

## Identical HLA Class II Alleles Predispose to Drug-triggered and Idiopathic Pemphigus Vulgaris

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In pemphigus vulgaris, a dermatological autoimmune disease, specific human leukocyte antigen (HLA) class II alleles, DR4 (*DRB1\*0402*) and DRw14 (*DRB1\*1401*, in linkage disequilibrium with *DQB1\*0503*), are thought to be susceptibility genes involved in the onset of the disease. We studied the HLA class II alleles (DQA1, DQB1, DRB1 and DPB1) of 6 patients with pemphigus, in whom the disease was "triggered" by drugs containing sulphhydryl or another sulphur-containing group. All patients carried the *DRB1\*0402* susceptibility allele, and one patient also carried the second susceptibility allele, namely *DQB1\*0503* (in linkage with *DRB1\*1401*). Bacterial, viral or environmental agents are suspected to trigger the onset of autoimmune diseases. Our study demonstrated the presence in patients with drug-triggered pemphigus vulgaris of the same HLA alleles thought to predispose to idiopathic pemphigus vulgaris. This finding strengthens the notion that these HLA alleles may be true disease susceptibility genes. *Key words:* autoimmune disease; drug-induced pemphigus.

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Pemphigus vulgaris (PV) is a chronic blistering autoimmune disease that affects the skin and mucous membranes. While autoantibodies directed against the intercellular cement substance appear in patients with PV and are believed to be the cause of the pathology (1), the primary causative agent(s) of the disease remain unknown. PV affects individuals that carry the HLA class II alleles *DRB1\*0402* and *DQB1\*0503* (2). The former is more abundant in Ashkenazi Jews than in other ethnic groups and may account for the observation that PV is more prevalent in Ashkenazim (3). Thus, HLA genes are thought to play a role in predisposition to this disease.

Although in most cases of PV the external etiological factors involved could not be identified, a small group of patients with PV exhibited clinical manifestations after exposure to certain drugs, many of which contain a sulphhydryl group (4-11). The onset and development of PV may result from the interaction between external agents (i.e., drugs, viruses) and genetic factors (involving HLA alleles and other as yet unknown genes).

The present study investigated the HLA class II allelic variants (DQA1, DQB1, DRB1 and DPB1) in unrelated Israeli Jewish PV patients who developed the disease after treatment with various drugs associated with the outbreak of the disease.

Table I. Description of patients with PV and their medications

Case No.	Age at onset/sex	Drug at diagnosis of pemphigus	Predisposing illness	Drug at exacerbations	Treatment	Fluocortolone therapy	Current disease status
1	48/F	Penicilline Dipyron	Upper respiratory tract infection	Cotrimoxazole for follicular tonsillitis	fluocortolone 120 mg/dl	10 mg/d	Remission
2	56/F	Amoxicillin Sulpride	Dental abscesses		fluocortolone 120 mg/dl	12.5 mg/d	Remission
3	25/M	Cloxacillin Tetracycline	Dental infection	Dipyron	fluocortolone 120 mg/dl	35 mg/d	Incomplete remission
4	80/F	Amoxicillin	Urinary tract infection	Naproxen, oxprenolol amoxicillin	fluocortolone 80 mg/dl	10 mg/d	Remission
5	79/F	Amoxicillin	Dental infection		fluocortolone 120 mg/dl	20 mg/d	Incomplete remission
6	39/F	Amoxicillin	Upper respiratory tract infection		fluocortolone 120 mg/dl azathioprine 100 mg/d	10 mg/d	Remission

Table II. ASO and SSO typing of amplified DRB1, DQA1, DQB1 and DPB1 DNA sequences from PV-triggered patients

Case No*	DRB	DQA	DQB	DPB
1	DRB1* 0402	DQA1*0301	DQB1*0302	DPB1*0201
2	DRB1* 0402	DQA1*0301	DQB1*0301 DQB1*0302	DPB1*0401
3	DRB1* 0402	DQA1*0301	DQB1*0301	DPB1*0401
4	DRB1* 0402	DQA1*0301	DQB1*0302	DPB1*0402
5	DRB1* 0402	DQA1*0301	DQB1*0302	DPB1*0402
6	DRB1* 0402 DRB1* 1401	DQA1*0301 DQA1*0101	DQB1*0301 DQB1*0503	DPB1*0401 DPB1*0801

\*Case numbers and order correspond to those in Table I.

## SUBJECTS AND METHODS

Six patients seen in the Department of Dermatology, Ichilov Hospital, Tel Aviv, were diagnosed as suffering from PV while being treated with various drugs (see Table I). In all the patients, histopathological examination of a biopsy specimen revealed a suprabasilar acantholytic cleft within the epidermis. Direct immunofluorescent microscopic studies of perilesional tissue and uninvolved skin showed intercellular deposition of IgG and C3. Indirect immunofluorescence showed an anti-intercellular-substance antibody at various titers.

Since the pemphigus lesions appeared within days of the drug therapy and did not completely resolve upon discontinuation of the drug, necessitating chronic steroid therapy, the disease entity was classified as "triggered" rather than "induced" by the drug.

All 6 patients were being treated with penicillin or penicillin derivatives previously reported to trigger PV (Table I and refs. 4–11). They were chosen for the study as a result of PV onset following drug treatment and were all Ashkenazi Jews (of West and East European origin). These 6 patients were HLA- and ethnically matched with 46 controls.

### Amplification and typing of genomic DNA

Peripheral blood samples were taken and DNA was purified from leukocytes as described previously (12). Amplification of genomic DNA from patients with PV and ethnically and HLA-matched controls was carried out by PCR (13). The first exon of the HLA-DRB1, HLA-DQA1, HLA-DQB1 and HLA-DPB1 genes was amplified by PCR using DRB1, DQA1, DQB1 and DPB1 specific primer pairs as described previously (14), and with the 11th HLA Workshop DNA reagents.

The allelic variants in the amplified DNAs were analyzed by filter dot hybridization with ASO and SSO probes as described previously (15).

## RESULTS

### HLA DRB allelic typing

The results (Table II) can be summarized as follows: 6 out of 6 patients carried the DRB1\*0402 allele; 1 of 6 carried in addition the DRB1\*1401 allele. The frequency of DRB1\*0402 (DR4, Dw10) among the 46 DR4 control haplotypes was observed to be 41% (3). In the present study we found that 6/6 (100%) of the drug-triggered patients with PV carried the HLA DRB1\*0402 haplotype and that 4/6, 67%, of the patients were homozygous for DRB1\*0402.

### HLA DQA and DQB typing

The results, summarized in Table II, indicate that all DR4 patients carried the DQA1\*0301 and DQB1\*302 and/or \*301 alleles (6/6). The patient who carried the DRB1\*1401 was typed as DQA1\*0101 and DQB1\*0503.

### HLA DPB typing

DPB typing showed that the distribution of DPB1 alleles in the PV-triggered patients were not different from the HLA-matched control group (control results not shown). This suggests that in triggered PV, as in idiopathic PV, the DPB1 locus does not show an association with the disease.

## DISCUSSION

Specific alleles at the human major histocompatibility (MHC) loci are strongly associated with various autoimmune diseases. For example, insulin-dependent diabetes mellitus is associated with DR3 and DR4, rheumatoid arthritis with DR4, celiac disease with DR3 and DR7, myasthenia gravis with DR3, multiple sclerosis with DR2, and PV with DR4 and DRw14 (16). Moreover, when these serologically determined HLA specificities were investigated at the genomic DNA sequence level, a specific sequence-defined allele in the  $\beta$ 1 domain of the DR $\beta$  and DQ $\beta$  polypeptide chain was strongly associated with the disease (2).

When the amino acid sequence of the alleles conferring susceptibility was compared to the several non-susceptible alleles, changes at codons 57 of the DQB, and codons at positions 68–72 of the DR $\beta$  chains were found (2, 15, 17). These substitutions change a charged amino acid to a non-charged one, or vice versa. It is speculated that a change in amino acid charge at positions 57 and 68–72 of the MHC  $\beta$  chain – which are believed to come in contact with the peptide and the T cell receptor during antigen presentation – may affect the structure of the MHC peptide-binding groove and alter recognition of the MHC peptide complex by the T cells. However, the primary events or agents that trigger the pathway leading to the development of most of the autoimmune diseases remain unknown.

In the present study we have shown that all patients (6/6) who developed PV after treatment with various drugs carried HLA alleles that are found in patients with idiopathic PV. Moreover, 4 of the 6 patients were homozygous for DRB1\*0402, suggesting a possible effect of gene dosage. These results are consistent with the view that certain HLA alleles predispose their carriers to autoimmune disease.

It is noteworthy that if additional drug-triggered PV patients prove to carry these HLA allelic variants, then it may be suggested that patients with PV, either idiopathic or "triggered" by drugs, should be advised to avoid certain medications. It may also point to the usefulness of typing the HLA alleles of relatives of patients with PV before exposing them to certain drugs.

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