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

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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 α6 and β4 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, it was observed that patients 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 leukocyte 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.

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
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.

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>C.P.</th>
<th>Int.</th>
<th>ICS ELISA</th>
<th>Dsg 1</th>
<th>Dsg 3</th>
<th>BMZ ELISA</th>
<th>Dsg 3</th>
<th>BMZ ELISA</th>
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<td>83</td>
<td>F</td>
<td>MuCu</td>
<td>97</td>
<td>IgG 1</td>
<td>160</td>
<td>110</td>
<td>167</td>
<td>80</td>
<td>17</td>
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<tr>
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<td>53</td>
<td>F</td>
<td>MuCu</td>
<td>82</td>
<td>IgG 2</td>
<td>640</td>
<td>165</td>
<td>369</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
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<td>M</td>
<td>MuCu</td>
<td>115</td>
<td>IgG 3</td>
<td>1120</td>
<td>115</td>
<td>236</td>
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<td>56</td>
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<tr>
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<td>M</td>
<td>Mu</td>
<td>146</td>
<td>IgG 4</td>
<td>320</td>
<td>6</td>
<td>193</td>
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<td>60</td>
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<tr>
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<td>M</td>
<td>MuCu</td>
<td>72</td>
<td>IgG 5</td>
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<td>201</td>
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<tr>
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<td>M</td>
<td>Mu</td>
<td>81</td>
<td>IgG 6</td>
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<td>F</td>
<td>MuCu</td>
<td>84</td>
<td>IgG 7</td>
<td>640</td>
<td>97</td>
<td>148</td>
<td>20</td>
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<tr>
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<td>67</td>
<td>M</td>
<td>MuCu</td>
<td>75</td>
<td>IgG 8</td>
<td>160</td>
<td>107</td>
<td>166</td>
<td>20</td>
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<td>Mu</td>
<td>36</td>
<td>IgG 9</td>
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<td>Mu</td>
<td>94</td>
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<td>171</td>
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<td>F</td>
<td>Mu</td>
<td>57</td>
<td>IgG 12</td>
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<td>12</td>
<td>127</td>
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<td>9</td>
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<td>M</td>
<td>Mu</td>
<td>2</td>
<td>IgG 13</td>
<td>160</td>
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<td>29</td>
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<td>16</td>
<td>IgG 14</td>
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<td>MuCu</td>
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<td>IgG 15</td>
<td>5120</td>
<td>17</td>
<td>158</td>
<td>20</td>
<td>25</td>
</tr>
</tbody>
</table>

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.

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:
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-DRb1*0402 and seven patients had HLA-DQb1*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 DQb1*0301 and DQb1*0602 associated with Pg.

In the same patients, MHC II genes associated with Pg were observed on the second haplotype. HLA-DQb1*0301 was present in seven patients. HLA-DQb1*0302 was present in nine patients, HLA-DQb1*0603 was present in one patient and HLA-DQb1*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 DQB1*0301 on one haplotype. One patient carried DQB1*0302, and two carried DQB1*0602. Also, five patients were homozygous for DQB1*0301.

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

| Table 2. Characterization of pemphigoid (Pg) patients with antibodies to (pemphigoid (Pg) and pemphigus vulgaris (PV)). |
|---|---|---|---|---|
| **Demographics** | **Anti-ICS** | **Anti-BMZ** |
| **Anti-ICS** | **Ind. IF** | **ELISA** | **Ind. IF** | **ELISA** |
| **Patient** | **Age** | **Sex** | **CP** | **Int.** | **IgG** | **Dsg 1** | **Dsg 3** | **IgG** | **BP1** | **BP2** | **1st haplotype** | **2nd haplotype** |
| 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 | 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 | Cu | 10 | 40 | 13 | 31 | 80 | 16 | 37 | 1303 | 0301 | 1104 | 0301 |
| BP7 | 69 | F | Cu | 36 | 20 | 16 | 29 | 4 | 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 | 1404 | 0503 | 1101 | 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]: $< 14 = \text{negative; } 14–20 = \text{indeterminate; } > 20 = \text{positive.}$ Dsg 3 ELISA index values [44]: $< 9 = \text{negative; } 9–20 = \text{indeterminate; } > 20 = \text{positive.}$ BP1 and BP2 index values [42,43]: $< 9 = \text{negative; } > 9 = \text{positive.}$ 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.
MHC class II genes and two autoantibodies

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 DRB1*0402 and DQB1*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 DQB1*0301. However, because of amino acid sequence homology in the critical 71–77 positions of the DQB1 gene, it has been demonstrated that DQB1*0302, DQB1*0303, DQB1*0305, DQB1*0602 and DQB1*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-DQB1*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 [16,52,53,55,61,64,67,75–77]. The alleles and haplotypes known to be associated with PV are DRB1*0402/DQB1*0302 and DRB1*1401/DQB1*0503. In one study there were several patients with these PV-associated haplotypes or phenotypes that also carried DQB1*0301 on the second haplotype [76]. In another study, two of nine PV patients had DQB1*0301 on the second haplotype, and this frequency was higher than in the control population [55]. In three of 10 patients with PV, DQB1*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 DQB1*0301 allele, they often carry DRB1*0402 or DQB1*0503 on the second haplotype [16,64,67]. Interestingly, in some studies the frequency of DRB1*04 is

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 DRB1*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 DQB1*0301 and DRB1*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

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 sera of a patient with dual diagnosis to both BMZ and ICS (indicated by the white arrows).
Bullous pemphigoid autoantigen BP180

Epitopes predicted to bind to DQ*0301 are represented as yellow. Epitopes predicted to bind to 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.

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) DRB1*0402 (green) or HLA-DQB1*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-DRB1*0402 and HLA-DQB1*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).
Integrin alpha chain, alpha 6 isoform a precursor (Homo sapiens).

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.

(b) Fig. 2. Continued
Matrix: HLA(DR4) DRB1*0402 Consensus sequence: ICFWHNHNM
Optimal Score: 44·604 Binding Threshold: 11·44
All rows highlighted in red represent predicted binders.

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<th>RANK</th>
<th>POS.</th>
<th>N</th>
<th>SEQUENCE</th>
<th>C MW (Da)</th>
<th>SCORE</th>
<th>% OPT.</th>
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<td>422</td>
<td>ASN V KY VM GR ND GGY 1063·23</td>
<td>14·554</td>
<td>32·63 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>83</td>
<td>TQK I TY RIS GV G ID Q 947·1</td>
<td>13·746</td>
<td>30·82 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>812</td>
<td>NDC LL IY DNE GA DAT 989·1</td>
<td>12·572</td>
<td>28·19 %</td>
<td></td>
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<tr>
<td>8</td>
<td>70</td>
<td>RNP I AK IT SD YQ AT Q 1020·15</td>
<td>12·438</td>
<td>27·89 %</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>359</td>
<td>QSV I SRY RV QST PVT 1091·24</td>
<td>11·643</td>
<td>26·10 %</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>303</td>
<td>WLA V YFFT SG NE G NW 1045·12</td>
<td>11·59</td>
<td>25·98 %</td>
<td></td>
<td></td>
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<tr>
<td>11</td>
<td>217</td>
<td>TGE V RT LT NS LD REQ 1000·11</td>
<td>11·507</td>
<td>25·80 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Homo sapiens desmoglein 3 (pemphigus vulgaris antigen) (DSG 3)
MMGLFPRTTGALAIFVVVILVHGELRIETKGQYDEEEMTMQQAKR
RQKREWVKFAKPCREGEDNSKRN
PIAKITSDYQATQKITYRISGVGIDQPPFGIFVVDKNTGDINITAIVDREETPSFLITCRALNAQGLDVEKP
LILTVKILDINDNPPVFSQQIFMGEIEENSASNSLVMILNATDADEPNHL
MMGLFPRTTGALAIFVVVILVHGELRIETKGQYDEEEMTMQQAKR
RQKREWVKFAKPCREGEDNSKRN
PIAKITSDYQATQKITYRISGVGIDQPPFGIFVVDKNTGDINITAIVDREETPSFLITCRALNAQGLDVEKP
LILTVKILDINDNPPVFSQQIFMGEIEENSASNSLVMILNATDADEPNHL

Epitopes predicted to bind to DRβ1*0402 are highlighted as yellow. Epitopes predicted to bind to DQβ1*0301 are represented 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. (c) Fig. 2.
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*0301 and anti-ICS antibodies. Collectively, these 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**


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