC2(1,2)	4	w3	2	44	SC31	7	w2
1795	11	52	SC(3,2)0	2	w1	26	38	S1C2(1,17)	7	w2
1771	2	35	SB41	w6	w1	25	18	S042	7	w2

The haplotypes are arranged with the DR4-bearing haplotype first, when present. Elements of the two specific HLA-DR4 extended or presumed extended haplotypes are underlined. DR and DQ types given in parentheses could not be unambiguously assigned.

frequency and common alleles, was found on haplotypes carried by an additional 6 patients. These two haplotypes or portions of them as SC21, DR4, DQw3 or SC31, DR4, DQw3 (with HLA-B different from B38 or B35) or DR4, DQw3 (with a complotype other than SC31 or SC21) were carried by 24 of the 26 patients.

Of the 24 patients positive for DR4, 3 were homozygotes and 21 were heterozygotes. One homozygote was heterozygous for [HLA-B38, SC21, DR4] and for HLA-B35, SC31, DR4; one was heterozygous for [HLA-B38, SC21, DR4] and HLA-B41, FC31, DR4 (nonextended and not a recognizable portion of an extended haplotype); the third was heterozygous for [HLA-B38, SC21, DR4] and HLA-B38, SC31, DR4 (presumed D-region segment of HLA-B35, SC31, DR4). The noncarriers of DR4 were DRw6/DR7 and DR2/DR7.

The frequencies of the two DR4, DQw3-containing haplotypes and their presumed segments in patients and Jewish controls are shown graphically in Fig. 1 and compared statistically in Table 3. It is evident that the intact haplotypes as well as complotype-DR4, DQw3-containing segments but not DR4, DQw3 alone (without SC21 or SC31) were increased among patients compared to controls. However, the increases in the complotype-DR, DQ portions were not significant when considered separately but were when combined with the complete haplotypes.

To determine whether the DQw3 found on patient DR4 haplotypes was of the subtypes DQw7 or DQw8, genomic DNA from patients and their immediate family members was digested separately with *Taq* I, *Bam*HI, *Kpn* I, or *Pvu* II and then subjected to Southern blot hybridization with a labeled DQB probe after agarose gel electrophoresis. DQw7 or DQw8 assignments were made from the presence or absence of fragments of specific size. Fig. 2 shows a representative

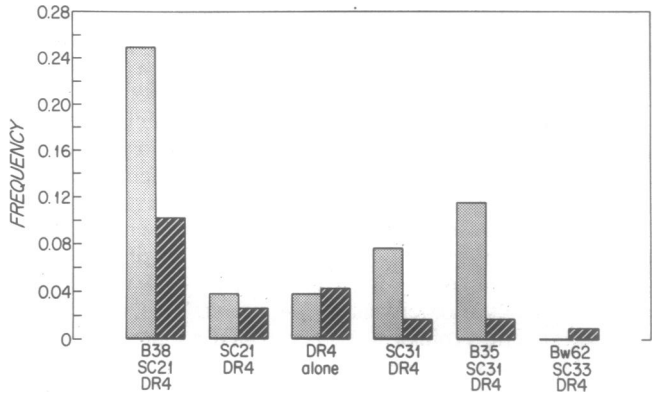

FIG. 1. Comparison between the frequencies of the extended haplotype [HLA-B38, SC21, DR4], its D-region segment SC31, DR4, the presumed extended haplotype HLA-B35, SC31, DR4, and its segment, SC31, DR4, among patient haplotypes on the left (stippled bars) and among normal control haplotypes from normal Jews on the right (hatched bars).

autoradiograph of such an analysis. Table 4 summarizes the results of assigning DQw7 and DQw8 specificities to DR4, DQw3 haplotypes in the patients and in Jewish controls. It is clear that every DR4, DQw3 haplotype among the patients was DQw8, whereas around 30% of DR4, DQw3 haplotypes among Jewish controls were DR4, DQw7. Although every example of [HLA-B38, SC21, DR4] carried DQw8 among Jewish controls (as well as patients), HLA-B35, SC31, DR4 usually carried DQw7 in Jewish controls, in contrast to DQw8 in patients. Four examples of the extended haplotype [HLA-B44, SC30, DR4] were found among Jewish controls but none among patients. All carried DQw7.

The results of analysis for mode of inheritance did not allow rejection of either mode of inheritance from the distributions of HLA-A26, HLA-B38, SC21, SC31, or DQw3 (or DQw8). The distribution of homozygotes for HLA-DR4, DQw8, on the other hand, allowed clear-cut rejection of recessive inheritance for HLA-DR4, DQw8 (or DR4, since all DR4 was linked with DQw8) and hence was consistent with dominant inheritance (Table 5).

DISCUSSION

All of the previously noted individual MHC markers found to be elevated in Jewish patients with pemphigus vulgaris, HLA-A26, B38 (17–19), DR4 (20, 21), and SC21 (22) can now be attributed to the increase among patients of two closely related haplotypes. One of these is an extended haplotype already known to be characteristic of normal Ashkenazi Jewish populations, [HLA-B38, SC21, DR4]; the other, HLA-B35, SC31, DR4, may also be an extended haplotype, and it contributes to the HLA-DR4 increase among patients.

Table 3. Frequencies of [HLA-B38, SC21, DR4], HLA-B35, SC31, DR4, and their D-region segments in Jewish patients with pemphigus vulgaris and Jewish controls

Haplotype or fragment	Frequency: In patient haplotypes	Frequency: In control haplotypes	*P*
[HLA-B38, SC21, DR4]	0.250	0.102	0.012
SC21, DR4	0.038	0.025	n.s.
[HLA-B38, SC21, DR4] + SC21, DR4	0.288	0.127	0.011
HLA-B35, SC31, DR4	0.115	0.017	0.011
SC31, DR4	0.077	0.017	n.s.
HLA-B35, SC31, DR4 + SC31, DR4	0.192	0.025	0.001

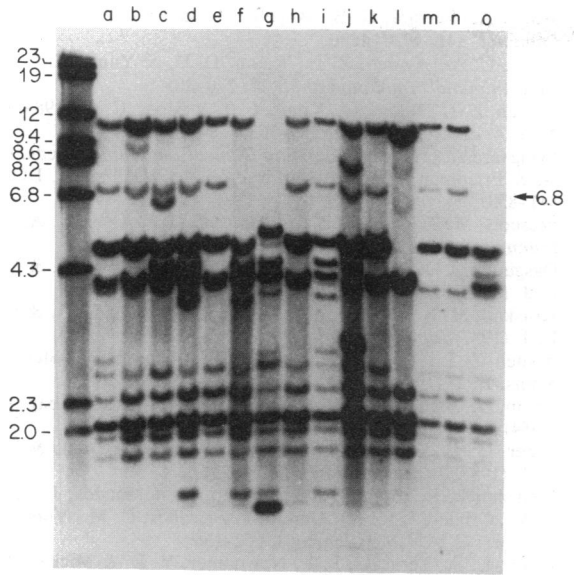

FIG. 2. Autoradiogram of *Pst* I RFLP patterns of DQB in pemphigus vulgaris Jewish patients who carry HLA-DR4, DQw3. Note the 6.8-kb band characteristic of DQw8. Lanes a–e are from patients; lanes f–k are from normal Jewish controls. Lanes l–o are from homozygous normal general controls. The leftmost lane contains size markers (kb). The haplotypes are as follows:

a.	A26	B38	SC21	DR4	DQw8	A26 B35	SC31	(DR4)	DQw8
b.	A26	B35	SC21	DR4	DQw8	A2 B51	SC31	DR2	DQw1
c.	A26	B35	SC31	DR4	DQw8	A2 Bw50	S1C2(1,17)	DR7	DQw2
d.	A26	B38	SC21	DR4	DQw8	A24 B14	SC2(1,2)	DR1	DQw1
e.	A26	B38	SC21	(DR4)	DQw8	A26 B38	SC31	(DR4)	DQw8
f.	A26	B35	SC31	DR4	DQw7	A3 B18	SB41	DRw6	DQw1
g.	A2	B35	SC31	DR4	DQw7	A24 B27	SC31	DR5	DQw7
h.	A26	B38	SC21	DR4	DQw8	A24 B35	SC31	DR5	DQw7
i.	A26	B38	SC21	DR4	DQw8	A33 B14	SC30	DR1	DQw1
j.	A24	B35	SC31	DR4	DQw8	A2 B8	SC01	DR3	DQw2
k.	A26	B38	SC21	DR4	DQw8	A21 B44	SC30	DR4	DQw7
l.	A31	B35		DR4	DQw7				
m.	A23	Bw65		DR4	DQw8				
n.	A26	B38	SC21	DR4	DQw8				
o.	A2	B44		DR4	DQw7				

In addition to the intact haplotypes, which account for most (70.3%) of the HLA-DR4-bearing haplotypes of patients, segments with SC21 or SC31 make up an additional 22.2% of HLA-DR4, DQw8-bearing haplotypes. Only 1 of the DR4-bearing patients has no HLA-B or complotype markers of the two related extended or presumably extended haplotypes on the DR4 haplotype. The susceptibility gene may have arisen, possibly by mutation or gene conversion, on a common ancestor of [HLA-(A26), B38, SC21, DR4] and HLA-(A26), B35, SC31, DR4. Alternatively, this event occurred independently on each of the haplotypes after they diverged. We prefer the first, simpler, interpretation. If so,

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

	No. Jewish patients		No. Jewish controls	
Haplotype or fragment	DQw7	DQw8	DQw7	DQw8
[HLA-B38, SC21, DR4]	0	10	0	15
SC21, DR4	0	2	2	1
HLA-B35, SC31, DR4	0	1	4	1
SC31, DR4	0	2	0	2
DR4	0	1	1	6
[HLA-B44, SC30, DR4]	0	0	4	0
[HLA-Bw62, SC33, DR4]	0	0	0	0
Total	0	16	11	25

Table 5. Distribution of homozygotes and heterozygotes for HLA-DR4 and non-DR4 in relation to mode of inheritance

HLA-DR4	No. observed	No. expected for dominant	χ^2	P	No. expected for recessive	χ^2	P
Homozygotes	3	3.6	0.52		7.0	2.28	
Heterozygotes	21	20.5	0.00		13.0	4.92	
Non-HLA-DR4	2	1.9	0.72		6.0	2.67	
			$\Sigma = 1.24$	n.s.		$\Sigma = 9.87$	<0.01

the two haplotypes may share similar or identical class II regions in general and thus the susceptibility allele specifically. Our data suggest but certainly do not demonstrate that the susceptibility gene is in the class II region of the MHC because most portions of the extended or presumed extended haplotypes found in patients have DR4, and few have only the telomeric markers HLA-A26, B38, or 35 and SC21 or SC31 without DR4. Since all DR4 haplotypes have DQw3, this marker alone is not informative in our study. The demonstration that all DR4 in patients is linked to DQw8, whereas around 30% of DR4 is linked to DQw7 in normal Jews, is consistent with a single major DR4, DQw8-region susceptibility gene for pemphigus vulgaris among Jewish patients. We cannot, however, further localize this gene.

The fact that one or the other of two closely related haplotypes is found in almost every Jewish patient with pemphigus and that there is a relative dearth of homozygotes suggests the possibility that MHC-mediated pemphigus susceptibility is expressed as a dominant trait. Formal analysis for conformity to numbers of homozygotes and heterozygotes for DR4, DQw8 predicted by recessive and dominant inheritance confirms this impression. Because we studied families, it was not necessary to analyze our data by the Thomson–Bodmer method, devised for phenotype data (33).

Our evidence that the MHC susceptibility gene for pemphigus vulgaris in Jews is inherited as a dominant trait is entirely consistent with its being an immune response gene for the intraepidermal intercellular cement substance antigen. Certainly, in experimental animals, immune responses are determined by dominantly expressed class II genes (34). Our own observations suggest that this is also true for the human immune response to the hepatitis B surface antigen (35). What is unclear is whether the class II molecules in the DR/DQ region of [HLA-B38, SC21, DR4] and HLA-B35, SC31, DR4 have identical structures in patients with pemphigus compared with the same haplotypes in normal individuals. If so, genes at another locus (loci) or relatively rare environmental factors must participate to produce disease, since one almost never finds more than one patient in a family and these haplotypes are common among healthy Jews.

Our results are related to those of Szafer *et al.* (36), who found common restriction fragments in *DQB* genes of DQw1 and DQw3 in DRw6- and DR4-positive Jewish Israeli and non-Jewish Austrian patients with pemphigus. Their finding of increased HLA-DR4 among Israeli Jews confirmed our previous observation and those of others in American Jews (19–21). However, we have not found a significant increase in HLA-DRw6 in the present study or in previous studies. Nevertheless, because we did note that DRw6, DR2, and DR7 haplotypes were increased or not decreased among patients, they may represent susceptibility markers of low frequency in our Jewish patient population. Because the study of Szafer *et al.* (36) is of phenotypes, assignment of restriction fragment variants to specific *DQ* genes is problematic. Furthermore, if the susceptibility gene is dominantly expressed, as our present study suggests, half of the *DR*, *DQ* genes of the patients should be the same as ethnically

matched normal *DR, DQ* genes, diluting the disease genes by half. In any case, our results suggest that Szafer *et al.* (36) were observing RFLP characteristic of DQw8, more common in patients than controls, that marks the two haplotypes with DR4, DQw8 observed in the present study. Since the DR4, DQw8 region is part of a larger fixed segment of DNA on the haplotypes present in most patients, the fact that the restriction fragment is detected in the *DQB* gene does not at all mean that the latter must be the susceptibility gene. The susceptibility gene may be anywhere in the fixed region and may be either a single allele or a haplotypic combination of alleles. The failure of Todd *et al.* (37) to find a specific amino acid to be "protective" against pemphigus may also relate to the dominant mode of inheritance of the MHC-linked susceptibility gene. Half the sequences, those of the irrelevant haplotype, would be expected to be normal, diluting the positive information. Similar considerations apply to the study of Scharf *et al.* (38), who noted specific *DRBI* oligonucleotide sequences associated with DR4 and Dw10 in pemphigus patients in Israel. It is known that [HLA-B38, SC21, DR4] carries the Dw10 specificity, and our preliminary, unpublished, observations indicate that this is also true for HLA-B35, SC31, DR4. Since at least one and probably both haplotypes have fixed alleles (are extended), one cannot conclude that any specific oligonucleotide sequences on these haplotypes are specifically involved in susceptibility without other evidence.

Our results emphasize the importance of studying a well-defined ethnic group in comparison with an ethnically matched set of controls. They also point up the necessity for doing family studies rather than phenotype studies. We would not have been able to recognize the presence of HLA-B35, SC31, DR4, nor would we be able to assess its presence or absence in a number of instances (families 4, 5, 7, and 9, to name a few) without family studies. It is of interest that the elements (except for HLA-DR4) of this haplotype, all common in normal Jews, are not statistically elevated among patients, whereas the haplotype is.

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