T Cell Recognition of Desmoglein 3 Peptides in Patients with Pemphigus Vulgaris and Healthy Individuals

Christian M. Veldman, Kerstin L. Gebhard, Wolfgang Uter, Ralf Wassmuth, Joachim Grötzinger, Erwin Schultz and Michael Hertl

*J Immunol* 2004; 172:3883-3892; ;
doi: 10.4049/jimmunol.172.6.3883
http://www.jimmunol.org/content/172/6/3883

Why The *JI*?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

References

This article cites 46 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/172/6/3883.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
T Cell Recognition of Desmoglein 3 Peptides in Patients with Pemphigus Vulgaris and Healthy Individuals

Christian M. Veldman, Kerstin L. Gebhard, Wolfgang Uter, Ralf Wassmuth, Joachim Grötzinger, Erwin Schultz, and Michael Hertl

Pemphigus vulgaris (PV) is considered as a paradigm of an autoantibody (autoAb)-mediated autoimmune disease of the skin associated with a loss of epidermal cell-cell adhesion caused by autoAb against the desmosomal adhesion molecules, desmoglein 3 (Dsg3) and desmoglein 1 (Dsg1) (1, 2). The pathogenic role of Dsg-specific autoAb has been clearly established by different investigators in several in vitro and in vivo models (3–5). Moreover, Dsg3 deficient mice express a phenotype with mucosal blisters/erosions that clearly resembles the clinical findings seen in PV (6). Recently, additional autoantigens that are recognized by PV sera have been identified; their pathogenic relevance is not yet fully clear. Two potential new target Ags of PV belong to the group of cholinergic receptors. Pemphaxin is an annexin homologue that binds acetylcholine (7) and the choline receptor is also present on keratinocytes (8). In addition, a novel member of the desmoglein family, Dsg4, was identified which shows great homology with Dsg3 for the first three extracellular domains (ECD) and was found to be recognized by PV sera (9). The precise role that autoAb against Dsg4 play in the pathogenesis of PV needs to be elucidated.

Clinically, PV is a potentially life threatening autoimmune bullous disorder characterized primarily by mucosal lesions (generally when IgG autoAb against Dsg3 are present), and mucocutaneous blisters and erosions (when IgG autoAb against both Dsg3 and Dsg1 are present) (10, 11). The titers of autoAb against Dsg3 and Dsg1 strongly correlate with activity of disease in individual patients (10, 12). AutoAb production in PV is polyclonal and most autoAb are of the IgG4 subclass in patients with active disease, while patients with chronic disease often have autoAb of the IgG1 and IgG4 subtypes (13, 14). AutoAb against Dsg1 and Dsg3 are capable, independent of complement activation, to induce the pathology of pemphigus by interfering with the function of desmosomes that mediate adhesion between epidermal keratinocytes (3, 15).

PV is associated with the HLA class II allele, HLA-DRB1*0402, particularly in Jewish patients (16), whereas DRB1*14/DQB1*0503 is prevalent in non-Jewish patients (17, 18). Recent evidence suggests that these HLA class II alleles are involved in the presentation of Dsg3 peptides to autoreactive T cells in PV patients (19–22). Autoreactive Th cells are presumably critical for the induction and regulation of Ab production (23). Dsg3-reactive Th1 and Th2 cells have been identified by several studies in PV patients. However, their phenotype, cytokine profile, immunogenetic restriction, and epitope specificity varied (19–21). In PV patients, both Dsg3-reactive Th1 and Th2 cells were identified that recognized portions of the ECD of Dsg3 in the context with PV-associated HLA class II alleles (20–22). Autoreactive Th1 cells reactive to Dsg3 were also isolated from the blood of healthy carriers of the PV-associated HLA class II alleles, HLA-DRB1*0402 and DQB1*0503 (21, 22).

The purpose of this study was to identify Dsg3 peptides that are...
recognized by autoreactive Th cells of PV patients. Because T cell recognition of Dsg3 was also detected in HLA class II-matched healthy individuals, the peptide specificity of Dsg3-reactive T cell clones (TCC) from these control donors was also studied. In a cohort of 16 PV patients and 12 healthy donors, autoreactive Th cells recognized a limited set of peptides located in the ECD of Dsg3. Two of these peptides were recognized by the majority of TCC of patients and healthy controls. Moreover, T cell recognition of Dsg3 peptides was predominantly restricted by HLA-DRB1*0402 and DQB1*0503. These observations strongly suggest that T cell recognition of Dsg3 peptides both in PV patients and in healthy individuals is tightly restricted by distinct HLA class II alleles and is directed against a limited set of epitopes located in the ECD of Dsg3.

Materials and Methods
Patients and healthy controls
Heparinized blood samples (40–80 ml) were obtained from 16 PV patients who were seen at the Department of Dermatology at the University of Erlangen, Germany and from 12 healthy controls. Both patients and healthy donors gave written informed consent to participate in this study. The clinical diagnosis of PV was confirmed by direct immunofluorescence microscopy (epidermal intercellular IgG and/or C3 deposits in perilesional skin), histopathology, indirect immunofluorescence microscopy (intercellular IgG binding to epithelial cells of monkey esophagus), and the presence of circulating autoAb against Dsg3, tested by a commercial Dsg3-ELISA (MDL, Naka-ku Nagoya, Japan). Patients were divided into three groups depending on the stage of disease (Table I). Acute onset PV was defined as the de novo development of blisters/erosions on previously unaffected mucosal surfaces and/or skin; all of these patients had not yet received immunosuppressive therapy. Chronic active PV was defined as the expansion/persistence of existing blisters or erosions on mucosal surfaces and/or skin; some of these patients had already received immunosuppressive treatment. Patients with remittent PV had not experienced new mucosal blisters/erosions for 6 mo or more before the study. Some of the studied PV patients had been already included in a recent study (22). However, the Dsg3-specific TCC that were characterized in the present investigation were newly generated for this purpose.

HLA class II genotyping was performed in all patients and controls. The determination of HLA-DRB1 and DQB1 alleles was conducted at high resolution by enzyme-linked-probe-hybridization-assay (Biotest Diagnostics, Dreieich, Germany) using locus-specific PCR products as templates.

Human recombinant Dsg3
The recombinant protein PVHis used in this study is a fusion protein, consisting of the ECD1–5 of Dsg3 linked to an E-tag and a histidine-tag (12). For the production of Dsg3 protein, recombinant PVHis baculovirus was amplified in SF21 insect cells as described previously (21, 24). For protein production, High-Five insect cells were inoculated for 4 days with the baculovirus at a multiplicity of infection of 10. Dsg3 protein was purified from culture supernatants by affinity chromatography over Ni-NTA-linked (nickel-nitrilotriacetic) agarose (Qiagen, Hilden, Germany) as recently described (22).

Isolation of Dsg3 reactive Th1 and Th2 cells by MACS cytokine secretion assay
PBMC (4–8 × 10^6) of PV patients and controls were cocultured with Dsg3 (10 μg PVHis/ml) for 16 h in vitro. For isolation and quantification of Dsg3-autoreactive Th1 and Th2 cells, a MACS cytokine secretion assay for IFN-γ and IL-4 (Miltenyi Biotec, Bergisch-Gladbach, Germany) was performed according to standardized procedures (25). After 16 h in vitro culture, high affinity anti-cytokine Ab were added that bound to the surface of in vitro activated cytokine-secreting T cells. Afterward, the cells were labeled with a secondary magnetic bead-coupled Ab for specific enrichment of IL-4 and IFN-γ-secreting T cells. The labeled cells were separated with MACS columns into IL-4 and IFN-γ-secreting (Th2) and Th1 T cells and finally counted with a hemocytometer as recently described (25).

Following MACS secretion assay, isolated autoreactive T cells were cloned by limiting dilution in 96-well plates (BD-Falcon, Heidelberg, Germany), according to a standard protocol (26). T cells were cultured and expanded with RPMI 1640 (Life Technologies, Karlsruhe, Germany), supplemented with 2% heat-inactivated PHS (ICN Pharmaceuticals, Irvine, CA), 8% heat-inactivated FCS (PAA, Cölbe, Germany), 100 U/ml penicillin, 100 μg/ml streptomycin, 20 mU γ-glutamine, nonessential amino acids, and 100 U/ml IL-2 (Biotest Pharma, Dreieich, Germany). For long term culture, TCC were stimulated periodically with 1% PHA (Sigma-Aldrich, Taufkirchen, Germany) and γ-irradiated (50 Gy) PBMC as APC.

Proliferative in vitro assays with Dsg3-responsive TCC
A total of 5 × 10^5 TCC were cultured in duplicate with 5 × 10^4 γ-irradiated human EBV-transformed autologous B lymphoblastoid cell lines (B-LCL) (80 Gy) or PBMC (50 Gy) as APC and Dsg3 (100 nM) or Dsg3 peptides (5 μM each) in RPMI 1640 with 10% FBS in 96-well round-bottom microplates (BD-Falcon) for 48 h at 37°C in 5% CO₂. For the final 18 h, 0.6 μCi [³H]thymidine (DuPont, Mechelen, Belgium) were added to each microculture. T cell proliferation was counted in a cell harvester (BD-Falcon) and expressed as stimulation index (SI), which is the ratio of [³H]thymidine uptake (cpm) in cultures with Ag and cultures without Ag. SI values >2 were considered to represent a significant proliferative response.

Identification of immunodominant T cell peptides of Dsg3
A total of 97 TCC were screened for Dsg3 peptide reactivity using 111 17mer peptides of Dsg3 that encompassed the entire ECD of Dsg3 and were staggered by 5 aa residues as previously described (21). These peptides were synthesized by pepset technique (Chiron Mimotopes, San Diego, CA) resulting in a purity greater than 65% as determined by HPLC. All 111 synthesized peptides were selected for the mapping of T cell epitopes. Identified immunodominant T cell peptides were re-synthesized using F-moc chemistry (Institute of Biochemistry, University of Erlangen, Germany) resulting in a purity of at least 95%. Lyophilized peptides were reconstituted in H₂O and 0.2% acetic acid at 2 mg/ml and stored at -80°C. Each individual peptide was used in vitro at 5 μM, which was found to be an optimal stimulatory concentration for most of the TCC.

HLA restriction assay of Dsg3-responsive TCC
A total of 5 × 10^4 cloned T cells were cocultured with 5 × 10^4 γ-irradiated (80 Gy) autologous or HLA-matched B-LCL cells and 100 nM Dsg3 or 5 μM Dsg3 peptides for 72 h. T cell proliferation was determined by the incorporation of [³H]thymidine (DuPont) that was added for the final 18 h of the culture (26). For HLA blocking experiments, mAb against HLA-DR (clone L243), HLA-DQ (clone SK10), or HLA- DP (clone B7/21) (BD PharMingen, Heidelberg, Germany) were added at 250 ng/ml to the cultures with Dsg3-reactive TCC and B-LCL as APC. Again, the proliferative T cell response to Dsg3 was determined by the incorporation of [³H]thymidine (DuPont) that was added for the final 18 h of culture.

Analysis of potential HLA class II binding motifs of identified Dsg3 peptides
Three-dimensional models of the two HLA class II alleles, HLA-DRB1*0402 and HLA-DQB1*0503, were built by using the x-ray structure of the MHC II/peptide complex (Protein Data Bank accession code 1seb) as a template. Sequence alignments of the molecules were generated and according to this alignment, amino acid residues were exchanged in the template using a database-search approach included in the software package WHATIF (27). Finally, the structural models were energy-minimized using the steepest descent algorithm implemented in the GRÖMOS force field (28). The structural representations were generated with the Grasp program (29). All programs were run on a Silicon Graphics Indigo (Silicon Graphics, Mountain View, CA) (29).

Analysis of the TCR VB region (TCRBV)
Total RNA was extracted from TCC using the RNeasy kit according to the manufacturer’s specifications (Qiagen). First-stranded cDNA was synthesized by reverse transcription using AMV reverse transcriptase following the manufacturer’s protocol (Promega, Mannheim, Germany). Complementary DNA was quantified using ultraviolet spectrometry, and diluted to 50 ng/μl with dH₂O. TCR were amplified by PCR using a 5′-sense primer specific for each of the 24 TCRBV families (and two subfamilies) and 11 β′-antisense Vβ-specific primers (27). Two and a half microliters (100 ng) of cDNA were combined with 22.5 μl of reaction mix containing 1× PCR buffer (Roche, Mannheim, Germany), 160 mM desoxyribonucleoside triphosphate (Amersham Pharmacia Biotech, Freiburg, Germany), 0.4 mM of the appropriate TCRBV-specific primer, 0.4 μM of TCRBV-specific primer, and 5 U Ampli Taq DNA polymerase (Roche, Mannheim, Germany). PCR amplification was conducted using 25 cycles of 94°C for 1 min, 68°C for 2 min, and 72°C for 3 min in a DNA
Table I. Clinical presentation, HLA class II alleles, autoAb titers and T cell response to Dsg3 peptides of the studied PV patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA Class II Alleles&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Clinical Phenotype&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Medication&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Anti-Dsg3-IgG&lt;sup&gt;e&lt;/sup&gt;</th>
<th>T Cell Response to Dsg3-Peptide&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Number of Reactive Th Clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV 1</td>
<td>0401, 1401 0301, 0503</td>
<td>None</td>
<td>Erosions of the oral mucosa</td>
<td>7</td>
<td>DG3(342-358) 0 2</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 2</td>
<td>1301, 1404 0603, 0503</td>
<td>Flaccid blisters on trunk</td>
<td>Erosions of the oral mucosa</td>
<td>8</td>
<td>DG3(189-205) 0 2</td>
<td>2 0</td>
</tr>
<tr>
<td>PV 3</td>
<td>0401, 1303 0301</td>
<td>Erosions on the trunk</td>
<td>Discrete buccal erosions</td>
<td>1</td>
<td>DG3(205-221) 0 1</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 4</td>
<td>0701 0303</td>
<td>Extensive vegetating ulcers</td>
<td>None</td>
<td>2</td>
<td>DG3(78-94) 0 ND</td>
<td>0 1</td>
</tr>
<tr>
<td>PV 5</td>
<td>0402, 1104 0302, 0301</td>
<td>Chronic erosions of the buccal mucosa</td>
<td>100 mg CY, 8 mg MP</td>
<td>168</td>
<td>DG3(96-112) 0 1</td>
<td>2 0</td>
</tr>
<tr>
<td>PV 6</td>
<td>0402, 1501 0302, 0602</td>
<td>None</td>
<td>None</td>
<td>108</td>
<td>DG3(205-221) 0 0</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 7</td>
<td>0804, 1411 0402, 0503</td>
<td>Chronic erosions on the face</td>
<td>None</td>
<td>172</td>
<td>DG3(205-221) 0 1</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 8</td>
<td>0804, 1411 0402, 0503</td>
<td>Trunk, axillar and facial erosions</td>
<td>Buccal ulcerations</td>
<td>172</td>
<td>DG3(205-221) 0 0</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 9</td>
<td>0402, 1104 0302, 0301</td>
<td>None</td>
<td>Discrete buccal erosions</td>
<td>167</td>
<td>DG3(250-266) 0 1</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 10</td>
<td>0701, 1401 02, 0503</td>
<td>None</td>
<td>Chronic oral erosions</td>
<td>58</td>
<td>DG3(96-112) 0 1</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 11</td>
<td>1401, 0405 0503, 0302</td>
<td>None</td>
<td>None (buccal erosions/ ulcers)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>181</td>
<td>DG3(96-112) 0 1</td>
<td>0 2</td>
</tr>
<tr>
<td>PV 12</td>
<td>0401, 1303 0301</td>
<td>None</td>
<td>None (discrete buccal erosions)</td>
<td>20 mg LF 1 DG3(342-358) 0 3</td>
<td>0 2</td>
<td></td>
</tr>
<tr>
<td>PV 13</td>
<td>0401, 1401 0301, 0503</td>
<td>None</td>
<td>None (discrete oral erosions)</td>
<td>25 mg AZA 8 DG3(96-112) 0 3</td>
<td>0 2</td>
<td></td>
</tr>
<tr>
<td>PV 14</td>
<td>0402, 1104 0302, 0301</td>
<td>None</td>
<td>None (buccal ulcers)</td>
<td>2 DG3(96-112) 0 3</td>
<td>0 2</td>
<td></td>
</tr>
<tr>
<td>PV 15</td>
<td>1401, 1502 0503, 0601</td>
<td>None</td>
<td>None (erosions of the hard palate)</td>
<td>0 DG3(96-112) 0 3</td>
<td>0 2</td>
<td></td>
</tr>
<tr>
<td>PV 16</td>
<td>0301, 0402 02*, 0302</td>
<td>None</td>
<td>None (vegetating erosions of the inguinal folds)</td>
<td>4 mg MP, 100 mg AZA 123</td>
<td>0 2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> PV1–4, acute onset PV; PV5–10, chronic active PV; PV11–16, remittent PV; as classified in Materials and Methods.
<sup>b</sup> PV-associated HLA class II alleles (bold) and homologous alleles (bold italic).
<sup>c</sup> At time of study.
<sup>d</sup> AZA, azathioprine; CY, cyclophosphamide; DADPS, diamino-dipenylsulfone; LF, leflunomide; MP, methylprednisolone; MPM, mycophenolate mofetil; PD, prednisolone.
<sup>e</sup> AutoAb to Dsg3, determined by ELISA with recombinant Dsg3. Values are expressed as the index value ((A<sub>405</sub> sample − A<sub>405</sub> negative control))/A<sub>405</sub> (positive control) − A<sub>405</sub> (negative control) × 100). Values > 20 (anti-Dsg3) were considered significant.

<sup>f</sup> As determined by proliferative in vitro response of MACS-selected TCC to recombinant Dsg3 and Dsg3 peptides (see Materials and Methods).
<sup>g</sup> Clinical description of mucosal or cutaneous lesions in parentheses refers to the initial clinical phenotype.
status, was tested with logistic regression analysis. The strength of association was quantified with the odds ratio; accompanying 95% confidence intervals was calculated using the profile likelihood method. For data analysis, the statistical software package SAS (version 8.2; SAS Institute, Cary, NC) was used.

**Results**

**Dsg3-reactive Th1 and Th2 cells in PV patients and healthy individuals**

A total of 16 patients and 12 healthy controls were included in this study. The majority (81%) of the studied PV patients expressed either HLA-DRB*0402 (31%) or HLA-DQB1*0503 (50%), which represent HLA class II alleles known to be prevalent in PV (Table I). All of the PV patients mounted detectable Th1 and Th2 responses against Dsg3 ranging from 2.8 to 37.6 cells/10^5 PBMC and 3.2 to 9.6 cells/10^5 PBMC, respectively (Fig. 1), which is in line with recent observations from our group (22): Statistical analysis revealed that there was no significant difference between the Th1 (chronic vs acute; p = 0.06) and Th2 frequency in acute onset, chronic active or remittent PV (Fig. 1).

Seven of 12 (58%) healthy donors carried the PV-prevalent HLA class II alleles, HLA-DRB1*0402 and/or HLA-DQB1*0503, while the five remaining individuals expressed PV-unrelated HLA class II alleles (Table II). Although none of the healthy donors had circulating autoAb against Dsg3, all of the carriers of the PV-associated HLA class II alleles, HLA-DRB1*0402 and HLA-DQB1*0503, exhibited T cell reactivity against Dsg3, which was predominantly of Th1 type (Fig. 1) since Dsg3-reactive Th2 cells were not identified above the detection limit of 0.8 T cells/10^5 PBMC (Fig. 1). In a few controls, MACS-derived Th2 cells which were below the detection limit of the assay were grown up in bulk cultures before limiting dilution resulting in the derivation of a total of 13 TCC of the Th2 type.

**Mapping of T cell epitopes of Dsg3**

A total of 97 Th1 and Th2 clones that were responsive to Dsg3 were isolated by MACS cytokine secretion assay from both PV patients and healthy controls (Table I and II). Dsg3 T cell reactivity was detected in all of the studied PV patients, independent from their clinical activity of disease and their immunosuppressive treatment, as well as in healthy individuals (Table I and II). Noteworthy, the majority (86%) of the TCC recognized a limited set of only eight Dsg3 peptides (Table III); the epitope specificity of the remaining 14 Dsg3-responsive TCC was not identified by this approach. Five Dsg3 peptides, DG3(78-94), DG3(96-112), DG3(189-205), DG3(205-221), and DG3(250-266), residing within the first ECD1–2 of Dsg3 were recognized by autoreactive Th1 and Th2 clones from both PV patients and healthy donors (Fig. 2, A and B; Table I and II). Two additional Dsg3 peptides, DG3(342-358) and DG3(376-392) residing in the ECD3 were recognized by TCC (n = 13) from three PV patients but not by the healthy donors. Peptide DG3(483-499) residing in the ECD5 of Dsg3 was exclusively recognized by TCC (n = 3) from a healthy donor.

In addition, a total of 21 Dsg3-responsive TCC were isolated from three PV patients and five healthy donors who did not carry any of the PV-associated HLA class II alleles (Table I and II). In these PV patients, eight TCC responded to Dsg3 peptide DG3(96-112) while the remaining four were specific for DG3(250-266). In the group of the healthy controls, seven TCC from the donors Co7 and Co12 were specific for DG3(96-112) while two TCC from donor Co5 were responsive to DG3(483-499) (Table II). Noteworthy, all the healthy controls that were responsive to DG3(96-112) were positive for HLA-DQB*B0301 which is homologous to DQB1*0503 (Table II). In the following analyses, the TCC were considered as observational unit.

**Identification of Dsg3 T cell peptides**

The majority of the PV patients and healthy controls responded to the Dsg3 peptides, DG3(96-112) and DG3(250-266), located in the ECD1–2 of Dsg3, respectively (Fig. 2, A and B). Noteworthy, DG3(96-112) and DG3(250-266) were also recognized by most of the generated Dsg3-reactive TCC. In detail, 46 of 97 (47.4%) TCC responded to peptide DG3(96-112), 11 of 97 TCC (11%) to DG3(250-266); 7 of 97 TCC (7%) each to DG3(205-221), DG3(342-358), or DG3(376-392); 5 of 97 TCC (5%) to DG3(189-205); and 7 of 97 TCC (7%) to DG3(78-94) and DG3(483-499), respectively (Fig. 2, A and B). There was no association between the case status (related here to a TCC being derived from a PV patient vs a healthy control) and global Dsg3 peptide recognition, controlling for HLA status as tested with logistic regression analysis (data not shown). A summary for all 97 TCC is shown in Tables I and II.

A significant association between the proliferative responses of TCC to distinct Dsg3 peptides and case status of TCC, controlling for HLA status, was found only for peptide DG3(96-112) (odds ratio 2.68, 95% confidence intervals: 1.08–6.94); as mentioned this refers to clones as observations/observational units, and not subjects (Fig. 3, A and B). This finding strongly suggests that this particular Dsg3 peptide is indeed preferentially recognized by autoreactive T cells. In contrast, there was no association between T cell reactivity to Dsg3 and responsiveness to any of the other identified Dsg3 peptides (Fig. 3A).

**Dsg3 peptide recognition of autoreactive Th1 and Th2 cells**

The peptides DG3(78-94), DG3(189-205), and DG3(205-221) were only recognized by three TCC of the Th1 type isolated from three controls, while peptides DG3(78-94), DG3(189-205), and DG3(376-392) were only recognized by seven Th2 clones isolated from three PV patients (Fig. 2A). However, no significant association between these Th1 or Th2 cells and Dsg3 peptide recognition to one of these peptides was observable due to the low numbers of TCC and thus limited power of the analysis. All other peptides were recognized equally by TCC of Th1 or Th2 type isolated from patients as well as from healthy individuals.
Differential T cell responsiveness to Dsg3 peptides

Even though all the Dsg3-responsive TCC were MACS-selected after short term in vitro stimulation with recombinant Dsg3, their responsiveness toward Dsg3 and Dsg3 peptides varied upon prolonged in vitro culture (1–2 mo). Three differential patterns of T cell recognition were found (Fig. 4): 1) TCC responsiveness to both, recombinant Dsg3 and a specific Dsg3 peptide, 2) TCC responsive to a specific Dsg3 peptide but not to recombinant Dsg3, and 3) TCC responsive only to recombinant Dsg3 but not to one of 111 17mer peptides that represent regions within the ECD1–5 of Dsg3.

Fig. 4 shows the distinct patterns of proliferative responses of three representative TCC PV7.1, PV10.2, and PV11.2, derived from three different PV patients. Like the majority (58 of 97) of the generated Dsg3-responsive TCC, TCC PV7.1 showed a vigorous proliferative response to both recombinant Dsg3 (100 nM) and to Dsg3 peptide DG3(342-358) with a maximal proliferation at a peptide concentration of 10–25 μM (Fig. 4A). A significant fraction of the TCC (39 of 97), as shown with the DG3(96-112)-responsive TCC PV10.2, did not proliferate in response to recombinant Dsg3 protein (100 nM) despite responsiveness to a distinct Dsg3 peptide (derived from two different manufacturers); their proliferative response usually reached a maximum at a lower peptide concentration, i.e., 2.5–5 μg/ml (Fig. 4B). Finally, a total of 14 additional Dsg3-specific TCC, stimulated by recombinant Dsg3 at 100 nM, were not responsive to any of the used 17mer peptides that span the entire ECD1–5 of Dsg3, as shown for the representative TCC PV 11.2 (Fig. 4C). These findings strongly suggest that autoreactive T cell responses against Dsg3 are tuned not only by differential epitope recognition but also by differential affinity to Dsg3 peptides.

HLA class II restriction of Dsg3 peptide recognition

In light of the preferential detection of HLA-DRB1*0402 and HLA-DQB1*0503 in the studied PV patients and Dsg3-reactive healthy donors, we assessed whether these PV-associated HLA class II alleles restricted T cell recognition of the identified Dsg3 peptides. First, HLA class II restriction of the peptide DG3(96-112) was studied by coculturing a total of six TCC from three patients (clones PV7.3, PV10.1, PV10.2, and PV11.1) and a healthy control (Co1.1 and Co1.2) with HLA-matched B-LCL as APC and DG3(96-112) at a concentration of 10 μg/ml (Fig. 5). TCC PV7.3, PV10.1, and PV10.2 were stimulated by DG3(96-112) in the presence of DRB1*0402 but not DQB1*0503 or irrelevant (DRB1*0701) APC (Fig. 5A). This proliferative response was uniformly inhibited by anti-DR moAb (Fig. 5B). In contrast, proliferation to DG3(96-112) of the TCC PV10.1, PV11.1, Co1.1, and Co1.2 was restricted to the presence of HLA-DQB1*0503 APC (Fig. 5C) and was inhibited by anti-DQ moAb

Table II. HLA class II alleles and T cell response to Dsg3/Dsg3 peptides of the studied healthy individuals

<table>
<thead>
<tr>
<th>Donor</th>
<th>DRB1</th>
<th>DQB1</th>
<th>T Cell Response</th>
<th>Number of Dsg3-Responsive T Cell Clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co1</td>
<td>1401, 1501</td>
<td><strong>0503</strong>, 0602</td>
<td>+ DG3(96-112)</td>
<td>0 0</td>
</tr>
<tr>
<td>Co2</td>
<td><strong>0402</strong>, 1501</td>
<td>0303, 0602</td>
<td>+ DG3(96-112)</td>
<td>6 0</td>
</tr>
<tr>
<td>Co3</td>
<td><strong>0402</strong>, 1401</td>
<td>0302, <strong>0503</strong></td>
<td>+ DG3(96-112)</td>
<td>6 0</td>
</tr>
<tr>
<td>Co4</td>
<td><strong>0402</strong>, 1102</td>
<td>0301, 0302</td>
<td>+ DG3(483-499)</td>
<td>0 0</td>
</tr>
<tr>
<td>Co5</td>
<td>0101, 0801</td>
<td>0402, 0501</td>
<td>+ DG3(483-499)</td>
<td>2 0</td>
</tr>
<tr>
<td>Co6</td>
<td>1104, 1401</td>
<td>0301, <strong>0503</strong></td>
<td>+ DG3(250-266)</td>
<td>1 0</td>
</tr>
<tr>
<td>Co7</td>
<td>1101, 1501</td>
<td><strong>0301</strong>, 0602</td>
<td>+ DG3(96-112)</td>
<td>3 0</td>
</tr>
<tr>
<td>Co8</td>
<td>1101, 1401</td>
<td>0301, <strong>0503</strong></td>
<td>+ DG3(250-266)</td>
<td>1 1</td>
</tr>
<tr>
<td>Co9</td>
<td>0301, 1401</td>
<td>02, <strong>0503</strong></td>
<td>+ DG3(96-112)</td>
<td>0 0</td>
</tr>
<tr>
<td>Co10</td>
<td>0301, 1302</td>
<td>02, 0609</td>
<td>– –</td>
<td>– –</td>
</tr>
<tr>
<td>Co11</td>
<td>0701, –</td>
<td>02, –</td>
<td>– –</td>
<td>– –</td>
</tr>
<tr>
<td>Co12</td>
<td>1319, 1501</td>
<td><strong>0301</strong>, 0602</td>
<td>+ DG3(96-112)</td>
<td>0 4</td>
</tr>
</tbody>
</table>

a PV-associated HLA class II alleles (bold) and homologous alleles (bold italic).

b As determined by proliferative in vitro response of MACS-selected TCC to recombinant Dsg3 and Dsg3 peptides (see Materials and Methods).

Table III. Identified Dsg3 T cell peptides

<table>
<thead>
<tr>
<th>Dsg3-Peptide</th>
<th>P1</th>
<th>P4</th>
<th>P6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG3(78-94)</td>
<td>Q  A  T  Q  K  I  T  Y  R  I  S  G  V  G  I  D  Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3(96-112)</td>
<td>P  F  G  I  F  V  V  D  K  N  T  G  D  I  N  I  T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3(189-205)</td>
<td>H  L  N  S  K  I  A  F  K  I  V  Q  S  E  P  A  G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3(205-221)</td>
<td>G  T  P  M  F  L  L  S  N  T  G  E  V  R  T  L  L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3(250-266)</td>
<td>Q  C  E  C  N  I  K  V  K  D  V  N  D  N  F  P  M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3(342-358)</td>
<td>S  V  K  L  S  I  A  V  K  N  K  A  E  F  H  Q  S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3(376-392)</td>
<td>N  V  R  E  G  I  A  F  R  P  A  S  K  T  F  T  V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3(483-499)</td>
<td>R  D  S  F  I  V  N  K  T  I  T  A  E  V  L  A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a According to published sequence of Dsg3 (3).

b Highlighted amino-acid sequences refer to conserved putative HLA class II binding motifs (33).

Dsg3 peptides were exclusively recognized by TCC from three PV patients.

d Dsg3 peptide was recognized by TCC from a healthy individual.
Noteworthy, TCC PV10.2 recognized peptide DG3(96-112) in the presence of both HLA-DRB1*0402/H11001 and DQB1*0503/H11001 APC (Fig. 5, A and C).

Identification of potential HLA class II binding motifs of the identified Dsg3 peptides

All of the Dsg3 peptides identified in the present study carried a positively charged amino acid (i.e., arginine or lysine) at the p4 anchor position while p1 contained a hydrophobic amino acid (iso-leucine or valine). Most of the Dsg3 peptides carried another small...
hydrophilic (serine or threonine) or hydrophobic amino acid (valine) at p6 (Table III). Three-dimensional model structures for HLA-DRB1*0402 and DQB1*0503 in complex with the DG3(96-112) peptide were generated as described in Materials and Methods. Fig. 6 shows an electrostatic potential map projected onto the surface of these HLA class II molecules. In case of HLA-DRB1*0402, the DG3(96-112) peptide is displayed demonstrating the interaction of the positively charged aa (lysine) at position p4 with the negatively charged surface (P4 pocket) of the HLA class II allele; these negative charges arise from residues DRB 70 and 71 of HLA-DRB1*0402 (Fig. 6A). Although the electrostatic potential of this binding pocket of DQB1*0503 is different compared with DRB1*0402, peptide DG3(96-122) could be accommodated easily into the binding groove of this allele (Fig. 6B). The missing charges may be compensated by the amino acids Glu111 and Asp106 that are in the vicinity of this binding pocket.

TCR Vβ repertoire of Dsg3-reactive Th cell clones

In light of the differential responsiveness of autoreactive TCC to distinct Dsg3 peptides the TCRBV usage of a panel of DG3(96-112) responsive TCC was investigated by RT-PCR. Noteworthy, 2 of 5 TCC responsive to DG3(96-112) were TCRBV5 positive while the remaining TCC used several other TCRBV chains, i.e., Vβ15, 18, and 19 (Table V). These findings suggest that the TCRBV usage of autoreactive TCC specific for a single Dsg3 peptide is rather heterogeneous.

Discussion

In this study, T cell recognition of defined epitopes of the ECD of Dsg3 was studied in PV patients and in Dsg3-responsive healthy individuals. Upon selection by MACS cytokine secretion assay, Dsg3-specific Th responses were not only detected in a cohort of PV patients (irrespective of their clinical status) but also in healthy carriers of the PV-associated HLA class II alleles, DRB1*0402 and DQB1*0503. This finding confirms previous studies from our group supporting the idea that carriers of distinct HLA class II alleles are genetically at risk to develop Dsg3-specific T cell responses independent from the development of PV (21, 22).

Our findings demonstrate that a limited set of only eight Dsg3 peptides was recognized by the majority of the Dsg3-responsive TCC from both PV patients and healthy individuals that were characterized in the present study. Five peptides residing in the ECD 1–3 of Dsg3 were recognized by autoreactive Th1 and Th2 cells from both PV patients and healthy carriers of PV-associated HLA class II alleles (Fig. 2A). There was no direct relationship between T cell reactivity to a distinct epitope of Dsg3 and the clinical status of the PV patients. Noteworthy, peptide DG3(96-112) residing in the ECD1 of Dsg3 was recognized by the majority of TCC derived from both PV patients and Dsg3-responsive healthy donors. Two of the Dsg3 peptides identified in this study, DG3(205-221) and

---

Table IV. Sequence homology of identified Dsg3 T cell peptides with previously identified Dsg3 peptides

<table>
<thead>
<tr>
<th>Dsg3 Peptide</th>
<th>Dsg3 Peptide</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG3(78-94)</td>
<td>aa 78-93</td>
<td>19</td>
</tr>
<tr>
<td>DG3(96-112)</td>
<td>aa 97-111</td>
<td>19</td>
</tr>
<tr>
<td>DG3(189-205)</td>
<td>aa 190-204</td>
<td>19</td>
</tr>
<tr>
<td>DG3(205-221)</td>
<td>aa 206-220</td>
<td>19</td>
</tr>
<tr>
<td>DG3(250-266)</td>
<td>aa 210-226</td>
<td>43</td>
</tr>
<tr>
<td>DG3(342-358)</td>
<td>aa 251-265</td>
<td>19</td>
</tr>
<tr>
<td>DG3(376-392)</td>
<td>aa 240-303</td>
<td>20</td>
</tr>
<tr>
<td>DG3(483-499)</td>
<td>aa 380-396</td>
<td>30</td>
</tr>
</tbody>
</table>

---

Figures:

**Figure 5.** HLA class II restriction of the proliferative T cell response to the immunodominant Dsg3 peptide DG3(96-112). Shown is the proliferation of six DG3(96-112)-responsive TCC upon coculture with DRB1*0402, DQB1*0503, and DRB1*0701 homozygous PBMC as APC (A and C) expressed as a SI which is the ratio of cpm in cultures with/without DG3(96-112). A SI >2 was considered to represent a significant proliferation; background proliferation ranged from 1361-2105 cpm. In addition, mAb against HLA-DR, -DQ, and -DP were added to cultures of the TCC with DG3(96-112) and either HLA-DRB1*0402 APC (B) or HLA-DQB1*0503 APC (D). Shown is the percentage of inhibition of T cell proliferation to DG3(96-112).

**Figure 6.** Potential binding sites to HLA-DRB1*0402 (A) and HLA-DQB1*0503 (B) of the identified Dsg3 peptides. Electrostatic potential maps of the HLA-DRB1*0402 (A) and HLA-DQB1*0503 (B) model structures. In case of the HLA-DRB1*0402 allele, peptide DG3(96-112) is represented as a rod model. Nitrogen, oxygen, and carbon atoms are colored blue, red, and white. Red and blue-colored regions on the surface representation of the two alleles indicate negative and positive charged regions, respectively. Binding pockets for the different positions in the peptide (P1, P4, and P6) are indicated. In the HLA-DQB1*0503 molecule, the p4 pocket exhibits a neutral charge.

---

The Journal of Immunology
DG3(376-392), had already been identified by our group in previous studies: DG3(205-221) is highly homologous to peptide p33 (aa 210–226) which was recognized by T cells from a Dsg3-responsive healthy donor (21) and peptide DG3(376-392) is highly homologous to peptide p67 (aa 380–396) which was recognized by T cells from a DRB1*0402 PV patient (30) (Table IV). The finding that most of the T cell peptides of Dsg3 are located in the first three ECD of Dsg3 is of interest since this region of the Dsg3 ectodomain harbors the major Ab epitopes (31). Two recent studies identified the NH2 terminus of Dsg3 (aa 1–161) as the major binding site for pathogenic autoAg (31, 32); within this region a stretch consisting of aa 25–88 was found to represent an immunodominant epitope for circulating autoAb (31).

An independent study by Lin et al. (20) identified a distinct Dsg3 peptide. These TCC fulfill the criteria of “type A” peptide-specific T cells according to a recent classification (40). In contrast, a considerable fraction (40.2%) of the TCC showed only response to a distinct Dsg3 peptide (at lower concentrations than the aforementioned TCC) but did not mount a significant proliferative response to recombinant Dsg3 protein like “type B” T cells (40). Moreover, despite a vigorous response to the recombinant Dsg3 protein, some Dsg3-specific TCC were not stimulated by any of the used Dsg3 peptides. This may have several reasons: 1) a particular T cell epitope was not fully contained within a single peptide, 2) posttranslational modifications of Dsg3 were not conserved in the synthetic peptides (41), or 3) an immunodominant peptide-induced anergy of autoreactive high affinity TCC (42). This different Ag responsiveness of the generated TCC has to be kept in mind when interpreting the potential significance of the identified immunodominant T cell epitopes of Dsg3 which may be under-/overestimated. Moreover, T cell epitopes may have been missed due to this experimental approach.

Table V. TCR Vβ usage of T cell clones responsive to DG3(96-112) from three PV patients and a healthy individual

<table>
<thead>
<tr>
<th>T Cell Clone</th>
<th>Donor</th>
<th>TCR Vβ Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co 1.1</td>
<td>Control #1</td>
<td>Vβ5</td>
</tr>
<tr>
<td>PV 7.5</td>
<td>Patient PV7</td>
<td>Vβ18</td>
</tr>
<tr>
<td>PV 8.3</td>
<td>Patient PV 8</td>
<td>Vβ5</td>
</tr>
<tr>
<td>PV 10.1</td>
<td>Patient PV10</td>
<td>Vβ15</td>
</tr>
<tr>
<td>PV 10.2</td>
<td>Patient PV10</td>
<td>Vβ19</td>
</tr>
</tbody>
</table>

The present study demonstrates that all of the identified Dsg3 epitopes share common anchor residues at relative positions 1, 4, and 6. These amino acid motifs were previously identified by Wucherpfennig and coworkers (19, 33) to be potential HLA class II binding sites. Based on putative binding motifs of DRB1*0402 (which is prevalent in PV) (34), Wucherpfennig and colleagues (19, 33) proposed seven candidate T cell epitopes of Dsg3 that share amino acid residues at p1, 4, and 6 anchor positions (Table III). In their study, PBMC from four DRB1*0402 PV patients were only stimulated by two of these Dsg3 peptides, aa 190–204 and aa 206–220 (19). Dsg3 peptide aa 190–204 is homologous to peptide DG3(189-205) of the current study, the later Dsg3 peptide (aa 206–220) is homologous to peptide DG3(205-221) of this study and peptide aa 210–226 of the aforementioned study (30) (Table IV). Thus, the findings of the present study clearly extend the previous observations of Wucherpfennig and coworkers by demonstrating that autoaggressive T cells of PV patients do indeed recognize a limited set of Dsg3 peptides. Based on the findings of the present study with a larger cohort of PV patients, DG3(96-112) seems to be preferentially recognized by autoaggressive T cells and not DG3(206-220) as suggested by their previous findings (Fig. 3).

An independent study by Lin et al. (20) identified T cell responses from PV patients to three polypeptides (aa 145–192, aa 240–303, and aa 570–614) representing stretches of the ECD1–5 of Dsg3. Noteworthy, polypeptide aa 240–303 contains a peptide DG3(250-266) which was identified in the present study and by Wucherpfennig and coworkers (19). The Dsg3 peptide DG3(342-358) identified in the present investigation has not been described before as well as the peptide DG3(483-499) which was recognized by T cells from two healthy donors.

Epitope spreading is a common phenomenon found in chronic relapsing autoimmune disorders (35). Our findings suggest that intramolecular epitope spreading of Dsg3 T cell peptides does not occur once the disease is clinically apparent since there was no direct relationship between Dsg3 peptide reactivity and a distinct clinical phenotype (i.e., active vs remittent disease). A recent study (36) clearly demonstrated that epitope spreading occurs in pemphigus foliaceus (PF), a related autoimmune disorder associated with autoAb against Dsg1. Individuals who later developed fogo selvagem, an endemic variant of PF, were found to exhibit autoAb against nonpathogenic epitopes of Dsg1 in the preclinical stage. Once full-blown disease occurred, they had developed autoAb directed against a distant, pathogenic epitope of the ECD1 of Dsg1 (36). There is also evidence for intermolecular epitope spreading in pemphigus since patients with PV may develop PF later in the course of disease (37, 38). Also, PV patients with only mucosal lesions (associated with anti-Dsg3 IgG) may later on develop cutaneous lesions that are associated with autoAb against Dsg1, the autoantigen of PF (11). Along this line, it is possible that intermolecular epitope spreading in PV, as shown for the subsequent development of Dsg3- and Dsg1-specific autoAb can be explained by the cross-reactivity of autoreactive Th cells specific for distinct epitopes of Dsg3 with similar or identical epitopes of the Dsg1 ectodomain (39).

Noteworthy, despite the uniform generation of Dsg3-responsive TCC by in vitro stimulation of PBMC with recombinant Dsg3 protein followed by MACS separation, the proliferative response of the established TCC to recombinant Dsg3 protein and Dsg3 peptides varied (Fig. 4). Most (60%) TCC recognized both, Dsg3 and a distinct Dsg3 peptide. These TCC fulfill the criteria of “type A” peptide-specific T cells according to a recent classification (40). In contrast, a considerable fraction (40.2%) of the TCC showed only response to a distinct Dsg3 peptide (at lower concentrations than the aforementioned TCC) but did not mount a significant proliferative response to recombinant Dsg3 protein like “type B” T cells (40). Moreover, despite a vigorous response to the recombinant Dsg3 protein, some Dsg3-specific TCC were not stimulated by any of the used Dsg3 peptides. This may have several reasons: 1) a particular T cell epitope was not fully contained within a single peptide, 2) posttranslational modifications of Dsg3 were not conserved in the synthetic peptides (41), or 3) an immunodominant peptide-induced anergy of autoreactive high affinity TCC (42). This different Ag responsiveness of the generated TCC has to be kept in mind when interpreting the potential significance of the identified immunodominant T cell epitopes of Dsg3 which may be under-/overestimated. Moreover, T cell epitopes may have been missed due to this experimental approach.

Another major finding of this study was the demonstration of Dsg3 peptide T cell recognition in association with HLA–DRB1*0402 and DQB1*0503 (Fig. 5). These findings are consistent with epidemiological studies showing a strong association of PV with HLA–DRB1*0402 (16) and HLA–DQB1*0503 (17, 18) and a recent study from our group that identified Th cell responses to Dsg3 not only in PV patients but also in healthy carriers of the aforementioned PV-associated HLA class II alleles (22). These findings are in line with three independent studies demonstrating that Dsg3-specific TCC were restricted by HLA–DRB1*0402 (19, 20, 43). The DGS(96-112)-responsive TCC Co1.1 stained positive with a DG3(96-112)/DRB1*0402 tetramer but not with the DG3(250-266)/DRB1*0402 control tetramer providing direct evidence for the presentation of DG3(96-112) by HLA–DRB1*0402 (C. Veldman, R. Eming, and M. Hertl, unpublished observation).
In the present study, 3 of 4 PV patients (Table I) and 2 of 3 of the Dsg3-responsive healthy donors that did not express DRB1*0402 or DQB1*0503 were positive for HLA-DRB1*0301 (Table II). A previous study demonstrated that non-PV-associated HLA class II alleles such as DQB1*0301 restricted Dsg3-driven T cell responses (43). DQB1*0301 is homologous to the PV-associated DQB1*0503 which differs from the common DQB1*0501 allele only by a valine to aspartic acid substitution at putative peptide binding position DQB-chain position 57 (44, 45). These findings may explain why PV patients and healthy individuals who do not carry the “classical” PV-associated HLA class II alleles develop autoreactive T cell responses against Dsg3 (Table I and II).

Dsg3-reactive Th1 (21) and Th2 (20) cells were identified that recognized identical epitopes of the ECD of Dsg3 in the context of PV-associated HLA class II alleles. In a recent study, both Dsg3-autoreactive Th1 and Th2 cells were isolated from patients with acute onset, chronic active and remittent PV. The appearance of Dsg3-reactive Th2 was constant at the different disease stages while Dsg3-reactive Th1 cells were detected at a significantly higher frequency in chronic active PV which was associated with the highest autoAb titers. Noteworthy, the titers of serum autoAb against Dsg3 were directly correlated with the ratio of autoreactive Th1/Th2 cells suggesting that both, Th1 and Th2 cells may be critically involved in the regulation of autoAb production.

Peptide Dsg3(96-112) was recognized by several TCC in association with either, DRB1*0402 or DQB1*0503. Noteworthy, the TCC PV01.02 recognized D3C(96-112) in association with both, DRB1*0402*+ and DQB1*0503*+ APC. This finding suggests that both HLA class II alleles possess at least similar peptide binding motifs which is supported by the three dimensional model structures of these two alleles with the D3C(96-112) peptide. All of the identified Dsg3 peptides of the present study fit into the peptide binding groove of DRB1*0402 based on their positive charge at p4 which is complementary to the negatively charged P4 pocket of DRB1*0402 (Fig. 6a). In DQB*0503, there is not such a negative charge at the P4 pocket (Fig. 6b). Based on the potential interactions of amino acid residues of the Dsg3 peptides at p1 and p6, peptide Dsg3(96-112) may act as a heteroclitic peptide in association with DQB1*0503. Cross-reactivity to similar or identical amino acid residues of D3C(96-112) which are presented by HLA-DQB1*0503 is also a possibility.

The heterogeneous TCR Vβ usage of Dsg3 peptide-specific T cells found in the present study strongly suggests that a highly selective usage of a single TCR Vβ-chain by T cells responsive to a distinct Dsg3 peptide is unlikely. This is also supported by the finding that the peptide affinity of the generated Dsg3-responsive TCC varied considerably (Fig. 4). In an independent study, TCC that responded to aa 145–192 of Dsg3 used the TCR Vβ13 while TCC responsive to peptides aa 240–303 and aa 570–614 preferentially used TCR Vβ7 and Vβ17 genes, respectively (46). In a recent study with patients suffering from PF, a related autoimmune disorder directed against Dsg1, 10 of 17 autoreactive Dsg1-specific TCC expressed oligoclonal TCR Vβ chains (47).

In summary, the findings of the present study strongly suggest that 1) autoreactive Th cells from PV patients (and healthy donors) using different TCR Vβ recognize a limited set of identical Dsg3 peptides, 2) that T cell recognition of Dsg3 is restricted by distinct, i.e., PV-associated HLA class II alleles, and 3) that the identified peptides share potential HLA class II binding motifs. Thus, PV is the consequence of a loss of T cell rather than B cell tolerance. This is supported by the recent evidence for epitope spreading at the Ab level but not at the T cell level as demonstrated in the present study.

Acknowledgments

We thank Dr. Masayuki Amagai (Department of Dermatology, Keio University, Tokyo, Japan) for recombinant PVHis (Dsg3) baculovirus, and Rüdiger Eming and Manfred Lutz for critical reading of the manuscript. We are also grateful to Astrid Mainka for expert help with the preparation of recombinant Dsg3 and the ELISA analysis of Dsg3 autoAb.

References

10. line receptor regulating keratinocyte adhesion is targeted by pemphigus vulgaris autoimmunity. Am. J. Pathol. 157:1377.
14. Amagai, M., T. Hashimoto, K. Natarajan, N. Nagarwalla, A. Okami, and A. R. Ahmed. 1995. Cross-reactivity to similar or identical amino acid residues of D3C(96-112) which are presented by HLA-DQB1*0503 is also a possibility.


