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The molecular basis for the presence of two autoimmune diseases occurring simultaneously – Preliminary observations based on computer analysis

LABIB R. ZAKKA[1], PEDRO A. RECHE[2], & A. RAZZAQUE AHMED[1]

[1]*Center for Blistering Diseases, Boston, Massachusetts, USA, and* [2]*Immunomedicine Group, Department of Immunology, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain*

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Abstract
Specific Human Leukocyte Antigen Class II (HLA II) molecules associated with pemphigus vulgaris (PV), mucous membraine pemphigoid (MMP), and mixed connective tissue disease (MCTD) may react with multiple T cell epitopes within desmoglein 3 (Dsg 3), bullous pemphigoid antigen 2 (BPAG 2), and 70 kDa polypeptide small nuclear ribonucleoproteins (snRNP70) in autoantibody production. We report a group of patients with simultaneous occurrences of PV with MCTD, and MMP with MCTD. In one patient group, we performed serological studies to show presence of antibodies to Dsg 3, Dsg 1, and snRNP70 simultaneously. In the second group, we performed serological studies to show presence of antibodies to BPAG 1, BPAG 2, β4 integrin, and snRNP70 simultaneously. In both groups, HLA II genes were analyzed and the observations were consistent with previously described associations with PV, MMP, and MCTD. It is possible that HLA-DQβ1*0301 allele, present in 10 of 17 patients and DRβ1*04 in some of the others, may have the ability to bind to several relevant T cell epitopes in the snRNP70 molecule. We have utilized a computer model to demonstrate that HLA II-restricted T cell epitopes present within the known autoantigens may be capable of eliciting an immune response. While other explanations and mechanisms exist, the authors suggest that epitope spreading may be one possible mechanism, amongst others, that may result in the simultaneous presence of two separate pathogenic autoantibodies.

Keywords: *MHC Class II Genes, Epitope Spreading, Pemphigus Vulgaris, Mucous Membrane Pemphigoid, Mixed Connective Tissue Disease*

Introduction

Pemphigus Vulgaris (PV) is a potentially fatal autoimmune mucocutaneous blistering disease (AMBD). Patients with PV present with flaccid blisters, affecting the skin as well as the mucosal surfaces, that rupture easily leaving denuded epithelium [1–4]. Histology shows an intraepidermal vesicle, and keratinocyte cell surface antibody deposition is seen on immunopathology [1–7]. The target antigens in PV are Desmoglein 1 (Dsg 1), Desmoglein 3 (Dsg 3) [8], and possibly the acetylcholine receptor [9]. Disease severity and activity may correlate with serum autoantibody titers [8,10–12].

Mucous Membrane Pemphigoid (MMP), Also known as Cicatricial Pemphigoid (CP), is an AMBD that affects the elderly. Patients present with a mean age of onset of 73 years [13]. MMP affects the mucous membranes of the conjunctiva, nose, oral mucosa, oesophagus, larynx, pharynx, genitalia, anal canal, and the skin [2–4,6,7,14,15]. Upon healing, the lesions produce irreversible scaring [2–4,6,7,14,15]. The antigens targeted by autoantibodies in MMP are a 230 kDa protein called Desmoplakin, also referred to as Bullous Pemphigoid Antigen 1 (BPAG 1), a 180 kDa hemidesmosome protein, also referred to as Bullous Pemphigoid Antigen 2 (BPAG 2), and human β4 integrin subunit [16,17].

Mixed Connective Tissue Disease (MCTD) is a systemic autoimmune disease with overlapping features of systemic lupus erythematosus (SLE),

Correspondence: A. Razzaque Ahmed, Center for Blistering Diseases, 70 Parker Hill Avenue, Boston, MA, USA 02120,
E-mail: arahmedmd@msn.com

dermatomyositis, rheumatoid arthritis, scleroderma, and polymyositis [18–21]. Primary symptoms of MCTD include arthralgias, Reynaud's Phenomenon, esophageal reflux or dysmotility, and finger swelling or diffuse hand swelling among others [19–32] (Table I). The primary cause of death in patients with MCTD is pulmonary disease [19,22,23]. The target antigen is a complex of small nuclear ribonucleoproteins (snRNP), including a 70 kDa polypeptide (snRNP70), that are non-covalently linked with the U1 ribonucleoprotein (U1-RNP) of the spliceosome complex [19,33,34]. It has been reported that the snRNP70 contains the major antigenic epitopes to which MCTD sera react [35–37].

Antibodies directed against protein antigens are produced by B cells engaging CD4 helper T cells. The mechanism occurs as follows: Protein antigens recognized by B cells through their B cell receptor (BCR) are internalized and processed [38]. Peptide antigens resulting from such processing are loaded onto Major Histocompatibility Complex Class II (MHC II) molecules, Human Leukocyte Antigen Class II (HLA II) molecules in humans, and expressed on the cell surface of B cells [38].

CD4 T cells bearing the appropriate T cell receptors (TCR) can then recognize these cell surface expressed HLA II-peptide antigens [38]. Only those B cells displaying CD4 T cell epitopes (HLA II-peptide antigens that are recognized by CD4 T cells) are subsequently capable of producing antibodies against the original antigen [38]. By this same mechanism, CD4 helper T cells lead to the production of pathogenic autoantibodies after recognizing T cell epitopes from self-antigens [39].

Earlier clinical studies have demonstrated that certain patients who have clinical, histological, and immunopathological features of PV have MCTD present simultaneously [40]. Likewise, certain patients clinically presenting as MMP have MCTD simultaneously [18]. The treatment and the clinical outcomes of these patients have been described in those publications [18,40]. The purpose of this study was to identify potential autoreactive HLA II-restricted helper T cell epitopes shared among PV, MMP, and MCTD that could explain the simultaneous occurrence of these relatively rare three diseases.

Table I. Major clinical features of MCTD*.

	% involvement
• Arthritis/arthralgia	95
• Raynauds	85
• Oesophageal involvement	67
• Impaired lung diffusion	67
• Swollen hands	66
• Myositis	63
• Scleroderma	33
• Serositis	27
• Renal disease	10
• Cerebral involvement	10

*Venables PJW. Mixed connective tissue disease. *Lupus* 2006;15(4):132–137. © 2011 by Sage Publications. Reprinted by permission of SAGE.

Methods

Patients

This study reports on two groups of patients. These patients presented to the Center for Blistering Diseases (CBD) in Boston between March 2001 and November 2009. An Institutional Review Board (IRB) approval was obtained as well as a written consent from each patient. It is important to note that the patients were diagnosed prior to their evaluation at the CBD and were referred to the CBD for the management of their complex disease. During the diagnostic work-up, a thorough serological evaluation was done. During this evaluation, it was observed that the patients had antibodies to snRNP70. In addition, in a review of systems, patients were found to have symptoms of MCTD. Since two relatively rare diseases were present in a group of patients, HLA typing for HLA Class II genes was performed.

Inclusion criteria

(1) Upon their initial diagnosis, the patients had histology, confirmed by direct immunofluorescence, for MMP and PV.
(2) Careful history of medications was taken and patients were not included if they were on medications known to be associated with inducing either MCTD, MMP or PV [41–45].
(3) All these patients with PV had antibodies to keratinocyte cell surface as determined by indirect immunofluorescence (IIF) using monkey esophagus as substrate. In addition, on Enzyme-Linked Immunosorbent Assay (ELISA), patients demonstrated high levels of antibodies to Dsg 1 and Dsg 3.
(4) Patients with MMP had antibodies to human β4 integrin subunit as determined by an immunoblot assay using Bovine Gingival Lysate as substrate. The presence of a 205 kDa band was considered the binding of the antibody in the patient sera to human β4 integrin subunit. The positive control was UMA6 antibody. The sera was negative for presence of antibody to human α6 integrin subunit. In addition, IIF was done to determine binding of patient sera to proteins in the Basement Membrane Zone (BMZ) and the antibody titers in the patient sera to BPAG1 and BPAG2 were determined by a standard ELISA.
(5) The patients met the criteria set by Alarcon-Segovia [46,47] to be considered as having MCTD.

(6) The presence of antibodies to snRNP70 in the sera of the patients tested on at least three different days at monthly intervals.

Patients

Eleven patients with extensive mucocutaneous PV were studied. Each patient had minimal 25–30% or more of body surface involvement with at least 2 mucosae involved. These patients had not responded to a mean dose of 125 mg/day of Prednisone and were non-responsive to at least 2 more immunosuppressive therapies. One feature that distinguishes these patients from other PV patients is that their disease was considered severe, widespread and recalcitrant to conventional immunosuppressive therapy.

Six patients with MMP are presented. These 6 patients all had extensive erosions in the oral cavity, pharynx, and nasal disease. Three patients had Ocular Cicatricial Pemphigoid (OCP) and 2 had vaginal disease. Each of the 6 patients had been treated intermittently with systemic corticosteroids and at least 3 or more immunosuppressive agents. All had received Dapsone therapy. Like the PV subsets, these MMP patients had progressive worsening disease with minimal benefit from conventional immunosuppressive therapy.

Serological analysis

In both groups of patients, antibodies to Dsg 1, Dsg 3, BPAG 1, and BPAG 2 were measured using an ELISA [12,48–50]. The index values of the ELISAs for BPAG 1 and BPAG 2 are identical: fewer than 9 is negative and greater than 9 is positive. The Dsg 3 ELISA index values are: Fewer than nine is negative, 9–20 is intermediate, and greater than 20 is positive. The Dsg 1 ELISA index values are: Less than 14 is negative, 9–20 is intermediate, and more than 20 is positive. Antibodies to human β4 integrin subunit and α6 integrin subunit were done by an immunoblot using bovine gingival lysate as substrate [51].

Patient sera in both groups was evaluated by IIF using monkey esophagus as substrates which measured the titers of the antibodies to intercellular cement substance (ICS) and BMZ proteins. The serological tests for antibodies to Dsg 3, Dsg 1, BPAG 1, BPAG 2, and snRNP70 were performed by laboratories at hospitals from where the patients were referred, and some by Beutner Laboratories, Buffalo, NY.

HLA Class II genes

Peripheral blood leukocytes obtained from 11 patients with PV and MCTD, and six patients with MMP and MCTD were drawn during the active stage of their diseases and studied for HLA II genes. We used the Polymerase Chain Reaction with Sequence Specific Primers (PCR-SSP) to identify HLA II genes encoded by the DRβ1 and DQβ1 loci as described [52]. The PCR-SSP was performed by the referring institutions, and some by the American Red Cross, HLA Lab in Dedham, MA.

Prediction of HLA II-restricted CD4 T cell epitopes. CD4 helper T cell cooperation can only be delivered upon the recognition of peptides bound to HLA II molecules (CD4 T cell epitopes). Therefore, we used peptide binding predictions to HLA II molecules as the basis for anticipating CD4 T cell epitopes [53]. Thus, we anticipated HLA II-restricted CD4 T cell epitopes in Dsg 3, BPAG 2, and snRNP70 using RANKPEP (http://imed.med.ucm.es/Tools/rankpep.html) [54,55]. RANKPEP implements motif profiles (position specific scoring matrix) derived from peptides known to bind to MHC as the basis to predict peptide-MHC binding [54–56] and, subsequently, T cell epitopes.

Specifically, we selected the relevant motif profiles to predict HLA-DQ7 (HLA-DRQ1*0301) and HLA-DR4 (HLA-DRB1*0402) restricted CD4 T cell epitopes in Dsg 3, BPAG 2, and snRNP70. Only those peptides that had a binding score above the binding threshold (BT) were considered as CD4 T cell epitopes. The BT is a valuable index in determining the potential capability of the T cell epitope in triggering T cell response in-vivo (details elsewhere in Reche et al. [54–56]).

Results

High resolution HLA II gene analysis By PCR-SSP

Only patients were studied without their families. However, based on linkage disequilibrium studies from HLA international workshops, the data and their known associations have been written as haplotypic rather than phenotypic.

Group 1: Patients simultaneously having pemphigus vulgaris and mixed connective tissue disease. Data on HLA typing is presented in Table I. Patients in this group had a diagnosis of PV and MCTD. This group consisted of 11 patients.

Serology results. All 11 patients had high antibody titers to Dsg 1 and Dsg 3, as measured by ELISA, that were high. The IIF titers of anti-ICS antibodies were also very high. All 11 patients had presence of antibodies to snRNP70. This data is presented in Table II.

High resolution HLA II gene analysis by PCR-SSP demonstrated that 6 patients had HLA-DRβ1*0402 and 8 patients carried DQβ1*0503. Five patients did not carry the HLA-DR4 molecule. Also, 6 patients carried HLA-DQβ1*0301 and of those patients, 4 did

Table II. Human Leukocyte Antigen Class II gene analysis of patients with *Pemphigus Vulgaris* (PV) and Mixed Connective Tissue Disease (MCTD).

	Group 1							
	HLA II							
	1st Haplotype		2nd Haplotype		Serology			
Patient	DRβ1	DQβ1	DRβ1	DQβ1	Anti-Dsg 1	Anti-Dsg 3	IIF anti-ICS antibodies	Anti-snRNP70
1	402	302	1401	503	110	177	640	+
2	402	302	1401	503	168	335	320	+
3	402	302	1401	503	110	201	160	+
4	402	302	1101	301	88	143	160	+
5	402	302	1104	301	41	171	320	+
6	402	302	1301	603	77	127	160	+
7	1101	301	1401	503	81	156	320	+
8	701	301	1401	503	79	148	320	+
9	1103	301	1401	503	82	63	640	+
10	1101	301	1401	503	107	189	80	+
11	1501	609	1401	503	121	233	160	+

(HLA II = Human Leukocyte Antigen Class II; Dsg = Desmoglein; IIF = Indirect immunofluorescence; ICS = Intercellular Cement Substance; RNP = Ribonucleoprotein; Dsg 1 ELISA Index Values: <14 = Negative; 14–20 = Indeterminate; >20 = Positive [12,45]; Dsg 3 ELISA Index Values: <9 = Negative; 9–20 = Indeterminate; >20 = Positive [12,45]).

not carry the HLA-DR4 molecule. Also, four patients carried HLA-DQβ1*0302 and 1 patient carried the HLA-DQβ1*0603. One patient did not carry neither HLA-DR4 nor HLA-DQβ1*0301.

Group 2: Patients simultaneously having mucous membrane pemphigoid and mixed connective tissue disease. Data on HLA typing is presented in Table II. Patients in this group had a diagnosis of MMP and MCTD. This group consisted of 6 patients.

Serology results. All 6 patients had antibody titers to BPAG 1 and BPAG 2, as measured by ELISA, that were high. The IIF titers for anti-BMZ antibodies were also very high. Antibodies to the subunits of human α6 were negative, and all 6 patients had antibodies to the subunit of human β4 integrin. All 6 patients had presence of antibodies to snRNP70. This data is presented in Table III.

High resolution HLA II gene analysis by PCR-SSP demonstrated that 3 patients carried the HLA-DR4 molecule. Four patients carried HLA-DQβ1*0301, and of those patients 2 were homozygous for HLA-DQβ1*0301, and 2 did not carry the HLA-DR4 molecule. One patient carried HLA-DQβ1*0302 allele with no HLA-DR4 molecule, and one patient carried both HLA-DQβ1*0302 and HLA-DQβ1*0603.

CD4 T cell epitopes associated with HLA Class II DRβ1*0402 and DQβ1*0301-genes

The HLA II genes associated with PV, MMP, and MCTD and the relevant antigens, when subjected to the RANKPEP program, demonstrate that there are potential T cell epitopes within snRNP70 (Figure 1), Dsg 3 (Figure 2), and BPAG 2 (Figure 3) that are predicted to bind to DRβ1*0402. Also, the RANKPEP program demonstrates potential T cell epitopes within snRNP70 (Figure 1), Dsg 3 (Figure 2), and BPAG 2 (Figure 3) that are predicted to bind to DQβ1*0301.

One study reported increased binding of T cells within amino acid sequence 374–437 of the snRNP70 in patients with MCTD [37]. However, the study did not report the HLA II allele of the patients studied. The RANKPEP program shows binding of snRNP70 to HLA-DQ7 on amino acid 373–384 (Figure 1). Also, the RANKPEP program showed binding of BP180 to HLA-DQ7 on amino acid 505–513, 635–643, 765–773, 841–849, 1055–1063, 1192–1210, 1283–1291, and 1320–1328 among others (Figure 3), in accordance with the literature [57–60]. Moreover, the RANKPEP program shows binding of Dsg 3 to HLA (DR4)DRβ1*0402 on amino acid 193–200, 217–225, 765–774, 605–613 in accordance with the literature [61–63]. Therefore, it should be noted that the RANKPEP program is reliable in predicting antigen-MCH II binding.

Discussion and conclusion

In this report we are presenting 11 patients who simultaneously had the clinical and serological diagnosis of PV and MCTD. We also report 6 patients who simultaneously had the clinical and serological features of MMP and MCTD. Clinical data on some

Table III. Human Leukocyte Antigen Class II gene analysis of patients with mucous membrane pemphigoid (MMP) and Mixed Connective Tissue Disease (MCTD). ((HLA II = Human Leukocyte Antigen Class II; BPAG = Bullous Pemphigoid Antigen; IIF = Indirect immunofluorescence; BMZ = Basement Membrane Zone; RNP = Ribonucleoprotein; BPAG1 and BPAG2 Index Values: <9 = Negative; >9 = Positive [43,44]).

Group 2											
	HLA II				Serology						
	1st Haplotype		2nd Haplotype								
Patient	DRβ1	DQβ1	DRβ1	DQβ1	Anti-BPAG 1	Anti-BPAG 2	IIF anti-BMZ antibodies	Anti-β4 antibodies	Anti-α6 antibodies	Anti-snRNP70
1	701	501	101	302	27	149	160	+	–	+
2	408	301	408	301	47	130	320	+	–	+
3	402	302	1104	603	88	320	640	+	–	+
4	301	201	1104	301	121	132	80	+	–	+
5	407	302	4011	301	76	87	160	+	–	+
6	1101	301	1101	301	74	82	320	+	–	+

of these patients have been reported previously [18,40].

All the patients in Group 1 had either the HLA II alleles DRβ1*0402 or DQβ1*0503 that have been reported in patients with PV in several studies [64–80]. However, only six patients had HLA-DR4 molecule, while the remaining 5 patients did not carry any allele known to be associated with MCTD [23,32,81–88]. Moreover, ten patients carried either DQβ1*0301, DQβ1*0302, or DQβ1*0603 alleles, four of whom did not carry HLA-DR4 molecule. Nonetheless, these 4 patients had the clinical features and serological markers typically associated with MCTD. It has been reported by Delgado et al. that DQβ1*0302, *0303, *0305, *0602 and *0603 allele share amino acid sequences 71–77 (Thr-Arg-Ala-Glu-Leu-Val-Thr) with *0301 [89]. Therefore, it is possible that these alleles have peptide binding specificities similar to DQβ1*0301.

All of the patients in group 2 carried either the HLA II alleles DQβ1*0301, *0302, or *0603 that have been reported in patients with MMP [17,89–92]. However, only three of six patients also carried the HLA-DR4 molecule, while the remaining 3 patients did not carry any allele known to be associated with MCTD [23,32,81–88]. Nonetheless, these 3 patients had clinical features and serological markers typically associated with MCTD.

The authors suggest that an immunogenetic basis may exist to explain the unique observations presented, while highlighting that there could be several other explanations. Although no other studies exist showing the presence of MMP and MCTD or PV and MCTD simultaneously in a patient, MMP and PV have been reported with many other autoimmune diseases. MMP has been reported in patients with SLE and Rheumatoid Arthritis [93–96]. Also, PV has been reported with SLE, Sjogren's Syndrome, and Systemic Sclerosis [97–105]. Moreover, there are multiple reports in the literature describing patients with PV and MMP simultaneously [8]. One study reported that DQβ1*0301 homozygous typing T cell lines from patients with PV proliferated in reaction to Dsg 3 peptide [106].

In the opinion of the authors, based on the computer modeling and several reports in the literature documenting the presence of these 2 diseases simultaneously in one patient, the phenomenon of epitope spreading may provide a possible explanation for the simultaneous presence of 2 autoimmune diseases in these 2 groups of patients presented in this report. Four possible scenarios of epitope spreading exist. The first scenario may be that one large antigen molecule may contain at least 2 similar epitopes with possible sequence overlap, and when 2 different HLA II molecules present them, they stimulate two different T cells [107].

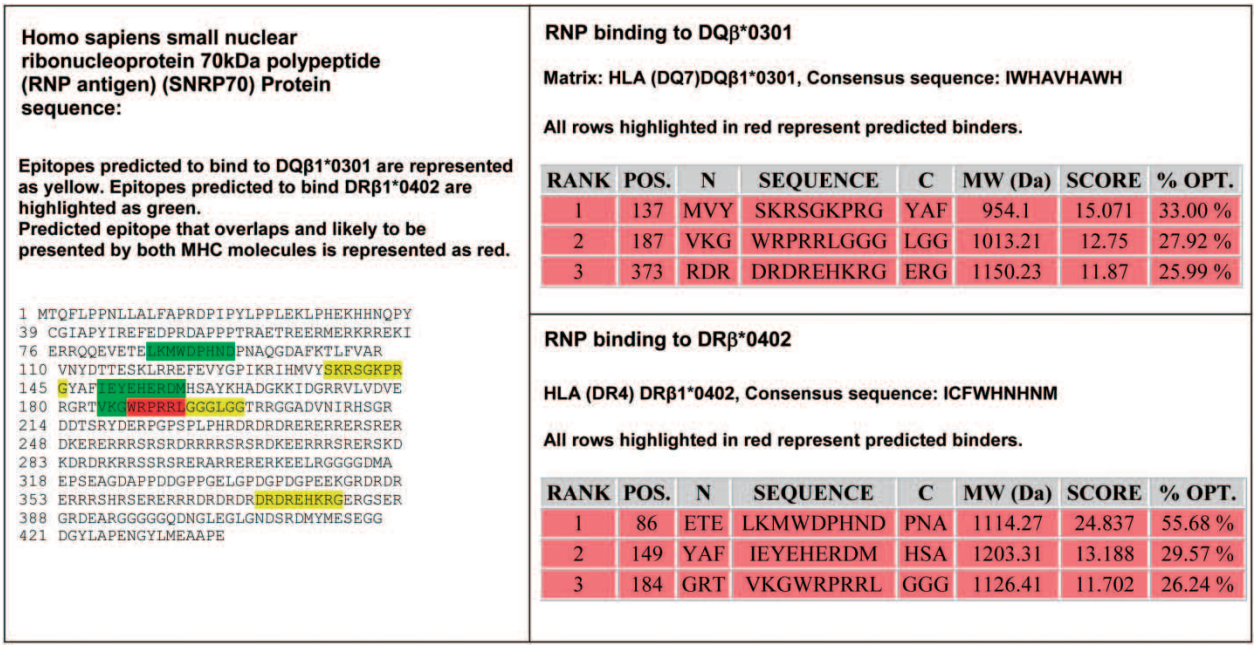


Figure 1. Predicted CD4 T cell epitopes in small nuclear ribonucleoproteins 70kDa polypeptide (snRNP70). Potential snRNP70-specific CD4 T cell epitopes restricted by HLA-DQ7(DQB1*0301) and HLA-DRβ1*0402 are shown in yellow and green, respectively. T cell epitopes that are predicted to bind both HLA-DRβ1*0402 and HLA-DQ7(DQB1*0301), are show in red. All shown peptides exceed the Binding Threshold (see Methods for details). POS. = Position; N = N-terminal; C = C-terminal; MW = Molecular Weight (Daltons); %OPT. = Optimal Score.

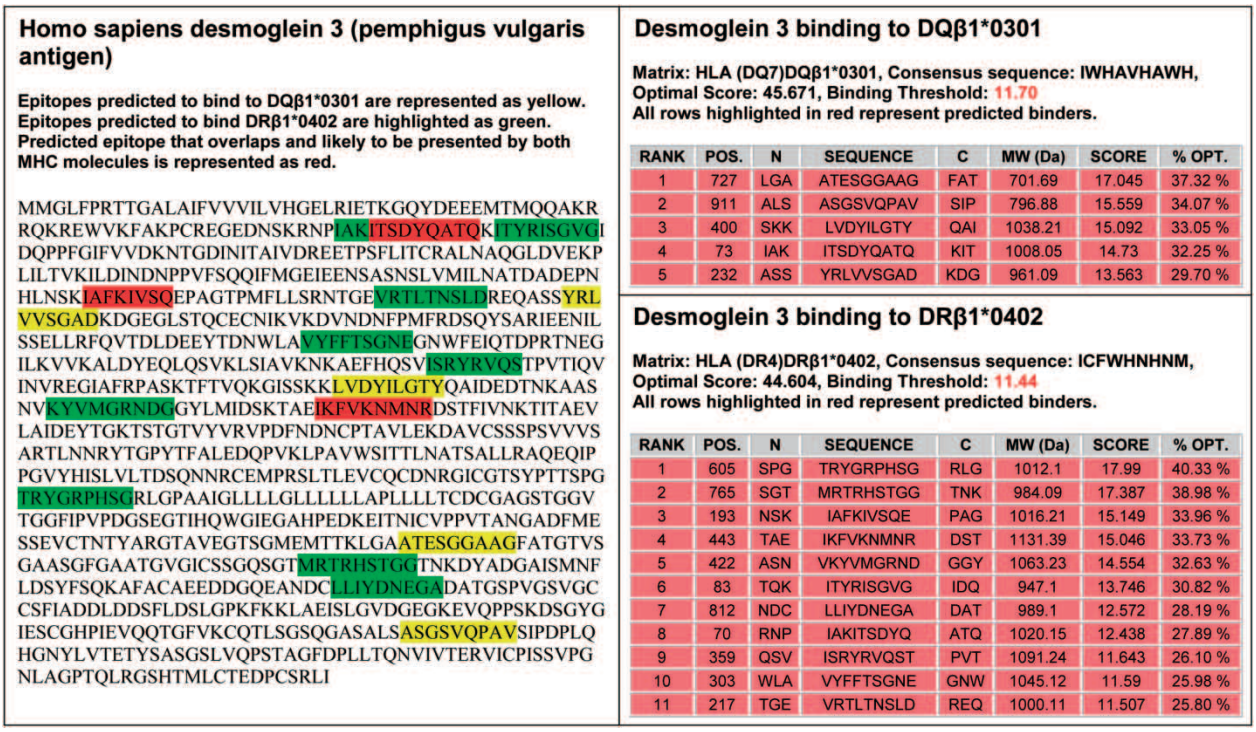
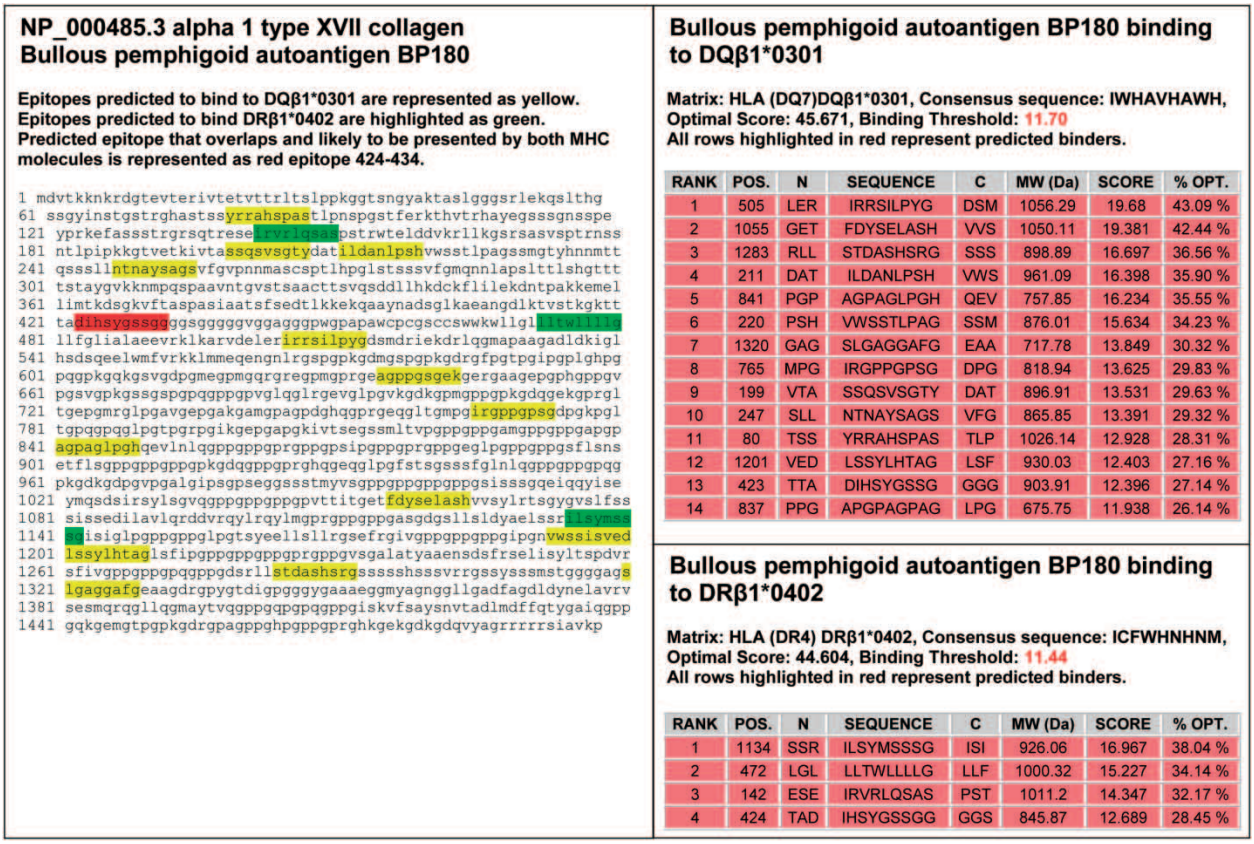


Figure 2. Predicted CD4 T cell epitopes in Desmoglein 3 (Dsg 3). Potential Dsg 3-specific CD4 T cell epitopes restricted by HLA-DQ7(DQB1*0301) and HLA-DRβ1*0402 are shown in yellow and green, respectively. T cell epitopes that are predicted to bind both HLA-DRβ1*0402 and HLA-DQ7(DQB1*0301), are show in red. All shown peptides exceed the Binding Threshold (see Methods for details). POS. = Position; N = N-terminal; C = C-terminal; MW = Molecular Weight (Daltons); %OPT. = Optimal Score.



NP_000485.3 alpha 1 type XVII collagen Bullous pemphigoid autoantigen BP180

Epitopes predicted to bind to DQβ1*0301 are represented as yellow. Epitopes predicted to bind DRβ1*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 mdvtkknkrdgtevterivtetvttrltslppkggtsngyaktaslgggsrlekqsltha
61 ssgyinstgstrghastssyrrahspastlpnspgstferkthvtrhayegssssgnsspe
121 yprkefassstrgrsqtreselrvrlqsaspstrwteldvkrllkgsrsasvsptrnss
181 ntlpipkkgtvetkivtassqsvsgtydatildanlpshvwsstlpagssmgtyhnnmtt
241 qsssllntnaysagsvfgvpnnmascsptlhpglstsssvfgmqnnlapslttlshgttt
301 tstaygvkknmpqspaavntgvstsaacttsvqsddllhkdckflilekdntpakkemel
361 limtkdsgkvftaspasiaatsfsedtlkkekqaaynadsglkaeangdlktvstkgktt
421 tadihsygssggggsgggggvggagggpwgpapawcpcgsccswwkwllgllltwllllg
481 llfglialaeevrklkarvdelerirrsilpygdsmdriekdrlqgmapaagadldkigl
541 hsdsqeelwmfvrkklmmeqengnlrgspgpkgdmgspgpkgdrgfpgtpgipgplghpg
601 pqgpkgqkgsvgdpgmegpmgqrgreqpmgprgeagppgsgekgergaagepgphgppgv
661 pgsvgpkgssgspgpqgppgpvglqglrgevglpgvkgdkgpmgppgpkgdqgekgprgl
721 tgepgmrglpgavgepgakgamgpagpdghqgprgeqqltgmpgirgppgpsgdpgkpgl
781 tgpqgpqglpgtpgrpgikgepgapgkivtsegssmltvpgppgppgamgppgppgapgp
841 agpaglpghqevlnlqgppgppgprgppgpsipgppgprgppgeglpgppgppgsflsns
901 etflsgppgppgppgpkgdqgppgprghqgeqglpgfstsgsssfglnlqgppgppgpqg
961 pkgdkgdpgvpgalgipsgpseggssstmyvsgppgppgppgppgpsisssgqeiqqyise
1021 ymqsdsirsylsgvqgppgppgppgpvttitgetfdyselashvvsylrtsgygvslfss
1081 sissedilavlqrddvrqylrqylmgprgppgppgasgdgslllsldyaelssrilsymss
1141 sgisiglpgppgppglpgtsyeellsllrgsefrgivgppgppgppgipgnvwssisved
1201 lssylhtaglsfipgppgppgppgprgppgvsgalatyaaensdsfrselisyltspdvr
1261 sfivgppgppgpqgppgdsrllstdashsrgsssshsssvrrgssyssmstggggags
1321 lgaggafgeaagdrgpygtdigpgggygaaaeggmyagnggllgadfagdldynelavrv
1381 sesmqrqgllqgmaytvqgppgqpgpqgppgiskvfsaysnvtadlmdffqtygaiqgpp
1441 gqkgemgtpgpkgdrgpagppghpgppgprghkgekgdkgdqvyagrrrrrsiavkp
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Bullous pemphigoid autoantigen BP180 binding to DQβ1*0301

Matrix: HLA (DQ7)DQβ1*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 %

Bullous pemphigoid autoantigen BP180 binding to DRβ1*0402

Matrix: HLA (DR4) DRβ1*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 %

Figure 3. Predicted CD4 T cell epitopes in Bullous Pemphigoid Antigen 2 (BP180). Predicted BP180-specific CD4 T cell epitopes restricted by HLA-DQ7(DQB1*0301) and HLA-DRβ1*0402 are shown in yellow and green, respectively. T cell epitopes that are predicted to bind both HLA-DRβ1*0402 and HLA-DQ7(DQB1*0301), are show in red. All shown peptides exceed the Binding Threshold (see Methods for details). POS. = Position; N = N-terminal; C = C-terminal; MW = Molecular Weight (Daltons); %OPT. = Optimal Score.

These T cells, through CD40-CD40L interaction, then stimulate two different B cells to produce two different antibodies. The second scenario may be that an Antigen Presenting Cell (APC) may internalize two separate antigens. The APC then stimulates 2 different T cells, each specific to either of the antigens [107–109]. The third scenario may be that a macromolecule complex of 2 or more antigens is internalized by an APC that stimulates two different T cells, making each specific for one antigen within the macromolecule [107,110–112].

Through CD40-CD40L interaction, B cells are enlisted resulting in the production of two different autoantibodies. Finally, the fourth scenario may be that the surrounding tissue gets damaged as a result of the presence of one autoimmune disease [107,113,114]. The damage exposes the immune system to a previously sequestered epitope, which is then internalized by an APC that stimulates a T cell that, in turn, recruits a B cell resulting in the production of a second antibody.

In patients with Stevens-Johnson syndrome, it has been demonstrated that if extensive inflammation and damage to surrounding tissue ensues, the patients often develop OCP [115]. Moreover, it has been found that the amount and type of antibodies produced are influenced by the MHC II genes [116]. Furthermore, studies indicate that binding groove flanking residues of core nonameric peptides play a role in peptide selection because the perfect fitting of the peptide residues within the HLA II allele binding groove cannot ensure that the whole peptide will fit completely [66].

Based on the studies by Reche et al. [54–56] using RANKPEP program, we have shown that MMP, PV, and MCTD antigens bear CD4 T cell epitopes restricted by DRβ1*0402 and DQβ1*0301 alleles that can stimulate an immune response (Figures 1, 2, and 3). There are patients who simultaneously have PV and MCTD and MMP and MCTD but lack the HLA II genes associated with MCTD. Nonetheless, the sera of these patients contain antibodies to snRNP70 and have the clinical features of MCTD. Therefore, within the four scenarios, it is theoretically possible for the patients who lack the HLA II genes associated with MCTD to benefit from the presence of DRβ1*0301 to produce autoantibodies to snRNP70 based on epitope spreading. This explanation provides a possible molecular basis to the presence of these two autoimmune diseases occurring

in the group of patients described in this manuscript. Also, Since DQβ1*0302 and *0603 alleles have peptide binding specificities similar to DQβ1*0301, it is also hypothetically possible for those alleles to produce a similar effect as *0301.

Although the model presented in this manuscript is hypothetical, it provides a basis in which it demonstrates that the molecular structure and the binding properties of the HLA II genes and the possible T cell epitopes within the relevant antigens may play a critical role in the simultaneous presence of two autoimmune diseases in the patients described in the study. Also, it follows that the T and B cells stimulated are specific leading to the production of specific autoantibodies. It is important to highlight that the authors are aware that this is not the only explanation to the observed phenomena, but are utilizing the available data in order to propose a possible explanation. It is also possible that other unknown genetic or non-genetic factors may have resulted in the production of two autoimmune diseases simultaneously in the same patient.

The genetic factors were emphasized because the patients are all unrelated. The authors acknowledge that the major limitation of this study is the fact that it lacks in-vitro studies in which T cells from these patients were simultaneously stimulated with the relevant T cell epitopes described in the manuscript, and proliferative responses were demonstrated. However, the authors felt that providing the information from this computer model to the readership would be of significant benefit to investigators in this field, because they have provided a rational basis for designing and conducting future experiments.

These observations are of clinical and biological importance. These studies demonstrate that, in some individuals with an inherited genetic predisposition towards autoimmune disease, the immune system may be aversely stimulated to produce two autoimmune diseases. Conventional therapy may then be less effective, and the course of the illness may become more chronic. This combination of two autoimmune diseases occurring simultaneously has the potential to influence the patient's morbidity and possible mortality, and poses a challenge to the treating physicians.

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References

[1] Ahmed AR, Graham J, Jordon RE, et al. Pemphigus: current concepts. Ann Intern Med 1980;92:396–405.

[2] Nousari HC, Anhalt GJ. Pemphigus and bullous pemphigoid. Lancet 1999;354:667–672.

[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;100:219–227.

[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;43:571–591; quiz 91–4.

[7] Scully C. A review of common mucocutaneous disorders affecting the mouth and lips. Ann Acad Med Singapore 1999; 28:704–707.

[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;201:290–295.

[10] Weissman V, Feuerman EJ, Joshua H, et al. The correlation between the antibody titers in sera of patients with *Pemphigus vulgaris* and their clinical state. J Invest Dermatol 1978;71: 107–109.

[11] Amagai M, Tsunoda K, Zillikens D, et al. The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile. J Am Acad Dermatol 1999;40: 167–170.

[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;159:2010–2017.

[13] Sami N, Yeh SW, Ahmed AR. Blistering diseases in the elderly: diagnosis and treatment. Dermatol Clin 2004;22: 73–86.

[14] McCuin JB, Hanlon T, Mutasim DF. Autoimmune bullous diseases: diagnosis and management. Dermatol Nurs 2006; 18:20–25.

[15] Scott JE, Ahmed AR. The blistering diseases. Med Clin North Am 1998;82:1239–1283.

[16] Wojnarowska F, Venning VA, Burge SM. Immunobullous Diseases. In: Burns T, Breathnach S, Cox N, Griffiths C, editors. Rook's Textbook of Dermatology (Seventh Edition). 7th ed. Malden, MA: Blackwell Science Ltd; 2008. p 60.

[17] 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;91:7747–7751.

[18] Malik M, Gurcan HM, Ahmed AR. Coexistence of mucous membrane pemphigoid and connective-tissue disease. Clin Exp Dermatol 2009;35:156–159.

[19] Hoffman RW, Maldonado ME. Immune pathogenesis of Mixed Connective Tissue Disease: a short analytical review. Clin Immunol 2008;128:8–17.

[20] Aringer M, Steiner G, Smolen JS. Does mixed connective tissue disease exist? Yes. Rheum Dis Clin North Am 2005;31: 411–420, v.

[21] Swanton J, Isenberg D. Mixed connective tissue disease: still crazy after all these years. Rheum Dis Clin North Am 2005; 31:421–436, v.

[22] Sullivan WD, Hurst DJ, Harmon CE, et al. A prospective evaluation emphasizing pulmonary involvement in patients with mixed connective tissue disease. Medicine (Baltimore) 1984;63:92–107.

[23] Burdt MA, Hoffman RW, Deutscher SL, et al. Long-term outcome in mixed connective tissue disease: longitudinal clinical and serologic findings. Arthritis Rheum 1999;42: 899–909.

[24] Sharp GC, Irvin WS, May CM, et al. Association of antibodies to ribonucleoprotein and Sm antigens with mixed connective-tissue disease, systematic lupus erythematosus and other rheumatic diseases. N Engl J Med 1976;295: 1149–1154.
[25] Lundberg I, Hedfors E. Clinical course of patients with anti-RNP antibodies. A prospective study of 32 patients. J Rheumatol 1991;18:1511–1519.
[26] Kotajima L, Aotsuka S, Sumiya M, et al. Clinical features of patients with juvenile onset mixed connective tissue disease: analysis of data collected in a nationwide collaborative study in Japan. J Rheumatol 1996;23:1088–1094.
[27] Frandsen PB, Kriegbaum NJ, Ullman S, et al. Follow-up of 151 patients with high-titer U1RNP antibodies. Clin Rheumatol 1996;15:254–260.
[28] Nimelstein SH, Brody S, McShane D, et al. Mixed connective tissue disease: a subsequent evaluation of the original 25 patients. Medicine (Baltimore) 1980;59: 239–248.
[29] Ginsburg WW, Conn DL, Bunch TW, et al. Comparison of clinical and serologic markers in systemic lupus erythematosus and overlap syndrome: a review of 247 patients. J Rheumatol 1983;10:235–241.
[30] Sanders DY, Huntley CC, Sharp GC. Mixed connective tissue disease in a child. J Pediatr 1973;83:642–645.
[31] Singsen BH, Swanson VL, Bernstein BH, et al. A histologic evaluation of mixed connective tissue disease in childhood. Am J Med 1980;68:710–717.
[32] Hoffman RW, Cassidy JT, Takeda Y, et al. U1-70-kd autoantibody-positive mixed connective tissue disease in children. A longitudinal clinical and serologic analysis. Arthritis Rheum 1993;36:1599–1602.
[33] Kramer A. The structure and function of proteins involved in mammalian pre-mRNA splicing. Annu Rev Biochem 1996; 65:367–409.
[34] Pettersson I, Hinterberger M, Mimori T, et al. The structure of mammalian small nuclear ribonucleoproteins. Identification of multiple protein components reactive with anti-(U1)ribonucleoprotein and anti-Sm autoantibodies. J Biol Chem 1984;259:5907–5914.
[35] Combe B, Rucheton M, Graafland H, et al. Clinical significance of anti-RNP and anti-Sm autoantibodies as determined by immunoblotting and immunoprecipitation in sera from patients with connective tissue diseases. Clin Exp Immunol 1989;75:18–24.
[36] Netter HJ, Guldner HH, Szostecki C, et al. A recombinant autoantigen derived from the human (U1) small nuclear RNP-specific 68-kd protein. Expression in Escherichia coli and serodiagnostic application. Arthritis Rheum 1988;31: 616–622.
[37] O'Brien RM, Cram DS, Coppel RL, et al. T-cell epitopes on the 70-kDa protein of the (U1)RNP complex in autoimmune rheumatologic disorders. J Autoimmun 1990; 3:747–757.
[38] Batista FD, Harwood NE. The who, how and where of antigen presentation to B cells. Nat Rev Immunol 2009;9: 15–27.
[39] Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 2001;1:147–153.
[40] Malik M, Ahmed AR. Dual diagnosis of pemphigus vulgaris and connective tissue disease. J Am Acad Dermatol 2006;55: 699–704.
[41] Valeyrie-Allanore L, Sassolas B, Roujeau JC. Drug-induced skin, nail and hair disorders. Drug Saf 2007;30: 1011–1030.
[42] Nigen S, Knowles SR, Shear NH. Drug eruptions: approaching the diagnosis of drug-induced skin diseases. J Drugs Dermatol 2003;2:278–299.
[43] Vassileva S. Drug-induced pemphigoid: bullous and cicatricial. Clin Dermatol 1998;16:379–387.
[44] Marzano AV, Vezzoli P, Crosti C. Drug-induced lupus: an update on its dermatologic aspects. Lupus 2009;18: 935–940.
[45] Katz U, Zandman-Goddard G. Drug-induced lupus: an update. Autoimmun Rev 2010;10:46–50.
[46] Alarcon-Segovia D. Mixed connective tissue disease: some statements. Clin Rheumatol 1982;1:81–83.
[47] Alarcon-Segovia D, Cardiel MH. Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. J Rheumatol 1989;16:328–334.
[48] 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;47:1245–1249.
[49] 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;30:224–232.
[50] 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;140:351–357.
[51] Rashid KA, Gurcan HM, Ahmed AR. Antigen specificity in subsets of mucous membrane pemphigoid. J Invest Dermatol 2006;126:2631–2636.
[52] Bunce M, Welsh K. PCR-SSP typing of HLA class I and class II alleles. In: AB Hahn, GA Land, RM Strothman, editors. ASHI Laboratory Manual. 4 ed. Mt. Laurel, NJ: ASHI Publications; 2000. p 19.
[53] Lafuente EM, Reche PA. Prediction of MHC-peptide binding: a systematic and comprehensive overview. Curr Pharm Des 2009;15:3209–3220.
[54] Reche PA, Glutting JP, Reinherz EL. Prediction of MHC class I binding peptides using profile motifs. Hum Immunol 2002;63:701–709.
[55] Reche PA, Glutting JP, Zhang H, et al. Enhancement to the RANKPEP resource for the prediction of peptide binding to MHC molecules using profiles. Immunogenetics 2004;56: 405–419.
[56] Reche PA, Keskin DB, Hussey RE, et al. Elicitation from virus-naive individuals of cytotoxic T lymphocytes directed against conserved HIV-1 epitopes. Med Immunol 2006;5:1.
[57] Thoma-Uszynski S, Uter W, Schwietzke S, et al. Autoreactive T and B cells from bullous pemphigoid (BP) patients recognize epitopes clustered in distinct regions of BP180 and BP230. J Immunol 2006;176:2015–2023.
[58] Lin MS, Fu CL, Giudice GJ, et al. Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. J Invest Dermatol 2000;115:955–961.
[59] Hacker-Foegen MK, Zillikens D, Giudice GJ, et al. T cell receptor gene usage of BP180-specific T lymphocytes from patients with bullous pemphigoid and pemphigoid gestationis. Clin Immunol 2004;113:179–186.
[60] Budinger L, Borradori L, Yee C, et al. Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. J Clin Invest 1998;102:2082–2089.
[61] Lin MS, Swartz SJ, Lopez A, et al. Development and characterization of desmoglein-3 specific T cells from patients with pemphigus vulgaris. J Clin Invest 1997;99: 31–40.
[62] Wucherpfennig KW, Yu B, Bhol K, et al. Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity: charged residues of a single MHC binding pocket confer selective presentation of self-peptides

in pemphigus vulgaris. Proc Natl Acad Sci USA 1995;92: 11935–11939.
[63] Riechers R, Grotzinger J, Hertl M. HLA class II restriction of autoreactive T cell responses in pemphigus vulgaris: review of the literature and potential applications for the development of a specific immunotherapy. Autoimmunity 1999;30: 183–196.
[64] Ahmed AR, Park MS, Tiwari JL, et al. Association of DR4 with pemphigus. Exp Clin Immunogenet 1987;4:8–16.
[65] 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: 107–110.
[66] Tong JC, Bramson J, Kanduc D, et al. Modeling the bound conformation of Pemphigus vulgaris-associated peptides to MHC Class II DR and DQ alleles. Immunome Res 2006;2:1.
[67] Delgado JC, Hameed A, Yunis JJ, et al. Pemphigus vulgaris autoantibody response is linked to HLA-DQB1*0503 in Pakistani patients. Hum Immunol 1997;57:110–119.
[68] 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.
[69] Miyagawa S, Higashimine I, Iida T, et al. HLA-DRB1*04 and DRB1*14 alleles are associated with susceptibility to pemphigus among Japanese. J Invest Dermatol 1997;109: 615–618.
[70] 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;67:125–139.
[71] Tron F, Gilbert D, Mouquet H, et al. Genetic factors in pemphigus. J Autoimmun 2005;24:319–328.
[72] Gazit E, Loewenthal R. The immunogenetics of pemphigus vulgaris. Autoimmun Rev 2005;4:16–20.
[73] 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;88:5056–5060.
[74] Sinha AA, Brautbar C, Szafer F, et al. A newly characterized HLA DQ beta allele associated with pemphigus vulgaris. Science 1988;239:1026–1029.
[75] 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;63:326–334.
[76] Glorio R, Rodriguez Costa G, Haas R, et al. HLA haplotypes and class II molecular alleles in Argentinian patients with pemphigus vulgaris. J Cutan Med Surg 2002;6:422–426.
[77] Brick C, Belgnaoui FZ, Atouf O, et al. Pemphigus and HLA in Morocco. Transfus Clin Biol 2007;14:402–406.
[78] Delgado JC, Yunis DE, Bozon MV, et al. MHC class II alleles and haplotypes in patients with pemphigus vulgaris from India. Tissue Antigens 1996;48:668–672.
[79] 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;48:662–667.
[80] Gonzalez-Escribano MF, Jimenez G, Walter K, et al. Distribution of HLA class II alleles among Spanish patients with pemphigus vulgaris. Tissue Antigens 1998;52:275–278.
[81] Hoffman RW, Rettenmaier LJ, Takeda Y, et al. Human autoantibodies against the 70-kd polypeptide of U1 small nuclear RNP are associated with HLA-DR4 among connective tissue disease patients. Arthritis Rheum 1990; 33:666–673.
[82] Genth E, Zarnowski H, Mierau R, et al. HLA-DR4 and Gm(1,3;5,21) are associated with U1-nRNP antibody positive connective tissue disease. Ann Rheum Dis 1987;46: 189–196.
[83] Kaneoka H, Hsu KC, Takeda Y, et al. Molecular genetic analysis of HLA-DR and HLA-DQ genes among anti-U1-70-kd autoantibody positive connective tissue disease patients. Arthritis Rheum 1992;35:83–94.
[84] Smolen JS, Steiner G. Mixed connective tissue disease: to be or not to be? Arthritis Rheum 1998;41:768–777.
[85] Black CM, Maddison PJ, Welsh KI, et al. HLA and immunoglobulin allotypes in mixed connective tissue disease. Arthritis Rheum 1988;31:131–134.
[86] Hoffman RW, Sharp GC, Deutscher SL. Analysis of anti-U1 RNA antibodies in patients with connective tissue disease. Association with HLA and clinical manifestations of disease. Arthritis Rheum 1995;38:1837–1844.
[87] Smolen JS, Klippel JH, Penner E, et al. HLA-DR antigens in systemic lupus erythematosus: association with specificity of autoantibody responses to nuclear antigens. Ann Rheum Dis 1987;46:457–462.
[88] Ruuska P, Hameenkorpi R, Forsberg S, et al. Differences in HLA antigens between patients with mixed connective tissue disease and systemic lupus erythematosus. Ann Rheum Dis 1992;51:52–55.
[89] 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;93:8569–8571.
[90] 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;145:406–414.
[91] 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; 145:805–808.
[92] 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;154:90–98.
[93] Redman RS, Thorne EG. Cicatricial pemphigoid in a patient with systemic lupus erythematosus. Arch Dermatol 1981; 117:109–110.
[94] Spigel GT, Winkelmann RK. Cicatricial pemphigoid and rheumatoid arthritis. Arch Dermatol 1978;114:415–417.
[95] Peyri J, Servitje O, Ribera M, et al. Cicatricial pemphigoid in a patient with rheumatoid arthritis treated with D-penicillamine. J Am Acad Dermatol 1986;14:681.
[96] Spigel GT. Association of cicatricial pemphigoid and rheumatoid arthritis. Arch Dermatol 1979;115:108–109.
[97] Malik M, Ahmed AR. Concurrence of systemic lupus erythematosus and pemphigus: coincidence or correlation? Dermatology 2007;214:231–239.
[98] Chan HL. Pemphigus vulgaris associated with systemic lupus erythematosus. Int J Dermatol 1999;38:948.
[99] Kuchabal DS, Kuchabal SD, Pandit AM, et al. Pemphigus vulgaris associated with systemic lupus erythematosus. Int J Dermatol 1998;37:636–638.
[100] Fong PH, Chan HL. Systemic lupus erythematosus with pemphigus vulgaris. Arch Dermatol 1985;121:26–27.
[101] Russell JG. Pemphigus, Sjogren's syndrome and mucosal pigmentation. Acta Stomatol Int 1987;8:25–29.
[102] Shapiro M, Jimenez S, Werth VP. Pemphigus vulgaris induced by D-penicillamine therapy in a patient with systemic sclerosis. J Am Acad Dermatol 2000;42:297–299.
[103] Nanda A, Kapoor MM, Dvorak R, et al. Coexistence of pemphigus vulgaris with systemic lupus erythematosus. Int J Dermatol 2004;43:393–394.

[104] Somorin AO, Agbakwu SN, Nwaefuna A. Systemic lupus erythematosus and pemphigus vulgaris preceded by depressive psychosis. Cent Afr J Med 1981;27:12–14.
[105] Hidalgo-Tenorio C, Sabio-Sanchez JM, Tercedor-Sanchez J, et al. Pemphigus vulgaris and systemic lupus erythematosus in a 46-y-old man. Lupus 2001;10:824–826.
[106] Hertl M, Karr RW, Amagai M, et al. Heterogeneous MHC II restriction pattern of autoreactive desmoglein 3 specific T cell responses in pemphigus vulgaris patients and normals. J Invest Dermatol 1998;110:388–392.
[107] McCluskey J, Farris AD, Keech CL, et al. Determinant spreading: lessons from animal models and human disease. Immunol Rev 1998;164:209–229.
[108] Mitchison NA. The carrier effect in the secondary response to hapten-protein conjugates. II. Cellular cooperation. Eur J Immunol 1971;1:18–27.
[109] Lake P, Mitchison NA. Regulatory mechanisms in the immune response to cell-surface antigens. Cold Spring Harb Symp Quant Biol 1977;41 Pt 2:589–595.
[110] Mamula MJ. Lupus autoimmunity: from peptides to particles. Immunol Rev 1995;144:301–314.
[111] Mamula MJ, Janeway CA, Jr. Do B cells drive the diversification of immune responses? Immunol Today 1993; 14:151–152; discussion 3–4.
[112] 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.
[113] Lehmann PV, Forsthuber T, Miller A, et al. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature 1992;358:155–157.
[114] Chan LS, Vanderlugt CJ, Hashimoto T, et al. Epitope spreading: lessons from autoimmune skin diseases. J Invest Dermatol 1998;110:103–109.
[115] Chan LS, Soong HK, Foster CS, et al. Ocular cicatricial pemphigoid occurring as a sequela of Stevens-Johnson syndrome. JAMA 1991;266:1543–1546.
[116] Paisansinsup T, Deshmukh US, Chowdhary VR, et al. 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: 5876–5884.