Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2010.04239.x

Relationship between target antigens and major histocompatibility complex (MHC) class II genes in producing two pathogenic antibodies simultaneously

L. R. Zakka,* D. B. Keskin,† P. Reche‡ and A. R. Ahmed*

*Center for Blistering Diseases, Department of Medicine, New England Baptist Hospital, †Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA, and ‡Immunomedine Group, Department of Immunology, Facultad de Medicina, Universidad Complutese de Madrid, Madrid, Spain

Accepted for publication 7 July 2010
Correspondence: A. R. Ahmed, Center for Blistering Diseases, New England Baptist Hospital, 70 Parker Hill Avenue, Boston, MA 02120, USA.
E-mail: arahmedmd@msn.com

Summary

In this report, we present 15 patients with histological and immunopathologically proven pemphigus vulgaris (PV). After a mean of 80 months since the onset of disease, when evaluated serologically, they had antibodies typical of PV and pemphigoid (Pg). Similarly, 18 patients with bullous pemphigoid (BP) and mucous membrane pemphigoid (MMP) were diagnosed on the basis of histology and immunopathology. After a mean of 60 months since the onset of disease, when their sera were evaluated they were found to have Pg and PV autoantibodies. In both groups of patients the diseases were characterized by a chronic course, which included several relapses and recurrences and were non-responsive to conventional therapy. The major histocompatibility complex class II (MHC II) genes were studied in both groups of patients and phenotypes associated typically with them were observed. Hence, in 33 patients, two different pathogenic autoantibodies were detected simultaneously. The authors provide a computer model to show that each MHC II gene has relevant epitopes that recognize the antigens associated with both diseases. Using the databases in these computer models, the authors present the hypothesis that these two autoantibodies are produced simultaneously due to the phenomena of epitope spreading.

Keywords: autoantibodies, bullous pemphigoid, epitope spreading, MHC class II genes, mucous membrane pemphigoid, pemphigus vulgaris

Introduction

Pemphigus vulgaris (PV) is a potentially fatal autoimmune mucocutaneous blistering disease (AMBD) characterized by flaccid blisters that can affect the skin and multiple mucous membranes [1–4]. An intra-epidermal vesicle is seen on histology, and the immunopathology is characterized by the deposition of antibodies on the keratinocyte cell surfaces [1–7]. The antigens are desmoglein 1 (Dsg 1) and desmoglein 3 (Dsg 3) [8], and possibly the acetylcholine receptor [9]. The titres of serum autoantibodies may correlate with disease activity and severity [8,10–12].

Pemphigoid (Pg) is principally a disease of the elderly and is associated with a high mortality rate. It has two major forms, bullous pemphigoid (BP) and mucous membrane pemphigoid (MMP) [8]. BP affects the skin [4], while MMP, also known as cicatricial pemphigoid (CP), affects predominantly the mucosa [2–4,6,7] and the skin [13,14]. The most important difference between the two subsets of pemphigoid is that when the blisters in MMP heal, they cause irreversible scarring [14]. A subepidermal/subepithelial blister with an infiltrate in the dermis or submucosa may be either predominantly eosinophilic, neutrophilic, or mixed [8,14,15]. The immunopathology shows deposition of immunoglobulins and/or complement along the basement membrane zone (BMZ) [4,8,14]. The target antigens in BP are desmoplakin-a 230 kDa protein also known as BP antigen 1 (BPAG 1) and a hemidesmosome protein, also known as BP antigen 2 (BPAG 2) of 180 kDa [8,15]. Antibodies to both BPAG 1 and BPAG 2 are present in the sera of many patients with BP. In MMP, ocular cicatricial pemphigoid (OCP) and oral pemphigoid (OP), the reported antigens include BPAG 1, BPAG 2, human integrin $\alpha 6$ and $\beta 4$ and epiligrin [15–20]. Patients with antibodies to epiligrin are referred to as anti-epiligrin cicatricial pemphigoid (AECP), with antibodies against laminin 5 [21]. The majority of patients with AECP have solid tumours and the mortality rate within the first 2 years is about 40–67% [22,23] and are not included in this study.

The simultaneous presence of PV and either BP or CP in the same patient has been reported by several authors [4,8,24–39]. In this study, we present two groups of patients. The first group consists of 15 patients who were diagnosed

initially to have PV based upon histology and immunopathology. However, when presenting at the Center for Blistering Diseases (CBD) in Boston, their sera demonstrated antibodies observed typically in both PV and Pg (BP and/or CP), as tested by indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA). The second group of 18 patients was diagnosed initially as having BP or CP. When presenting at the CBD in Boston, their serologies demonstrated antibodies typical of Pg but also of antibodies seen in patients with PV.

The production of antibodies by B cells requires the cooperation of CD4 helper T cells and is delivered on the T cell receptor (TCR)-mediated recognition of major histocompatibility complex class II (MHC II)-bound peptide antigens (T cell epitopes) displayed on the cell surface of B cells [40]. These helper cell epitopes are derived from the same antigens that are targeted by the antibodies after intracellular processing. Similarly, pathogenic autoantibody production is also contingent upon autoreactive $CD4^+$ helper T cells recognizing T cell epitopes from self-antigens [41]. In this context, the purpose of this study was to characterize the autoantibody profile in the two mentioned groups of patients, to determine the human leucocyte antigen class II (HLA II) – MHC II – genes and to identify potential autoreactive helper T cell epitopes that might be shared across disease models.

Methods

Patients

The patients in this study have not been reported in any earlier publications. These patients were seen between March 2005 and November 2009 at the Center for Blistering Diseases (CBD) in Boston. Institutional Review Board (IRB) approval was obtained to conduct the study. Written consent was obtained from each patient. It is important to highlight that, in all the patients, the initial diagnosis was made considerably earlier than their evaluation at the CBD. They were referred to the CBD because, in spite of high-dose long-term systemic corticosteroids and the use of multiple systemic immunosuppressive agents used over a period of several months or years, their diseases were not controlled and a sustained clinical remission had never been achieved. Hence, in these patients, the disease was chronic and characterized by multiple recurrences and relapses.

Inclusion criteria

1. At the time of initial diagnosis, the patients had histology, confirmed by direct immunofluorescence for PV, BP and CP.
2. The duration between the initial diagnosis and the time at presentation to the CBD was available.
3. Careful history of drugs being taken by each patient was obtained. Patients who were on medications that are known to be associated with triggering either PV or Pg were not included.
4. All these patients were referred to CBD for clinical management of recurrent disease. The presence of antibodies for two diseases occurring simultaneously was confirmed in each patient on serological evaluation at three different time intervals.

Serological analysis

Indirect immunofluorescence. The sera of the patients in both groups was evaluated by indirect immunofluorescence (IIF) using monkey oesophagus as substrates which measured the titres of the intercellular cement substance (ICS) antibodies and BMZ antibodies. Simultaneously, in both groups, antibodies to Dsg 1, Dsg 3, BPAG 1 and BPAG 2 were measured by an ELISA [12,42–44]. The index values for the ELISAs are as follows: for Dsg 1, fewer than 14 is negative, 9–20 is intermediate and greater than 20 is positive. The index values for Dsg 3 are: fewer than nine is negative, 9–20 is intermediate, and greater than 20 are positive. The index values of BPAG1 and BPAG2 are identical and as follows: fewer than nine is negative and greater than nine is positive. These serological tests were performed by laboratories at hospitals from where patients were referred, and some by Beutner Laboratories, Buffalo, NY.

HLA class II genes

HLA II genes encoded by the DRβ1 and DQβ1 loci were identified by polymerase chain reaction (PCR) with sequence-specific primers (PCR-SSP), as described previously [45], and were performed by laboratories in the institutions from where the patients were referred and some by the American Red Cross, HLA Laboratory in Dedham, MA.

Molecular analysis of the MHC II genes and their potential sites to bind to relevant antigens

Because T cell immune responses are triggered by MHC-bound peptide antigens (T cell epitopes), prediction of peptide-MHC binding is a basis to anticipate T cell epitopes [46]. MHC molecules bind peptides with a shared sequence similarity due to their binding pocket restrictions. Therefore, in this study we have position-specific scoring matrix (PSSMs), derived from epitopes that are known to bind to specific HLA II molecules [47–49], to predict potential T cell epitopes within the antigens of interest. T cell epitope predictions using PSSMs were executed using the RANKPEP server (http://imed.med.ucm.es/Tools/rankpep.html). Only those peptides that received scores above the binding threshold (BT) were considered to bind to the relevant HLA II

Table 1. Characterization of pemphigus vulgaris (PV) patients with antibodies to PV and pemphigoid (Pg).

Group 1														
					Serological studies						HLA			
					Anti-ICS			Anti-BMZ			MHC II			
Demographics					Ind. IF	ELISA		Ind. IF	ELISA		1st haplotype		2nd haplotype	
Patient	Age	Sex	C.P.	Int.	IgG	Dsg 1	Dsg 3	IgG	BP1	BP2	DRB1	DQB1	DRB1	DQB1
PV1	83	F	MuCu	97	160	110	167	80	17	20	1401	0503	1101	0301
PV2	53	F	MuCu	82	640	165	369	40	4	26	1454	0503	0302	0402
PV3	62	M	MuCu	115	1260	115	236	40	56	12	1454	0503	0402	0302
PV4	56	M	Mu	146	320	6	193	80	60	58	0402	0302	1301	0603
PV5	23	M	MuCu	72	640	15	201	40	n.d.	20	0402	0302	1101	0301
PV6	68	M	Mu	81	640	19	63	20	25	28	1401	0503	1101	0301
PV7	24	F	MuCu	84	640	97	148	20	n.d.	13	1401	0503	1101	0301
PV8	67	M	MuCu	75	160	107	166	20	n.d.	19	0402	0302	1104	0301
PV9	54	F	Mu	36	160	14	143	20	11	21	0801	0503	1104	0301
PV10	60	F	Mu	101	160	132	25	20	4	17	0402	0302	1304	0201
PV11	63	M	Mu	94	320	24	171	40	15	26	0402	0302	0102	0501
PV12	54	F	Mu	57	640	12	127	40	9	21	1454	0503	1301	0502
PV13	38	M	Mu	2	160	20	29	40	n.d.	14	0301	0201	1501	0602
PV14	47	F	Mu	16	640	11	141	20	14	19	0402	0302	1302	0602
PV15	39	M	MuCu	28	5120	17	158	20	25	18	0402	0302	1001	0501

Dsg 1 enzyme-linked immunosorbent assay (ELISA) index values [44]: < 14 = negative; 14–20 = indeterminate; > 20 = positive. Dsg 3 ELISA index values [44]: < 9 = negative; 9–20 = indeterminate; > 20 = positive. BP1 and BP2 index values [42,43]: < 9 = negative; > 9 = positive. Ind. IF: indirect immunofluorescence; M: male; F: female; J: Jewish; NJ: non-Jewish; Mu: mucous membrane; MuCu: mucocutaneous; BP1: BP antigen 1 (230 kDa); BP2: BP antigen 2 (180 kDa); CP: clinical profile; Dsg: desmoglein; Int.: time interval in weeks; n.d.: not done; IgG: immunoglobulin G; HLA: human leucocyte antigen; MHC: major histocompatibility complex; ICS: intercellular cement substance; BMZ: basement membrane zone.

molecule. Each PSSM has a specific BT that was defined after computing the binding scores of the same peptide epitopes used to derive them; ~85% of epitopes that are known to bind to a given MHC molecule receive a binding score that is above the BT. Details are reported by Reche *et al*. [47–49].

Results

Group 1

Patients with PV as initial diagnosis and subsequently having antibodies to PV and Pg antigens. These data are presented in Table 1. Patients in this group had a diagnosis of PV based on histology and direct immunofluorescence at the time of initial evaluation. When evaluated at CBD their sera contained antibodies to PV and Pg antigens. This group consisted of 15 patients: eight males and seven females. Ages ranged from 23 to 83 (mean 54·23). All patients were Caucasians. Eight patients had only mucosal disease and seven patients had mucocutaneous disease. The interval between the time of the initial histological and immunological diagnosis and the serological detection of two antibodies was 2–146 months (mean 80 months).

Serological testing demonstrated the following results:

1. On IIF, all patients had antibodies to the keratinocyte surface. The titres varied from 80 to 640.
2. On IIF, all the patients had high levels of anti-BMZ antibodies. The sera of all the patients bound only to the epidermal side of the basement membrane on salt split skin (SSS).
3. The ELISA for Dsg 1 was positive in seven of the 15 patients and Dsg 3 in all 15 patients.
4. The ELISA for BPAG 2 was positive in 15 patients.

Group 2

Patients diagnosed initially as Pg and subsequently demonstrating antibodies to both Pg and PV antigens. These data are presented in Table 2. Patients in this group had an initial diagnosis of Pg (BP/MMP) based on histology and direct immunofluorescence at the time of initial evaluation. When evaluated at the CBD, their sera demonstrated the presence of antibodies to both Pg and PV antigens. Seven patients had BP and 11 patients had MMP (four males and 14 females). The ages ranged from 28 to 91 (mean 64·94). All patients were Caucasians. Five patients had only cutaneous disease, six had only mucosal disease and seven had mucocutaneous disease. The interval between the immunopathological diagnosis of Pg and the date of the serology demonstrating the presence of two antibodies was 10–151 months (mean 60 months).

Serological testing demonstrated the following results:

Table 2. Characterization of pemphigoid (Pg) patients with antibodies to (pemphigoid (Pg) and pemphigus vulgaris (PV).

Group 2														
					Serological studies						HLA			
					Anti-ICS			Anti-BMZ			MHC II			
Demographics					Ind. IF	ELISA		Ind. IF	ELISA		1st haplotype		2nd haplotype	
Patient	Age	Sex	CP	Int.	IgG	Dsg 1	Dsg 3	IgG	BP1	BP2	DRB1	DQB1	DRB1	DQB1
BP1	91	M	Cu	16	40	18	26	640	18	13	0701	0202	1501	0602
BP2	79	F	Cu	72	40	25	15	160	n.d.	14	1104	0301	1101	0301
BP3	86	F	Cu	32	160	11	17	80	3	18	1101	0301	1104	0301
BP4	49	F	MuCu	79	20	23	2	1280	15	20	0701	0202	1301	0501
BP5	82	M	Cu	59	160	27	4	640	4	27	1104	0301	1101	0301
BP6	80	F	MuCu	10	40	13	31	80	16	37	1303	0301	1104	0301
BP7	69	F	Cu	36	20	16	29	40	12	132	1001	0501	0404	0302
CP1	28	F	Mu	42	160	32	65	20	18	n.d.	0901	0202	0404	0301
CP2	56	F	Mu	144	80	27	29	40	3	18	1104	0301	1101	0301
CP3	41	F	MuCu	151	160	18	26	40	18	13	1401	0503	0407	0301
CP4	57	F	Mu	66	20	23	34	40	n.d.	14	1501	0602	0401	0301
CP5	79	M	MuCu	62	20	18	20	40	4	20	0101	0503	0401	0301
CP6	67	F	MuCu	42	40	21	36	40	36	44	1401	0503	0404	0301
CP7	72	M	Mu	75	40	40	59	20	7	21	0101	0503	0401	0301
CP8	55	F	Mu	41	40	19	23	20	n.d.	28	0101	0503	1101	0301
CP9	47	F	MuCu	29	20	23	29	640	71	18	0101	0503	1201	0301
CP10	66	F	Mu	61	20	13	30	40	n.d.	24	1404	0503	1101	0301
CP11	52	F	MuCu	18	20	22	30	20	4	27	0103	0501	1101	0301

Dsg 1 enzyme-linked immunosorbent assay (ELISA) index values [44]: < 14 = negative; 14–20 = indeterminate; > 20 = positive. Dsg 3 ELISA index values [44]: < 9 = negative; 9–20 = indeterminate; > 20 = positive. BP1 and BP2 index values [42,43]: < 9 = negative; > 9 = positive. Ind. IF: indirect immunofluorescence; M: male; F: female; J: Jewish; NJ: non-Jewish; Mu; mucous membrane; MuCu: mucocutaneous; Cu, Cutaneous; BP1, BP Antigen 1 (230 kDa); BP2, BP Antigen 2 (180 kDa); CP: clinical profile; Dsg: desmoglein; Int.: time interval in weeks; n.d.: not done; IgG: immunoglobulin G; HLA: human leucocyte antigen; MHC: major histocompatibility complex; ICS: intercellular cement substance; BMZ: basement membrane zone.

1. On IIF, the sera of all 17 patients were positive for anti-BMZ antibodies. The sera of all the patients bound only to the epidermal side of the basement membrane on SSS.
2. The ELISA BPAG 1 was positive in eight of 18 patients, not performed in 18 patients and negative in the remaining six of 18 patients. For BPAG 2, the ELISA was positive for 18 patients.
3. On IIF, all patients had antibodies to the keratinocyte surface; the titre varied from 20 to 160.
4. The ELISA for Dsg 1 and/or Dsg 3 was positive in 16 of 18 patients, and two of 18 had indeterminate levels.

Indirect immunofluorescence results using monkey oesophagus as substrate are presented in Fig. 1 as a prototype example. Figure 1a represents binding of PV sera to keratinocyte cell surface antigens. Figure 1b represents BP or CP antibodies binding to BMZ. Figure 1c represents a sera in which antibodies to PV bind to ICS and antibodies to BP or CP bind to BMZ simultaneously. The binding to the keratinocyte cell surface is brighter because the antibody titre may be higher and the binding is sharp and defined.

Immunogenetic studies

Only patients, not families, were studied. Although the data are phenotypic, the results are expressed as presumed haplotypes because of their known associations based upon linkage disequilibrium.

Group 1: patients with PV as initial diagnosis and subsequently having antibodies to PV and Pg antigens. High-resolution MHC II gene analysis by PCR-SSP demonstrated that nine patients had HLA-DRβ1*0402 and seven patients had HLA-DQβ1*0503. Also, one patient carried only the PV HLA genes but no Pg genes; one patient did not carry any gene associated with PV, but had the DQβ1*0301 and DQβ1*0602 associated with Pg.

In the same patients, MHC II genes associated with Pg were observed on the second haplotype. HLA-DQβ1*0301 was present in seven patients. HLA-DQβ1*0302 was present in nine patients, HLA-DQβ1*0603 was present in one patient and HLA-DQβ1*0602 was present in two patients.

Group 2: patients diagnosed initially as Pg and subsequently demonstrating antibodies to both Pg and PV antigens. High-resolution MHC II gene analysis by PCR-SSP demonstrated that 15 patients carried DQβ1*0301 on one haplotype. One patient carried DQβ1*0302, and two carried DQβ1*0602. Also, five patients were homozygous for DQβ1*0301.

In the same patients, MHC II genes associated with PV were observed on the second haplotype. Seven patients

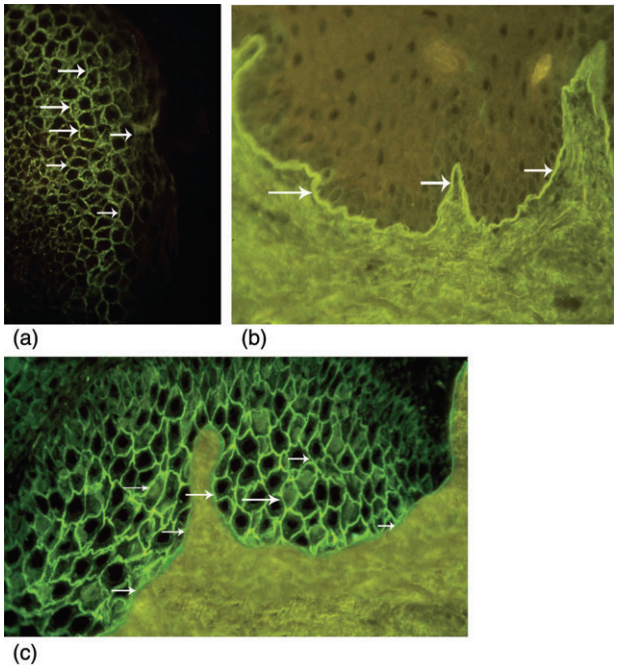

Fig. 1. Indirect immunofluorescence using monkey oesophagus as substrate. (a) Binding of sera of a pemphigus vulgaris (PV) patient to intercellular cement substance (ICS) (keratinocyte cell surface) (indicated by the white arrows). (b) Binding of sera of a pemphigoid (Pg) patient to basement membrane zone (BMZ) (indicated by the white arrows). (c) Binding of the sera of a patient with dual diagnosis to both BMZ and ICS (indicated by the white arrows).

carried DQβ1*0503 and those same seven patients also carried DQβ1*0301 on the second haplotype. None of the patients carried DRβ1*0402. Six patients carried only Pg genes but no PV genes. One patient did not carry any genes known to be associated with either PV or Pg.

Molecular analysis of the MHC II genes and their potential sites to bind to relevant antigens

The HLA II alleles associated with PV and Pg and the relevant antigens, when subjected to the RANKPEP program, demonstrate that there are potential T cell epitopes within BPAG 2 (Fig. 2a) and α6 integrin (Fig. 2b) that are predicted to bind to DRβ1*0402. For purposes of brevity only BPAG 2 and α6 data are presented, but these observations would be applicable to BPAG 1 and β4 integrin. When subjected to the RANKPEP program, there are potential T cell epitopes within Dsg 3 that are predicted to bind to DQβ1*0301 and DRβ1*0402, as presented in Fig. 2c.

Discussion

In this report we present 15 patients with a clinically, histologically and immunopathologically established diagnosis of PV. After a mean of 80 months following the initial diagnosis with persistent disease, when evaluated serologically patients demonstrated the presence of antibodies seen typically in PV patients, and in association with antibodies seen in patients with Pg. Similarly, we report a group of 18 patients with a clinical, histological and immunopathologically established diagnosis of Pg. After a mean period of 60 months with clinically active disease, their sera demonstrated presence of antibodies seen in Pg and in association with antibodies seen typically in PV. Thus, these patients could be labelled as having a dual diagnosis. The PV, BP and CP patients had long-term chronic disease characterized by repeated exacerbations and relapses. Hence, it appears that these patients are a distinct subset of patients in both disease groups, their distinctive features being chronicity of disease, recurrent relapses and remissions and lack of response to conventional therapy. Several patients are described in the literature who had PV and Pg simultaneously [8].

The patients in group 1 have the MHC II genes DRβ1*0402 and DQβ1*0503 that have been reported in patients with PV in several studies [50–66]. Many of these patients also had the MHC II genes associated with patients with Pg, which is DQβ1*0301. However, because of amino acid sequence homology in the critical 71–77 positions of the DQβ1 gene, it has been demonstrated that DQβ1*0302, DQβ1*0303, DQβ1*0305, DQβ1*0602 and DQβ1*0603 have largely overlapping peptide-binding repertoires and, thus, may have shared epitopes within Pg [67].

In group 2, there were 18 patients with subsets of MMP and BP, all of which are characterized by *in-vivo* deposition of anti-BMZ antibodies on direct immunofluorescence. These patients had MHC II gene HLA-DQβ1*0301 that is observed typically in patients with all the variants of Pg [16,67–74]. In addition, many of these patients also carry the MHC II genes associated typically with PV patients.

While the authors recognize completely that there could be several reasons that could account for these unique observations, the current data would suggest that one of the variables may be immunogenetically based. The presence of haplotypes or alleles associated with PV and Pg simultaneously in the same patient have been reported previously in several studies [16,52,53,55,61,64,67,69,75–77]. The alleles and haplotypes known to be associated with PV are DRβ1*0402/DQβ1*0302 and DRβ1*1401/DQβ1*05031. In one study there were several patients with these PV-associated haplotypes or phenotypes that also carried DQβ1*0301 on the second haplotype [76]. In another study, two of nine PV patients had DQβ1*0301 on the second haplotype, and this frequency was higher than in the control population [55]. In three of 10 patients with PV, DQβ1*0301 was present and all these patients had predominantly mucosal diseases [77]. Similarly, several studies on Pg which included OP, OCP, MMP and BP show that while patients carry the DQβ1*0301 allele, they often carry DRβ1*0402 or DQβ1*0503 on the second haplotype [16,64,67]. Interestingly, in some studies the frequency of DRβ1*04 is

(a)

NP_000485·3 alpha 1 type XVII collagen

Bullous pemphigoid autoantigen BP180
Epitopes predicted to bind to DQ*0301 are represented as yellow. Epitopes predicted to bind DR*0402 are highlighted as green. Predicted epitope that overlaps and likely to be presented by both MHC molecules is represented as red

epitope 424-434.

```
1 mdvtkknkrdgtevterivtetvttrltslppkggtsngyaktaslgggsrlekqslthg
61 ssgyinstgstrghastssyrrahspastlpnspgstferkthvtrhayegsssgnsspe
121 yprkefassstrgrsqtreseirvrlqsaspstrwtelddvkrllkgsrsasvsptrnss
181 ntlpipkkgtvetkivtassqsvsgtydatildanlpshvwsstlpagssmgtyhnnmtt
241 qsssllntnaysagsvfgvpnnmascsptlhpglstsssvfgmqnnlapslttlshgttt
301 tstaygvkknmpqspaavntgvstsaacttsvqsddllhkdckflilekdntpakkemel
361 limtkdsgkvftaspasiaatsfsedtlkkekqaaynadsglkaeangdlktvstkgktt
421 tadihsygssggggsgggggvggagggpwgpapawcpcgsccswwkwllgllltwllllg
481 llfglialaeevrklkarvdelerirrsilpygdsmdriekdrlqgmapaagadldkigl
541 hsdsqeelwmfvrkklmmeqengnlrgspgpkgdmgspgpkgdrgfpgtpgipgplghpg
601 pqgpkgqkgsvgdpgmegpmgqrgregpmgprgeagppgsgekgergaagepgphgppgv
661 pgsvgpkgsspgpgpqgppgpvglqglrgevglpgvkgdkgpmgppgpkgdqgekgprgl
721 tgepgmrglpgavgepgakgamgpagpdghqgprgeqgltgmpgirgppgpsgdpgkpgl
781 tgpqgpqglpgtpgrpgikgepgapgkivtsegssmltvpgppgppgamgppgppgapgp
841 agpaglpghqevlnlqgppgppgprgppgpsipgppgprgppgeglpgppgppgsflsns
901 etflsgppgppgppgpkgdqgppgprghqgeqglpgfstsgsssfglnlqgppgppgpqg
961 pkgdkgdpgvpgalgipsgpseggssstmyvsgppgppgppgppgsisssgqeiqqyise
1021 ymqsdsirsylsgvqgppgppgppgpvttitgetfdyselashvvsylrtsgygvslfss
1081 sissedilavlqrddvrqylrqylmgprgppgppgasgdgsllsldyaelssrilsymss
1141 sgisiglpgppgppglpgtsyeellsllrgsefrgivgppgppgppgipgnvwssisved
1201 lssylhtaglsfipgppgppgppgprgppgvsgalatyaaensdsfrselisyltspdvr
1261 sfivgppgppgpqgppgdsrllstdashsrgsssshsssvrrgssysssmstggggags
1321 lgaggafgeaagdrgpygtdigpgggygaaaeggmyagnggllgadfagdldynelavrv
1381 sesmqrqgllqgmaytvqgppgqpgpqgppgiskvfsaysnvtadlmdffqtygaiqgpp
1441 gqkgemgtpgpkgdrgpagppghpgppgprghkgekgdkgdqvyagrrrrrsiavkp
```

Bullous pemphigoid autoantigen BP180 binding to DRB1*0402

Matrix: HLA(DR4) DRB1*0402 Consensus sequence: ICFWHNHNM
Optimal Score: 44·604 Binding Threshold: 11·44
All rows highlighted in red represent predicted binders.

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	1134	SSR	ILSYMSSSG	ISI	926·06	16·967	38·04 %
2	472	LGL	LLTWLLLLG	LLF	1000·32	15·227	34·14 %
3	142	ESE	IRVRLQSAS	PST	1011·2	14·347	32·17 %
4	424	TAD	IHSYGSSGG	GGS	845·87	12·689	28·45 %

Bullous pemphigoid autoantigen BP180 binding to DQβ*0301

Consensus sequence: IWHAVHAWH Optimal Score: 45·671
Binding Threshold: 11·70 All rows highlighted in red represent predicted binders

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	505	LER	IRRSILPYG	DSM	1056·29	19·68	43·09 %
2	1055	GET	FDYSELASH	VVS	1050·11	19·381	42·44 %
3	1283	RLL	STDASHSRG	SSS	898·89	16·697	36·56 %
4	211	DAT	ILDANLPSH	VWS	961·09	16·398	35·90 %
5	841	PGP	AGPAGLPGH	QEV	757·85	16·234	35·55 %
6	220	PSH	VWSSTLPAG	SSM	876·01	15·634	34·23 %
7	1320	GAG	SLGAGGAFG	EAA	717·78	13·849	30·32 %
8	765	MPG	IRGPPGPSG	DPG	818·94	13·625	29·83 %
9	199	VTA	SSQSVSGTY	DAT	896·91	13·531	29·63 %
10	247	SLL	NTNAYSAGS	VFG	865·85	13·391	29·32 %
11	80	TSS	YRRAHSPAS	TLP	1026·14	12·928	28·31 %
12	1201	VED	LSSYLHTAG	LSF	930·03	12·403	27·16 %
13	423	TTA	DIHSYGSSG	GGG	903·91	12·396	27·14 %
14	837	PPG	APGPAGPAG	LPG	675·75	11·938	26·14 %

Fig. 2. Predicted T cell epitopes. Figure depicts potential T cell epitopes that were predicted to be restricted by either human leucocyte antigen D-related (HLA-DR)β1*0402 (green) or HLA-DQβ1*0301 (yellow) from bullous pemphigoid antigen 2 (BPAG 2, BP180) (a), integrin alpha chain, alpha 6 isoform (b) and Dsg 3 (c). T cell epitopes that are predicted to be restricted by both HLA II molecules, HLA-DRβ1*0402 and HLA-DQβ1*0301, are shown in red. All the T cell epitopes shown in the figure have a binding score above the binding threshold (see Material and methods for details).

Alpha 6 Integrin binding to DQβ*0301

Consensus sequence: IWHAVHAWH Optimal Score: 45-671

Binding Threshold: 11·70 All rows highlighted in red represent predicted binders

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	34	DNV	IRKYGDPGS	LFG	974·09	14·91	32·65 %
2	341	PQY	FDRDGEVGG	AVY	932·95	14·743	32·28 %
3	503	KSC	FEYTANPAG	YNP	951·01	14·403	31·54 %
4	495	PSG	ICLQVKSCF	EYT	1022·29	14·108	30·89 %
5	1050	YDD	SVPRYHAVR	IRK	1066·24	12·743	27·90 %
6	280	VSG	APRANHSGA	VVL	861·92	12·698	27·80 %
7	174	DWS	FCDGRLRGH	EKF	1042·19	12·271	26·87 %

Alpha 6 Integrin binding to DRβ*0402

Consensus sequence: ICFWHNHNM Optimal Score: 44-604

Binding Threshold: 11·44 All rows highlighted in red represent predicted binders.

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	216	WKG	IVRVEQKNN	TFF	1081·23	17·503	39·24 %
2	1081	KQW	ITKWNENES	YS	1079·16	16·5	36·99 %
3	941	RCP	LRGLDSKAS	LIL	928·06	16·022	35·92 %
4	951	ASL	ILRSRLWNS	TFL	1103·33	15·987	35·84 %
5	408	GKV	FIYHGSANG	INT	947·02	15·872	35·58 %
6	351	GGA	VYVYMNQQG	RWN	1083·22	15·005	33·64 %
7	34	DNV	IRKYGDPGS	LFG	974·09	13·6	30·49 %
8	291	AVV	LLKRDMKSA	HLL	1043·29	13·222	29·64 %
9	771	DIN	LKLETTSNQ	DNL	1015·12	12·786	28·67 %
10	174	DWS	FCDGRLRGH	EKF	1042·19	12·424	27·85 %
11	727	LSC	VANQNGSQA	DCE	869·88	12·33	27·64 %
12	724	EKQ	LSCVANQNG	SQA	886·97	12·035	26·98 %
13	179	DGR	LRGHEKFGS	CQQ	1012·14	11·879	26·63 %

(b)

Integrin alpha chain, alpha 6 isoform a precursor [*Homo sapiens*].

Epitopes predicted to bind to DQβ*0301 are represented asyellow. Epitopes predicted to bind DRβ*0402 are highlighted as green. Predicted epitope that overlaps and likely to be presented by both MHC molecules is represented as red.

MAAAGQLCLLYLSAGLLSRLGAAFNLDTREDNVIRKYGDPGSLFGFSLAMHWQLQP
EDKRLLLVGAPRAEALPLQRANRTGGLYSCDITARGPCTRIEFDNDADPTSESKED
QWMGVTVQSQGPGGKVVTCAHRYEKRQHVNTKQESRDIFGRCYVLSQNLRIEDDMD
GGDWSFCDGRLRGHEKFGSCQQGVAATFTKDFHYIVFGAPGTYNWKGIVRVEQKNN
TFFDMNIFEDGPYEVGGETEHDESLVPVPANSYLGFSLDSGKGIVSKDEITFVSGA
PRANHSGAVVLLKRDMKSAHLLPEHIFDGEGLASSFGYDVAVVDLNKDGWQDIVIG
APQYFDRDGEVGGAVYVYMNQQGRWNNVKPIRLNGTKDSMFGIAVKNIGDINQDGY
PDIAVGAPYDDLGKVFIYHGSANGINTKPTQVLKGISPYFGYSIAGNMDLDRNSYP
DVAVGSLSDSVTIFRSRPVINIQKTITVTPNRIDLRQKTACGAPSGICLQVKSCFE
YTANPAGYNPSISIVGTLEAEKERRKSGLSSRVQFRNQGSEPKYTQELTLKRQKQK
VCMEETLWLQDNIRDKLRPIPITASVEIQEPSSRRRVNSLPEVLPILNSDEPKTAH
IDVHFLKEGCGDDNVCNSNLKLEYKFCTREGNQDKFSYLPIQKGVPELVLKDQKDI
ALEITVTNSPSNPRNPTKDGDDAHEAKLIATFPDTLTYSAYRELRAPPEKQLSCVA
NQNGSQADCELGNPFKRNSNVTFYLVLSTTEVTFDTPDLDINLKLETTSNQDNLAP
ITAKAVVIELLLSVSGVAKPSQVYFGGTVVGEQAMKSEDEVGSLIEYEFRVINLG
KPLTNLGTATLNIQWPKEISNGKWLLYLVKVESKGLEKVTCEPQKEINSLNLTESH
NSRKKREITEKQIDDNRKFSLFAERKYQTLNCSVNVNCVNIRCPLRGLDSKASLITL
RSRLWNSTFLEEYSKLNYLDILMRAFIDVTAAAENIRLPNAGTQVRVTVFPSKTVA
QYSGVPWWIILVAILAGILMLALLVFILWKCGFFKRSRYDDSVPRYHAVRTRKEER
EIKDEKYIDNLEKKQWITKWNENESYS

Fig. 2. *Continued*

(c)

Homo sapiens desmoglein 3 (pemphigus vulgaris antigen) (DSG 3)

Epitopes predicted to bind to DQβ*0301 are represented as yellow.
Epitopes predicted to bind DRβ*0402 are highlighted as green. Predicted epitope that overlaps and likely to be presented by both MHC molecules is represented as red.
Area highlighted as pink with bold letters is the dominant published PV epitope.

MMGLFPRTTGALAIFVVVILVHGELRIETKGQYDEEEMTMQQAKR
RQKREWVKFAKPCREGEDNSKRNPIAKITSDYQATQKITYRISGVGI
DQPPFGIFVVDKNTGDINITAIVDREETPSFLITCRALNAQGLDVEKP
LILTVKILDINDNPPVFSQQIFMGEIEENSASNSLVMILNATDADEPN
HLNSKIAFKIVSQEPAGTPMFLLSRNTGEVRTLTNSLDREQASSYR
LVVSGADKDGEGLSTQCECNIKVKDVNDNFPMFRDSQYSARIEENI
LSSELLRFQVTDLDEEYTDNWLAVYFFTSGNEGNWFEIQTDPRTNE
GILKVVKALDYEQLQSVKLSIAVKNKAEFHQSVISRYRVQSTPVTIQ
VINVREGIAFRPASKTFTVQKGISSKKLVDYILGTYQAIDEDTNKAA
SNVKYVMGRNDGGYLMIDSKTAEIKFVKNMNRDSTFIVNKTITAE
VLAIDEYTGKTSTGTVYVRVPDFNDNCPTAVLEKDAVCSSSPSVVV
SARTLNNRYTGPYTFALEDQPVKLPAVWSITTLNATSALLRAQEQI
PPGVYHISLVLTDSQNNRCEMPRSLTLEVCQCDNRGICGTSYPTTSP
GTRYGRPHSGRLGPAAIGLLLLGLLLLLLAPLLLLTCDCGAGSTGG
VTGGFIPVPDGSEGTIHQWGIEGAHPEDKEITNICVPPVTANGADFM
ESSEVCTNTYARGTAVEGTSGMEMTTKLGAATESGGAAGFATGTV
SGAASGFGAATGVGICSSGQSGTMRTRHSTGGTNKDYADGAISMN
FLDSYFSQKAFACAEEDDGQEANDCLLIYDNEGADATGSPVGSVG
CCSFIADDLDDSFLDSLGPKFKKLAEISLGVDGEGKEVQPPSKDSGY
GIESCGHPIEVQQTGFVKCQTLSGSQGASALSASGSVQPAVSIPDPL
QHGNYLVTETYSASGSLVQPSTAGFDPLLTQNVIVTERVICPISSVP
GNLAGPTQLRGSHTMLCTEDPCSRLI

Matrix: HLA(DQ7)DQB1*0301 Consensus sequence: IWHAVHAWH
Optimal Score: 45·671 Binding Threshold: 11·70
All rows highlighted in red represent predicted binders.

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	727	LGA	ATESGGAAG	FAT	701·69	17·045	37·32 %
2	911	ALS	ASGSVQPAV	SIP	796·88	15·559	34·07 %
3	400	SKK	LVDYILGTY	QAI	1038·21	15·092	33·05 %
4	73	IAK	ITSDYQATQ	KIT	1008·05	14·73	32·25 %
5	232	ASS	YRLVVSGAD	KDG	961·09	13·563	29·70 %

Matrix: HLA(DR4) DRB1*0402 Consensus sequence: ICFWHNHNM

Optimal Score: 44·604 Binding Threshold: 11·44
All rows highlighted in red represent predicted binders.
Peptide highlighted in Yellow is the known dominant PV epitope.
Prediction of a known T cell auto-antigen using algorithm validates the method.

RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
1	605	SPG	TRYGRPHSG	RLG	1012·1	17·99	40·33 %
2	765	SGT	MRTRHSTGG	TNK	984·09	17·387	38·98 %
3	193	NSK	IAFKIVSQE	PAG	1016·21	15·149	33·96 %
4	443	TAE	IKFVKNMNR	DST	1131·39	15·046	33·73 %
5	422	ASN	VKYVMGRND	GGY	1063·23	14·554	32·63 %
6	83	TQK	ITYRISGVG	IDQ	947·1	13·746	30·82 %
7	812	NDC	LLIYDNEGA	DAT	989·1	12·572	28·19 %
8	70	RNP	IAKITSDYQ	ATQ	1020·15	12·438	27·89 %
9	359	QSV	ISRYRVQST	PVT	1091·24	11·643	26·10 %
10	303	WLA	VYFFTSGNE	GNW	1045·12	11·59	25·98 %
11	217	TGE	VRTLTNSLD	REQ	1000·11	11·507	25·80 %

Fig. 2. *Continued*

statistically significantly increased in Pg patients on the same haplotype as DQβ1*0301 [53,69]. Unfortunately, however, high- resolution typing of DRβ1*04 was not performed [53,69]. Should the DRβ1*04 be DRβ1*0402, it would have readily explained the presence of alleles linked strongly to PV. Another major handicap of these studies is that the authors did not study or report the presence of pathogenic autoantibodies to PV or Pg in their reports.

There are several reports in the literature to indicate that patients with PV and Pg have been associated with several other autoimmune diseases. BP has been reported in patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome, myasthenia gravis (MG) and multiple sclerosis (MS) [78–89]. MMP has been reported in patients with SLE, connective tissue disease (CTD) and RA [90–95]. Also, PV has been reported with SLE, CTD, MS, MG, alopecia universalis, insulin-dependent diabetes mellitus, autoimmune thyroid disease, Sjögren's syndrome and systemic sclerosis [96–113].

There are numerous examples in the literature of patients with one autoimmune disease who, during the course of their illness, develop a second autoimmune disease [89,114–131].

Epitope spreading provides four possible scenarios that may explain the presence of two simultaneous pathogenic autoantibodies in one patient. The first scenario may be that one large antigen molecule may contain at least two epitopes that have a certain degree of similarity or overlapping sequence [132]. However, when presented by two different MHC II genes, they stimulate two different T cells and resultantly activate two different B cells through CD40–CD40L interaction. These B cells then produce two different autoantibodies. The second scenario may be that antigen-presenting cells (APC), internalizing two separate antigens, then activate two different T cells that are specific to any one of these antigens [132–134]. The third scenario may be that two antigens are clustered in a single macromolecule complex, all of which are internalized by a single APC [132,135–137]. This APC then activates two different T cells, making each specific for one subset of the macromolecule. The T cells would then enlist B cells and result in the production of two different autoantibodies. Finally, the fourth scenario may be that an autoimmune disease causes tissue damage and inflammation in the surrounding tissue [132,138,139]. This exposes the immune system to a previously sequestered epitope. This epitope becomes internalized by an APC, presented to a T cell that enlists B cells and produces a second antibody. It has been demonstrated in patients with Stevens–Johnson syndrome that when there is extensive inflammation and tissue damage, patients often develop OCP [140]. Moreover, it has been found that MHC II genes are important in influencing the type of antibodies and the amount produced [141]. Furthermore, recent studies indicate that the perfect fitting of the core nonameric peptide residues within the binding groove within the MHC II alleles are not capable of guaranteeing a complete fitting of the entire peptide [52]. Indeed, flanking residues outside the binding groove could also play an important role in the selection of the peptide [52]. Based on the studies by Reche *et al.*, using the RANKPEP program, we have shown that PV and Pg antigens may potentially bind both the DRβ1*0402 and DQβ1*0301 genes to stimulate an immune response (Fig. 2a–c). Therefore, within the four scenarios, it is theoretically possible for a patient with only DRβ1*0402 to produce anti-BMZ antibodies if epitope spreading occurred to a Pg antigen, and the same is possible for DQβ1*0301and anti-ICS antibodies. Collectively, those hypothetical models would lead to the conclusion that key determinants in one patient producing two autoantibodies would lie in the molecular structure of the antigen and binding properties of the MHC II gene products. That said, the specificity of T cells to recognize specific epitopes and enlist B cells that produce only a specific autoantibody is a necessary corollary. While the authors do not claim that this may be the only mechanism to explain their observations, they are highlighting the above primarily because of the availability of these data and their possible utilization in providing an explanation. It is also possible that the production of two antibodies in the same patient could occur as a consequence of other genetic factors or non-genetic factors that have yet to be identified or described. The authors have highlighted the genetic factors because all these patients are unrelated.

While definitive experiments showing T cell proliferation are not performed in this study, there are preliminary reports in the literature to show that when homozygous typing cell lines that carry DQβ1*0301 are used for the purpose of antigen presentation, T cells from PV patients proliferate when stimulated with Dsg 3 peptides [75]. This is evidence that the DQβ1*0301 molecule, with Dsg 3, has the potential to give rise to or facilitate the process that can produce PV antibodies.

The importance of these observations is both clinical and biological. These studies provide a unique opportunity to demonstrate that, in rare instances when individuals inherit genes associated with enhanced susceptibility to developing an autoimmune disease, trigger(s) can activate the immune system to respond unfavourably and produce two autoantibodies. Such patients can have a chronic form of the disease that is recalcitrant to conventional therapy. The clinical scenarios presented pose a significant problem to the patient and the treating physician.

Disclosure

The authors have no conflicts of interest or competing interests to disclose.

References

1 Ahmed AR, Graham J, Jordon RE, Provost TT. Pemphigus: current concepts. Ann Intern Med 1980; **92**:396–405.

2 Nousari HC, Anhalt GJ. Pemphigus and bullous pemphigoid. Lancet 1999; **354**:667–72.
3 Lever WF. Pemphigus and pemphigoid. A review of the advances made since 1964. J Am Acad Dermatol 1979; **1**:2–31.
4 Sami N, Bhol KC, Beutner EH *et al.* Simultaneous presence of mucous membrane pemphigoid and pemphigus vulgaris: molecular characterization of both autoantibodies. Clin Immunol 2001; **100**:219–27.
5 Beutner EH, Jordon RE, Chorzelski TP. The immunopathology of pemphigus and bullous pemphigoid. 1968. J Invest Dermatol 1989; **92**:166S; discussion 7S–8S.
6 Fleming TE, Korman NJ. Cicatricial pemphigoid. J Am Acad Dermatol 2000; **43**:571–91; quiz 91–4.
7 Scully C. A review of common mucocutaneous disorders affecting the mouth and lips. Ann Acad Med Singapore 1999; **28**:704–7.
8 Sami N, Ahmed AR. Dual diagnosis of pemphigus and pemphigoid. Retrospective review of thirty cases in the literature. Dermatology 2001; **202**:293–301.
9 Grando SA. Autoimmunity to keratinocyte acetylcholine receptors in pemphigus. Dermatology 2000; **201**:290–5.
10 Weissman V, Feuerman EJ, Joshua H, Hazaz B. The correlation between the antibody titers in sera of patients with pemphigus vulgaris and their clinical state. J Invest Dermatol 1978; **71**:107–9.
11 Amagai M, Tsunoda K, Zillikens D, Nagai T, Nishikawa T. The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile. J Am Acad Dermatol 1999; **40**:167–70.
12 Ishii K, Amagai M, Hall RP *et al.* Characterization of autoantibodies in pemphigus using antigen-specific enzyme-linked immunosorbent assays with baculovirus-expressed recombinant desmogleins. J Immunol 1997; **159**:2010–17.
13 McCuin JB, Hanlon T, Mutasim DF. Autoimmune bullous diseases: diagnosis and management. Dermatol Nurs 2006; **18**:20–5.
14 Scott JE, Ahmed AR. The blistering diseases. Med Clin North Am 1998; **82**:1239–83.
15 Wojnarowska F, Venning VA, Burge SM. Immunobullous diseases, 7th edn. Malden, MA: Blackwell Science Ltd, 2008.
16 Yunis JJ, Mobini N, Yunis EJ *et al.* Common major histocompatibility complex class II markers in clinical variants of cicatricial pemphigoid. Proc Natl Acad Sci USA 1994; **91**:7747–51.
17 Bhol KC, Goss L, Kumari S, Colon JE, Ahmed AR. Autoantibodies to human alpha6 integrin in patients with oral pemphigoid. J Dent Res 2001; **80**:1711–15.
18 Tyagi S, Bhol K, Natarajan K, Livir-Rallatos C, Foster CS, Ahmed AR. Ocular cicatricial pemphigoid antigen: partial sequence and biochemical characterization. Proc Natl Acad Sci USA 1996; **93**:14714–19.
19 Bhol KC, Dans MJ, Simmons RK, Foster CS, Giancotti FG, Ahmed AR. The autoantibodies to alpha 6 beta 4 integrin of patients affected by ocular cicatricial pemphigoid recognize predominantly epitopes within the large cytoplasmic domain of human beta 4. J Immunol 2000; **165**:2824–9.
20 Kumari S, Bhol KC, Simmons RK *et al.* Identification of ocular cicatricial pemphigoid antibody binding site(s) in human beta4 integrin. Invest Ophthalmol Vis Sci 2001; **42**:379–85.
21 Rose C, Schmidt E, Kerstan A *et al.* Histopathology of anti-laminin 5 mucous membrane pemphigoid. J Am Acad Dermatol 2009; **61**:433–40.
22 Fukushima S, Egawa K, Nishi H *et al.* Two cases of anti-epiligrin cicatricial pemphigoid with and without associated malignancy. Acta Derm Venereol 2008; **88**:484–7.
23 Sadler E, Lazarova Z, Sarasombath P, Yancey KB. A widening perspective regarding the relationship between anti-epiligrin cicatricial pemphigoid and cancer. J Dermatol Sci 2007; **47**:1–7.
24 Ahmed AR, Workman S. Anti-intercellular substance antibodies. Presence in serum samples of 14 patients without pemphigus. Arch Dermatol 1983; **119**:17–21.
25 Cram DL, Griffith MR, Fukuyama K. Pemphigus-like antibodies in cicatricial pemphigoid. Arch Dermatol 1974; **109**:235–8.
26 Kumar V, Yarbrough C, Beutner EH. Complement-fixing intercellular antibodies in a case of cicatricial pemphigoid. Arch Dermatol 1980; **116**:812–14.
27 Roenigk HH Jr, Deodhar S. Pemphigus treatment with azathioprine. Clinical and immunologic correlation. Arch Dermatol 1973; **107**:353–7.
28 Buhac J, Bhol K, Padilla T Jr, Foster CS, Ahmed AR. Coexistence of pemphigus vulgaris and ocular cicatricial pemphigoid. J Am Acad Dermatol 1996; **34**:884–6.
29 Ead RD. Pemphigus-like antibodies: a report of two cases. Br J Dermatol 1979; **100**:723–5.
30 Smolle J, Kerl H. Pitfalls in the diagnosis of pemphigus vulgaris (early pemphigus vulgaris with features of bullous pemphigoid). Am J Dermatopathol 1984; **6**:429–35.
31 Leibovici V, Ron N, Goldenhersh M, Holubar K. Coexistence of pemphigus and bullous pemphigoid. Int J Dermatol 1989; **28**:259–60.
32 Matsubara K, Kanauchi H, Tanaka T, Imamura S. Coexistence of pemphigus and bullous pemphigoid. J Dermatol 1995; **22**:68–71.
33 Takahashi H, Wada T, Matsuo S, Iwatsuki K, Iizuka H. A case of bullous pemphigoid with antibodies against intercellular 130 kd antigen. J Dermatol 1995; **22**:576–81.
34 Bernard P, Catanzano G, Vignaud St Florent JD, Fayol J, Bonnetblanc JM. [Bullous pemphigoid with pemphigus type antibodies *in vivo*. 2 cases]. Ann Dermatol Venereol 1986; **113**:671–6.
35 Chorzelski TP, Maciejowski E, Jablonska S *et al.* Coexistence of pemphigus and bullous pemphigoid. Arch Dermatol 1974; **109**:849–53.
36 Kore-eda S, Horiguchi Y, Ohtoshi E *et al.* A case of autoimmune bullous dermatosis with features of pemphigus vulgaris and bullous pemphigoid. Am J Dermatopathol 1995; **17**:511–16.
37 Ninomiya J, Nakabayashi A, Sei Y, Takiuchi I. Bullous pemphigoid complicated with pemphigus vulgaris? Dermatology 1994; **189** (Suppl. 1):117–19.
38 Velthuis PJ, Hendrikse JC, Nefkens JJ. Combined features of pemphigus and pemphigoid induced by penicillamine. Br J Dermatol 1985; **112**:615–19.
39 Dobmeier LJ, Sams WM Jr, Beutner EH. Intercellular antibodies in a patient without clinical pemphigus. Ann NY Acad Sci 1971; **177**:218–23.
40 Batista FD, Harwood NE. The who, how and where of antigen presentation to B cells. Nat Rev Immunol 2009; **9**:15–27.
41 Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 2001; **1**:147–53.
42 Barnadas MA, Rubiales MV, Gonzalez MJ *et al.* Enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence testing in a bullous pemphigoid and pemphigoid gestationis. Int J Dermatol 2008; **47**:1245–9.
43 Kobayashi M, Amagai M, Kuroda-Kinoshita K *et al.* BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and

monitoring tool for bullous pemphigoid. J Dermatol Sci 2002; **30**:224–32.
44 Amagai M, Komai A, Hashimoto T *et al.* Usefulness of enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. Br J Dermatol 1999; **140**:351–7.
45 Bunce M, Welsh K. PCR-SSP typing of HLA class I and class II alleles, 4th edn. Mt. Laurel, NJ: ASHI Publications, 2000.
46 Lafuente EM, Reche PA. Prediction of MHC-peptide binding: a systematic and comprehensive overview. Curr Pharm Des 2009; **15**:3209–20.
47 Reche PA, Glutting JP, Reinherz EL. Prediction of MHC class I binding peptides using profile motifs. Hum Immunol 2002; **63**:701–9.
48 Reche PA, Glutting JP, Zhang H, Reinherz EL. Enhancement to the RANKPEP resource for the prediction of peptide binding to MHC molecules using profiles. Immunogenetics 2004; **56**:405–19.
49 Reche PA, Keskin DB, Hussey RE, Ancuta P, Gabuzda D, Reinherz EL. Elicitation from virus-naive individuals of cytotoxic T lymphocytes directed against conserved HIV-1 epitopes. Med Immunol 2006; **5**:1.
50 Ahmed AR, Park MS, Tiwari JL, Terasaki PI. Association of DR4 with pemphigus. Exp Clin Immunogenet 1987; **4**:8–16.
51 Lombardi ML, Mercuro O, Ruocco V *et al.* Common human leukocyte antigen alleles in pemphigus vulgaris and pemphigus foliaceus Italian patients. J Invest Dermatol 1999; **113**:107–10.
52 Tong JC, Bramson J, Kanduc D, Chow S, Sinha AA, Ranganathan S. Modeling the bound conformation of pemphigus vulgaris-associated peptides to MHC class II DR and DQ alleles. Immunome Res 2006; **2**:1.
53 Delgado JC, Hameed A, Yunis JJ *et al.* Pemphigus vulgaris autoantibody response is linked to HLA-DQB1*0503 in Pakistani patients. Hum Immunol 1997; **57**:110–19.
54 Loiseau P, Lecleach L, Prost C *et al.* HLA class II polymorphism contributes to specify desmoglein derived peptides in pemphigus vulgaris and pemphigus foliaceus. J Autoimmun 2000; **15**:67–73.
55 Miyagawa S, Higashimine I, Iida T, Yamashina Y, Fukumoto T, Shirai T. HLA-DRB1*04 and DRB1*14 alleles are associated with susceptibility to pemphigus among Japanese. J Invest Dermatol 1997; **109**:615–18.
56 Lee E, Lendas KA, Chow S *et al.* Disease relevant HLA class II alleles isolated by genotypic, haplotypic, and sequence analysis in North American Caucasians with pemphigus vulgaris. Hum Immunol 2006; **67**:125–39.
57 Tron F, Gilbert D, Mouquet H *et al.* Genetic factors in pemphigus. J Autoimmun 2005; **24**:319–28.
58 Gazit E, Loewenthal R. The immunogenetics of pemphigus vulgaris. Autoimmun Rev 2005; **4**:16–20.
59 Ahmed AR, Wagner R, Khatri K *et al.* Major histocompatibility complex haplotypes and class II genes in non-Jewish patients with pemphigus vulgaris. Proc Natl Acad Sci USA 1991; **88**:5056–60.
60 Sinha AA, Brautbar C, Szafer F *et al.* A newly characterized HLA DQ beta allele associated with pemphigus vulgaris. Science 1988; **239**:1026–9.
61 Loewenthal R, Slomov Y, Gonzalez-Escribano MF *et al.* Common ancestral origin of pemphigus vulgaris in Jews and Spaniards: a study using microsatellite markers. Tissue Antigens 2004; **63**:326–34.
62 Glorio R, Rodriguez Costa G, Haas R, Gruber M, Fainboim L, Woscoff A. HLA haplotypes and class II molecular alleles in Argentinian patients with pemphigus vulgaris. J Cutan Med Surg 2002; **6**:422–6.
63 Brick C, Belgnaoui FZ, Atouf O *et al.* Pemphigus and HLA in Morocco. Transfus Clin Biol 2007; **14**:402–6.
64 Delgado JC, Yunis DE, Bozon MV *et al.* MHC class II alleles and haplotypes in patients with pemphigus vulgaris from India. Tissue Antigens 1996; **48**:668–72.
65 Carcassi C, Cottoni F, Floris L *et al.* HLA haplotypes and class II molecular alleles in Sardinian and Italian patients with pemphigus vulgaris. Tissue Antigens 1996; **48**:662–7.
66 Gonzalez-Escribano MF, Jimenez G, Walter K *et al.* Distribution of HLA class II alleles among Spanish patients with pemphigus vulgaris. Tissue Antigens 1998; **52**:275–8.
67 Delgado JC, Turbay D, Yunis EJ *et al.* A common major histocompatibility complex class II allele HLA-DQB1*0301 is present in clinical variants of pemphigoid. Proc Natl Acad Sci USA 1996; **93**:8569–71.
68 Chan LS, Hammerberg C, Cooper KD. Significantly increased occurrence of HLA-DQB1*0301 allele in patients with ocular cicatricial pemphigoid. J Invest Dermatol 1997; **108**:129–32.
69 Setterfield J, Theron J, Vaughan RW *et al.* Mucous membrane pemphigoid: HLA-DQB1*0301 is associated with all clinical sites of involvement and may be linked to antibasement membrane IgG production. Br J Dermatol 2001; **145**:406–14.
70 Drouet M, Delpuget-Bertin N, Vaillant L *et al.* HLA-DRB1 and HLA-DQB1 genes in susceptibility and resistance to cicatricial pemphigoid in French Caucasians. Eur J Dermatol 1998; **8**: 330–3.
71 Haider N, Neuman R, Foster CS, Ahmed AR. Report on the sequence of DQB1*0301 gene in ocular cicatricial pemphigoid patients. Curr Eye Res 1992; **11**:1233–8.
72 Carrozzo M, Fasano ME, Broccoletti R *et al.* HLA-DQB1 alleles in Italian patients with mucous membrane pemphigoid predominantly affecting the oral cavity. Br J Dermatol 2001; **145**:805–8.
73 Oyama N, Setterfield JF, Powell AM *et al.* Bullous pemphigoid antigen II (BP180) and its soluble extracellular domains are major autoantigens in mucous membrane pemphigoid: the pathogenic relevance to HLA class II alleles and disease severity. Br J Dermatol 2006; **154**:90–8.
74 Ahmed AR, Foster S, Zaltas M *et al.* Association of DQw7 (DQB1*0301) with ocular cicatricial pemphigoid. Proc Natl Acad Sci USA 1991; **88**:11579–82.
75 Hertl M, Karr RW, Amagai M, Katz SI. Heterogeneous MHC II restriction pattern of autoreactive desmoglein 3 specific T cell responses in pemphigus vulgaris patients and normals. J Invest Dermatol 1998; **110**:388–92.
76 Miyagawa S, Amagai M, Niizeki H *et al.* HLA-DRB1 polymorphisms and autoimmune responses to desmogleins in Japanese patients with pemphigus. Tissue Antigens 1999; **54**:333–40.
77 Saenz-Cantele AM, Fernandez-Mestre M, Montagnani S, Calebotta A, Balbas O, Layrisse Z. HLA-DRB1*0402 haplotypes without DQB1*0302 in Venezuelan patients with pemphigus vulgaris. Tissue Antigens 2007; **69**:318–25.
78 Huang CY, Chen TC. Bullous pemphigoid associated with systemic lupus erythematosus: the discrimination of antibasement membrane zone antibody. Int J Dermatol 1997; **36**:40–2.
79 Stoll DM, King LE Jr. Association of bullous pemphigoid with systemic lupus erythematosus. Arch Dermatol 1984; **120**:362–6.
80 Clayton CA, Burnham TK. Systemic lupus erythematosus and

coexisting bullous pemphigoid: immunofluorescent investigations. J Am Acad Dermatol 1982; **7**:236–45.
81 Sant SM, O'Loughlin S, Murphy GM. Bullous pemphigoid and rheumatoid arthritis: is there disease association? Ir J Med Sci 1997; **166**:106–7.
82 Hsu VM, Krey PR, Schwartz RA. Bullous pemphigoid and rheumatoid arthritis. Cutis 1989; **43**:30–2.
83 Giannini JM, Callen JP, Gruber GG. Bullous pemphigoid and rheumatoid arthritis. J Am Acad Dermatol 1981; **4**:695–7.
84 Walker MJ. Bullous pemphigoid and rheumatoid arthritis with herpes simplex in the vesicular fluid. Cutis 1977; **19**:93–4.
85 Yamamoti T, Yokoyama A, Mamada A, Miyazaki Y, Nishioka K. Familial occurrence of coexistence of bullous pemphigoid and Sjogren's syndrome. Int J Dermatol 1998; **37**:475–6.
86 James WD. Bullous pemphigoid, myasthenia gravis, and thymoma. Arch Dermatol 1984; **120**:397.
87 Kirtschig G, Walkden VM, Venning VA, Wojnarowska F. Bullous pemphigoid and multiple sclerosis: a report of three cases and review of the literature. Clin Exp Dermatol 1995; **20**:449–53.
88 Masouye I, Schmied E, Didierjean L, Abba Z, Saurat JH. Bullous pemphigoid and multiple sclerosis: more than a coincidence? Report of three cases. J Am Acad Dermatol 1989; **21**:63–8.
89 Nielsen NM, Frisch M, Rostgaard K *et al.* Autoimmune diseases in patients with multiple sclerosis and their first-degree relatives: a nationwide cohort study in Denmark. Mult Scler 2008; **14**: 823–9.
90 Malik M, Gurcan HM, Ahmed AR. Coexistence of mucous membrane pemphigoid and connective-tissue disease. Clin Exp Dermatol 2010; **35**:156–9.
91 Redman RS, Thorne EG. Cicatricial pemphigoid in a patient with systemic lupus erythematosus. Arch Dermatol 1981; **117**:109–10.
92 Spigel GT, Winkelmann RK. Cicatricial pemphigoid and rheumatoid arthritis. Arch Dermatol 1978; **114**:415–17.
93 Olsen KE, Holland EJ. The association between ocular cicatricial pemphigoid and rheumatoid arthritis. Cornea 1998; **17**:504–7.
94 Peyri J, Servitje O, Ribera M, Henkes J, Ferrandiz C. Cicatricial pemphigoid in a patient with rheumatoid arthritis treated with d-penicillamine. J Am Acad Dermatol 1986; **14**:681.
95 Spigel GT. Association of cicatricial pemphigoid and rheumatoid arthritis. Arch Dermatol 1979; **115**:108–9.
96 Malik M, Ahmed AR. Concurrence of systemic lupus erythematosus and pemphigus: coincidence or correlation? Dermatology 2007; **214**:231–9.
97 Chan HL. Pemphigus vulgaris associated with systemic lupus erythematosus. Int J Dermatol 1999; **38**:948.
98 Kuchabal DS, Kuchabal SD, Pandit AM, Nashi HK. Pemphigus vulgaris associated with systemic lupus erythematosus. Int J Dermatol 1998; **37**:636–8.
99 Fong PH, Chan HL. Systemic lupus erythematosus with pemphigus vulgaris. Arch Dermatol 1985; **121**:26–7.
100 Russell JG. Pemphigus, Sjogren's syndrome and mucosal pigmentation. Acta Stomatol Int 1987; **8**:25–9.
101 Meiner Z, Zlotogorski A, Brautbar C. Pemphigus associated with multiple sclerosis. Clin Exp Dermatol 1992; **17**:217.
102 Friedel J, Jeandel C, Abensour M, Heid E. Multiple sclerosis and autoimmune skin bullae: a case of pemphigus vulgaris. Dermatologica 1987; **175**:159–60.
103 Hamlet KR, Stevens SR, Gushurst C, Karabin G, Cooper KD. Juvenile pemphigus vulgaris associated with Graves' disease. J Am Acad Dermatol 1995; **33**:132–4.
104 Shabbir SG, Hassan M, Kazmi SA, Suhail S. Myasthenia gravis and pemphigus vulgaris. J Pak Med Assoc 1984; **34**:349–51.
105 Noguchi S, Nishitani H. [Case of myasthenia gravis associated with pemphigus vulgaris following thymectomy. Immunological studies and a review of the literature]. Rinsho Shinkeigaku 1976; **16**:625–32.
106 Shapiro M, Jimenez S, Werth VP. Pemphigus vulgaris induced by d-penicillamine therapy in a patient with systemic sclerosis. J Am Acad Dermatol 2000; **42**:297–9.
107 Maize JC, Dobson RL, Provost TT. Pemphigus and myasthenia gravis. Arch Dermatol 1975; **111**:1334–9.
108 Wolf R, Feuerman EJ. Pemphigus in association with autoimmune thyroid disease. Cutis 1981; **27**:423–4, 31.
109 Grandhe NP, Dogra S, Kanwar AJ. Multiple autoimmune syndrome in a patient with pemphigus vulgaris. Acta Derm Venereol 2005; **85**:91–2.
110 Nanda A, Kapoor MM, Dvorak R, Al-Sabah H, Alsaleh QA. Coexistence of pemphigus vulgaris with systemic lupus erythematosus. Int J Dermatol 2004; **43**:393–4.
111 Somorin AO, Agbakwu SN, Nwaefuna A. Systemic lupus erythematosus and pemphigus vulgaris preceded by depressive psychosis. Cent Afr J Med 1981; **27**:12–14.
112 Hidalgo-Tenorio C, Sabio-Sanchez JM, Tercedor-Sanchez J, Leon-Ruiz L, Perez-Alvarez F, Jimenez-Alonso J. Pemphigus vulgaris and systemic lupus erythematosus in a 46-y-old man. Lupus 2001; **10**:824–6.
113 Malik M, Ahmed AR. Dual diagnosis of pemphigus vulgaris and connective tissue disease. J Am Acad Dermatol 2006; **55**:699–704.
114 Somers EC, Thomas SL, Smeeth L, Hall AJ. Autoimmune diseases co-occurring within individuals and within families: a systematic review. Epidemiology 2006; **17**:202–17.
115 Panczel P, Falus A, Meretey K *et al.* [Association between cumulative familial incidence of type I diabetes and rheumatoid arthritis]. Orv Hetil 1985; **126**:1281–4, 7–9.
116 Thomas DJ, Young A, Gorsuch AN, Bottazzo GF, Cudworth AG. Evidence for an association between rheumatoid arthritis and autoimmune endocrine disease. Ann Rheum Dis 1983; **42**:297–300.
117 Becker KL, Titus JL, Woolner LB, Ferguson RH. Thyroiditis and rheumatoid arthritis. Proc Staff Meet Mayo Clin 1963; **38**:125–9.
118 Shiroky JB, Cohen M, Ballachey ML, Neville C. Thyroid dysfunction in rheumatoid arthritis: a controlled prospective survey. Ann Rheum Dis 1993; **52**:454–6.
119 Pongratz R, Buchinger W, Semlitsch G, Meister E, Nadler K, Rainer F. [Increased occurrence of autoimmune thyroiditis in patients with chronic rheumatoid arthritis]. Acta Med Austriaca 2000; **27**:58–60.
120 Alpigiani MG, Cerboni M, Bertini I *et al.* Endocrine autoimmunity in young patients with juvenile chronic arthritis. Clin Exp Rheumatol 2002; **20**:565–8.
121 Wynn DR, Rodriguez M, O'Fallon WM, Kurland LT. A reappraisal of the epidemiology of multiple sclerosis in Olmsted County, Minnesota. Neurology 1990; **40**:780–6.
122 Baker HW, Balla JI, Burger HG, Ebeling P, Mackay IR. Multiple sclerosis and autoimmune diseases. Aust NZ J Med 1972; **2**:256–60.
123 Midgard R, Gronning M, Riise T, Kvale G, Nyland H. Multiple sclerosis and chronic inflammatory diseases. A case–control study. Acta Neurol Scand 1996; **93**:322–8.
124 De Keyser J. Autoimmunity in multiple sclerosis. Neurology 1988; **38**:371–4.
125 Broadley SA, Deans J, Sawcer SJ, Clayton D, Compston DA.

Autoimmune disease in first-degree relatives of patients with multiple sclerosis. A UK survey. Brain 2000; **123**:1102–11.
126 Barcellos LF, Kamdar BB, Ramsay PP *et al.* Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study. Lancet Neurol 2006; **5**:924–31.
127 Heinzlef O, Alamowitch S, Sazdovitch V *et al.* Autoimmune diseases in families of French patients with multiple sclerosis. Acta Neurol Scand 2000; **101**:36–40.
128 Laroni A, Calabrese M, Perini P *et al.* Multiple sclerosis and autoimmune diseases: epidemiology and HLA-DR association in North-east Italy. J Neurol 2006; **253**:636–9.
129 Neuhausen SL, Steele L, Ryan S *et al.* Co-occurrence of celiac disease and other autoimmune diseases in celiacs and their first-degree relatives. J Autoimmun 2008; **31**:160–5.
130 Reunala T, Collin P. Diseases associated with dermatitis herpetiformis. Br J Dermatol 1997; **136**:315–18.
131 Asakawa H, Kashihara T, Fukuda H, Yamamoto M. A patient with thymoma and four different organ-specific autoimmune diseases. Neth J Med 2002; **60**:292–5.
132 McCluskey J, Farris AD, Keech CL *et al.* Determinant spreading: lessons from animal models and human disease. Immunol Rev 1998; **164**:209–29.
133 Mitchison NA. The carrier effect in the secondary response to hapten–protein conjugates. II. Cellular cooperation. Eur J Immunol 1971; **1**:18–27.
134 Lake P, Mitchison NA. Regulatory mechanisms in the immune response to cell-surface antigens. Cold Spring Harb Symp Quant Biol 1977; **41** (Pt 2):589–95.
135 Mamula MJ. Lupus autoimmunity: from peptides to particles. Immunol Rev 1995; **144**:301–14.
136 Mamula MJ, Janeway CA Jr. Do B cells drive the diversification of immune responses? Immunol Today 1993; **14**:151–2; discussion 3–4.
137 Roth R, Gee RJ, Mamula MJ. B lymphocytes as autoantigen-presenting cells in the amplification of autoimmunity. Ann NY Acad Sci 1997; **815**:88–104.
138 Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature 1992; **358**:155–7.
139 Chan LS, Vanderlugt CJ, Hashimoto T *et al.* Epitope spreading: lessons from autoimmune skin diseases. J Invest Dermatol 1998; **110**:103–9.
140 Chan LS, Soong HK, Foster CS, Hammerberg C, Cooper KD. Ocular cicatricial pemphigoid occurring as a sequela of Stevens–Johnson syndrome. JAMA 1991; **266**:1543–6.
141 Paisansinsup T, Deshmukh US, Chowdhary VR, Luthra HS, Fu SM, David CS. HLA class II influences the immune response and antibody diversification to Ro60/Sjogren's syndrome-A: heightened antibody responses and epitope spreading in mice expressing HLA-DR molecules. J Immunol 2002; **168**:5876–84.