Autoimmune bullous disorders are a group of severe skin diseases characterized clinically by blisters and erosions of skin and/or mucous membranes. A hallmark of these disorders is the presence of IgG and occasionally IgA autoantibodies that target distinct adhesion structures of the epidermis, dermoepidermal basement membrane, and anchoring fibrils of the dermis. This Review focuses on the potential role of autoreactive T cells in the pathogenesis of these disorders. Pemphigus vulgaris (PV) and bullous pemphigoid (BP) are the best-characterized bullous disorders with regard to pathogenesis and T cell involvement. Activation of autoreactive T cells in PV and BP is restricted by distinct HLA class II alleles that are prevalent in individuals with these disorders. Autoreactive T cells are not only present in patients but can also be detected in healthy individuals. Recently, a subset of autoreactive T cells with remarkable regulatory function was identified in healthy individuals and to a much lesser extent in patients with PV, suggesting that the occurrence of autoimmune bullous disorders may be linked to a dysfunction of Tregs.

Autoimmune bullous skin disorders are characterized by the presence of autoantibodies that target distinct adhesion molecules of the epidermis and dermoepidermal basement membrane zone, leading to a loss of adhesive function of the target antigen(s) and, clinically, to the appearance of blisters and erosions (1, 2) (Figures 1 and 2). Desmogleins are transmembranous components of desmosomes, adhesion units specialized in conferring epidermal keratinocyte cohesion and linked to intercellular molecules of the desmosomal plaque, which in turn interact with components of the cytoskeleton (Figure 1). In pemphigus, IgG autoantibodies against desmoglein 3 (Dsg3) and Dsg1 lead to loss of desmosomal adhesion of epidermal keratinocytes and intraepidermal blister formation (Figure 2). In the pemphigoids, IgG autoantibodies against components of the dermoepidermal basement membrane such as bullous pemphigoid (BP) antigen 180 (BP180; also referred to as BP antigen 2 and type XVII collagen), BP antigen 230 (BP230; also referred to as BP antigen 1), and laminin 5 interfere with the adhesion of basal epidermal keratinocytes to the dermoepidermal basement membrane zone (Figure 1). BP180 and BP230 are transmembranous and intracellular components, respectively, of hemidesmosomes of basal epidermal keratinocytes, while laminin 5 is a ligand of BP180 located in the lamina lucida of the basement membrane zone (Figure 1). The autoantigen of epidermolysis bullosa, type VII collagen, is the major component of anchoring fibrils that connect the dermoepidermal basement membrane with interstitial collagen bundles of the dermis (Figure 1). The autoantigen of dermatitis herpetiformis, epidermal transglutaminase, is targeted by autoantibodies of the IgA class in the papillary dermis (Figure 2).

Based on the specificity of the targeted antigens, several clinically and immune serologically distinct bullous disorders have been defined (Figure 2). Pemphigus and pemphigoid are considered to be prototypic bullous disorders based on their well-characterized immune response–mediated pathogenesis (3–5). Apart from pemphigus and BP, there is only circumstantial evidence that autoreactive T cells are present and involved in the pathogenesis of the autoimmune bullous disorders epidermolysis bullosa acquista (6, 7) and dermatitis herpetiformis (8, 9). In pemphigus vulgaris (PV) and BP, autoreactive CD4+ T lymphocytes that are presumably crucial in initiating the autoimmune response recognize distinct epitopes of the extracellular portions of Dsg3 and BP180, components of desmosomal and hemidesmosomal adhesion complexes of human skin, respectively. Dsg3- and BP180-reactive T cells produce Th2 cytokines such as IL-4, IL-5, and IL-13 and presumably foster the production of autoantibodies of the Th2-dependent IgG4 subtype, which are preferentially seen in active stages of these disorders (Figure 3).

Autoreactive T lymphocytes in pemphigus

PV is a severe blistering disorder of the mucous membranes and skin associated with IgG autoantibodies against the desmosomal adhesion molecules Dsg3 and Dsg1 (10) (Figures 1 and 2). Autoantibodies against Dsg3 and Dsg1 are critical in the pathogenesis of PV since their transfer into newborn mice induces a phenotype resembling PV (11, 12). Peripheral CD4+ T cell responses, and occasionally CD8+ T cell responses, to the ectodomain of Dsg3 were identified in PV patients by several independent investigators (13–15); however, the phenotype, cytokine profile, immunogenetic restriction, and epitope specificity of these autoreactive T cell responses varied. Dsg3-reactive Th1 (13) and Th2 (15, 16) cells were identified that recognized portions of the extracellular domain of Dsg3 in the context of PV-associated HLA class II alleles. By ELISPOT assay, Dsg3-specific autoreactive Th1 and Th2 cells were detected at similar frequencies in acute onset PV (17). By magnetic cell sorting (MACS) cytokine secretion assay, Dsg3-reactive Th2 cells were detected at similar frequencies in acute onset, chronic active, and remittent PV, while the number of autoreactive Th1 cells exceeded that of Th2 cells in chronic active PV (18). Autoreactive Th1 and Th2 cells may be involved in the regulation of the production of pathogenic autoantibodies by B cells in PV since sera of patients

Nonstandard abbreviations used: BP, bullous pemphigoid; BP180, BP antigen 180; BP230, BP antigen 230; Dsg, desmoglein; NC16A, noncollagenous domain 16; PF, pemphigus foliaceus; PG, pemphigoid gestationis; PV, pemphigus vulgaris; Tr1, type 1 Treg.

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T cell control in autoimmune bullous skin disorders

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with PV contain Th1-regulated IgG1 and Th2-regulated IgG4 autoantibodies directed against Dsg3 (Figure 3) (19, 20).

PV is associated with HLA-DRβ1*0402 and HLA-DQβ1*0503, while the endemic variant of pemphigus foliaceus (PF), fogo selvagem, which is found in limited areas of South America, is characterized by a prevalence of the 2 HLA class II alleles, DRβ1*0402 and DRβ1*0101 (21–23). We and others found in patients with PV that Th1 and Th2 cell recognition of Dsg3 peptides was restricted by HLA-DRβ1*0402 and/or HLA-DQβ1*0503 and that the proliferative response of autoreactive Th cells was blocked by anti-DR and anti-DQ antibodies, respectively (15, 18, 24, 25). Occasionally, Dsg3-reactive Th cell clones were also restricted by non–PV-associated HLA class II alleles; however, these alleles were homologous to DRβ1*0402 with regard to peptide-binding motifs (25).

In several studies, we were able to confirm the hypothesis that distinct HLA class II alleles shape the autoimmune response to Dsg3 (13, 24, 26). Dsg3-reactive Th1 and Th2 clones from PV patients and HLA-matched healthy donors recognized a limited set of Dsg3

Figure 1
Autoantigens of human autoimmune bullous skin disorders and their role in epidermal and dermoepidermal adhesion. (A) Desmosomes. The integrity of epidermal cell cohesion is largely dependent on desmosomes, plaque-like intercellular adhesion structures that connect transmembranous adhesion molecules such as the desmogleins and desmocollins with keratins of the cytoskeleton through interaction with intracellular components of the desmosomal plaque such as desmoplakin, plakoglobin, plakophilin, envoplakin, and periplakin. In addition to Dsg3 and Dsg1, which are autoantigens in pemphigus, all the other aforementioned components of desmosomes have been identified as autoantigens of different clinical variants of pemphigus. (B) Hemidesmosomes and components of the dermoepidermal basement membrane. Basal keratinocytes adhere to the basement membrane zone by the interaction of cytoplasmic and transmembranous components of hemidesmosomes, such as BP230 and BP180 and α6β4 integrin, with ligands such as laminin 5 located in the lamina lucida and lamina densa of the dermoepidermal basement membrane zone. The intracellular hemidesmosomal components BP230 and plectin are linked to keratins of the cytoskeleton and interact with the cytoplasmic domains of BP180 and α6β4 integrin, which in turn interact with laminin 5 via their ectodomains. Type VII collagen is the major component of anchoring fibrils, which link the basement membrane to interstitial dermal collagen fibers by direct interaction with laminin 5 in the lamina densa of the basement membrane. Figure modified from ref. 58.
Immunological and clinical characteristics of autoimmune bullous skin disorders. (A–C) Pemphigus is characterized by the presence of IgG (and occasionally IgA) specific for desmosomal target antigens (visualized as an intercellular staining pattern by direct immunofluorescence; A) resulting in a loss of intraepidermal adhesion (as shown by histopathology; B) and blisters and/or erosions of the mucous membranes and skin (C). (D–F) In the pemphigoids, including linear IgA bullous dermatosis, IgG (or IgA) autoantibodies bind to antigens of hemidesmosomes and the lamina lucida of the dermoepidermal junction (D), resulting in a loss of subepidermal adhesion (E) and tense blisters (F). (G–I) Epidermolysis bullosa acquisita is associated with IgG (and sometimes IgA) binding to the anchoring fibrils underneath the lamina densa of the dermoepidermal junction (G), resulting in a subepidermal loss of adhesion (H) and tense blisters with a tendency toward scarring and milia formation (I). (J–L) Dermatitis herpetiformis is associated with deposits in the papillary dermis (dotted line indicates the dermoepidermal junction) of IgA reactive with epidermal transglutaminase (J), a subepidermal loss of adhesion (K), and herpetiform blisters or pruritic papules (L). Magnification, x100 (A, B, D, E, G, H, J, and K).

Peptides located within the Dsg3 ectodomain, namely DG3_96–112, DG3_199–205, DG3_280–291, and DG3_320–326, which were recognized in association with HLA-DRB1*0402 and HLA-DQB1*0503 (26). All the identified Dsg3 peptides carried a positive charge at position 4 (p4), which is critical for binding to the negatively charged p4 pockets of DRB1*0402 (aa residues at positions DRβ70 and DRβ71) and DQB1*0503 (aa position DQβ57; Figure 4) (14). T cells from healthy carriers of the PV-associated HLA class II molecules exhibited CD4+ T cell responses against identical epitopes of the Dsg3 ectodomain (26). This later finding supports the concept that PV is the consequence of a loss of tolerance on the B cell level rather than on the T cell level (26). TCR analysis revealed that Dsg3-reactive T cells were of oligoclonal origin and expressed a limited set of different TCRs (27), while an independent study found that autoreactive Th clones specific for the putative immunodominant epitope DG3_96–112 carried a greater spectrum of different TCRs (26).

Circumstantial evidence for a critical role of autoreactive T cells in fostering antibody production was provided by a previous study (28). By ELISPOT assay, IgG-secreting B cells specific for Dsg3 were detected upon in vitro stimulation of peripheral lymphocytes from PV patients with Dsg3. Depletion of the CD4+ T cell subset led to inactivation of IgG-secreting autoreactive B cells. IgG production by the autoreactive B cells was also abolished when anti–HLA-DR or anti–HLA-DQ monoclonal antibody was added to the cultures. These findings strongly suggest that circulating Dsg3-specific B cells are regulated by HLA class II–restricted autoreactive CD4+ T cells (Figure 3).

Autoreactive T cells were also identified in the endemic variant of PF, fogo selvagem (29). Peripheral CD4+ T cells from patients with endemic PF developed a proliferative response to the extracellular portion of Dsg1, the autoantigen of PF, and secreted Th2 cytokines. Using MACS cytokine secretion assay, we identified Dsg1-responsive Th1 and Th2 cells at similar frequencies in patients with PF and occasionally also in healthy individuals (30). These findings are in line with the aforementioned observations in PV, indicating that the presence of autoreactive Th cells is not restricted to patients (18). Other clinical pemphigus variants such as IgA pemphigus and paraneoplastic pemphigus have not been thoroughly characterized regarding the presence of autoreactive T cells, despite the identification of their autoantigens (Table 1). There is some evidence that paraneoplastic pemphigus is at least partly mediated by a CD8+ T cell infiltrate in affected epithelium since necrosis of epithelial cells next to a T cell infiltrate is a hallmark of paraneoplastic pemphigus (31).

**Autoreactive T lymphocytes in the pemphigoids**

The pemphigoids are a group of distinct autoimmune subepidermal blistering diseases of the skin associated with IgG antibodies against BP180 and BP230, 2 components of junctional adhesion complexes called hemidesmosomes that are critically involved in the maintenance of dermoepidermal adhesion (Figure 1) (3–5). BP180 is considered to be the major autoantigen of BP and IgG antibodies against the noncollagenous domain 16 (NC16A) of the BP180 ectodomain, which are detected in more than 90% of BP patients and have been shown to be pathogenic in vitro and in vivo (32, 33). Th2 and Th1 responses against the BP180 ectodomain were identified in the majority of the studied BP patients in 2 independent studies (34, 35).
Recent studies showed that BP180-reactive Th cells and IgG autoantibodies recognized similar or identical epitopes clustered in distinct regions of the BP180 ectodomain and BP230 (36–38). Specifically, the majority of autoreactive Th2 and Th1 cells and B cells recognized epitopes within the NH2-terminal portion, followed by reactivity against the COOH-terminal and central portions, of the BP180 ectodomain (37). Of note, T and B cell reactivity against the NH2-terminal portion of the BP180 ectodomain was associated with severe BP, with widespread blisters and erosions, while the central portion was more frequently recognized in limited BP, with few blisters and erosions (37). In contrast, less than 50% of the studied BP patients showed a combined T and B cell response against the COOH- and NH2-terminal globular domains of BP230 (38). This finding is of particular interest in light of the current discussion as to whether the transmembranous adhesion molecule BP180 or the intracellular hemidesmosomal component BP230 is the major autoantigen of BP (Figure 1) (38).

A considerable number of patients with BP and a clinical variant, pemphigoid gestationis (PG), displayed NC16A-specific peripheral T cell responses, which were mainly of the Th2 type and a mixed...
Th1/Th2 type, respectively (35, 39). T cell recognition of BP180-NC16A seems to be rather heterogeneous since BP180-NC16A–specific T cell clones from the BP patients preferentially expressed a TCR different from the TCR expressed by T cell clones derived from a PG patient (40). Recently, IFN-γ secretion by NC16A-specific autoreactive T cells was also detected in a subset of patients with mucous membrane pemphigoid, suggesting that this clinical variant of BP is also associated with autoreactive T cell responses to BP180 (41). Finally, a mixed Th1/Th2 response to BP180 was identified in patients with linear IgA bullous dermatosis. This disorder is clinically distinct from BP and is primarily associated with IgA autoantibodies against a proteolytically cleaved antigen located within the BP180 ectodomain (Figure 1) (42).

A common HLA class II allele, HLA-DQβ1*0301, seems to be associated with distinct clinical pemphigoid variants (43). Another study found that the association of BP with DQβ1*0301 was restricted to men (44). In our own experience, several BP180-specific Th1 and Th2 cell clones derived from BP patients were found to be restricted by HLA-DQβ1*0301 (45). A subset of healthy individuals who carried the BP-associated HLA class II allele DQB1*0301 also showed BP180-specific T cell responses that were predominantly of the Th1 type (45). Distinct peptide-binding motifs of HLA-DQβ1*0301 have not yet been defined, nor were the anchor motifs of T cell epitopes of BP180 characterized in detail (38). The presence of IgG autoantibodies against BP180 in the clinical BP variant mucous membrane pemphigoid seems to be associated with the presence of HLA-DQβ1*0301 (46).

In vivo evidence for pathogenic relevance of autoreactive T cells in pemphigus and pemphigoid

Results obtained from a knockout mouse with a targeted disruption of the Dsg3 gene (Dsg3–/– mice) support the concept that the absence or loss of the adhesive function of Dsg3 leads to fragility of the skin, oral erosions, and blisters accompanied by weight loss due to inhibited food uptake and a runted phenotype around day 18 of age (47). Amagai et al. used Dsg3–/– mice to establish an active in vivo model of PV by immunizing the Dsg3–/– mice with recombinant mouse Dsg3, which finally led to the production of anti-Dsg3 antibodies (48). Splenocytes from these Dsg3-immunized mice were then transferred into immunodeficient Rag2–/– mice that expressed Dsg3, resulting in the stable production of anti-Dsg3 IgG and the development of a phenotype resembling PV.

Table 1

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Autoantibodies</th>
<th>Autoantigens recognized by autoreactive T cells</th>
<th>T cell subset</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
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<td>PV</td>
<td>IgG4, IgG1, occasionally IgA (in IgA pemphigus)</td>
<td>Dsg3 peptides</td>
<td>PBMC</td>
<td>(14)</td>
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<tr>
<td></td>
<td></td>
<td>Dsg3</td>
<td>Th1, Th2</td>
<td>(15, 16, 18)</td>
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<td></td>
<td></td>
<td>Dsg3, Dsg1</td>
<td>Th1, Th2</td>
<td>(13, 25)</td>
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<td>Th1, Th2</td>
<td>(26)</td>
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<tr>
<td></td>
<td></td>
<td>Dsg3</td>
<td>Th1, Th2, Tr1</td>
<td>(54, 56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dsg3</td>
<td>CD8:CD28:Treg</td>
<td>(55)</td>
</tr>
<tr>
<td>PF, fogo selvagem</td>
<td>IgG4, IgG1</td>
<td>Dsg3</td>
<td>Th1, Th2</td>
<td>(29, 30)</td>
</tr>
<tr>
<td>BP</td>
<td>IgG1, IgG4, occasionally IgA</td>
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<td>Th1, Th2</td>
<td>(34, 35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BP180</td>
<td>Th1, Th2</td>
<td>(38)</td>
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<td>(39)</td>
</tr>
<tr>
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<td>BP180</td>
<td>ND</td>
<td>(41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BP230</td>
<td>ND</td>
<td>(42)</td>
</tr>
<tr>
<td>Mucous membrane pemphigoid</td>
<td>IgG1, IgG4, occasionally IgA</td>
<td>Laminin 5, β1 integrin, BP230</td>
<td>Th1, Th2</td>
<td>(43)</td>
</tr>
<tr>
<td>Linear IgA bullous dermatosis</td>
<td>IgG1, IgG4, occasionally IgA</td>
<td>BP180</td>
<td>ND</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>BP230</td>
<td>ND</td>
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</tr>
</tbody>
</table>

ND, not determined.
immunization of the DRB0402-DQ8–transgenic mice with human Dsg3 recognize epitopes of Dsg3 that exhibit HLA class II anchor motifs identical to the Dsg3 epitopes previously identified in humans (26, 52).

Thus the present mouse model will hopefully facilitate the study of the regulation of HLA class II–restricted T cell recognition of Dsg3 and T cell help for the production of pathogenic antibodies in vivo.

**Potential regulatory network of autoaggressive T cells in pemphigus**

There is now compelling evidence that CD4+ T cells specialized in suppressing immune responses play a critical role in immune regulation (53). Tregs exert a dominant effect in controlling autoimmunity and maintaining peripheral tolerance. The best described of these cells are the naturally occurring CD4+CD25+ Tregs that inhibit Th activation in a cell contact–dependent and non–Ag-specific manner; this T cell subset represents up to 10% of peripheral CD4+ T cells (53). In addition to the naturally occurring CD4+CD25+ Tregs, which have been shown to be continuously produced within the thymus, other T cell subsets bearing suppressive capacity have been described. Among those the most prominent are type 1 Tregs (Tr1s), which have been shown to be induced upon antigen exposure under certain tolerogenic conditions and are characterized by the production of the immunosuppressive cytokines IL-10 and TGF-β (53). Recent progress has been made in the characterization of Tr1s in terms of isolation and induction.

At present, a potential imbalance of CD4+CD25+ Tregs has not been extensively investigated in pemphigus. In a recent study, a subset of Dsg3-reactive, IL-10–secreting T cells was identified that was present in the majority of healthy carriers of PV-associated HLA class II alleles but in less than 20% of the studied PV patients (Figure 5). This distinct T cell subset fulfilled the criteria of Tr1s and suppressed the proliferative response of Dsg3-specific autoreactive Th cells by secretion of IL-10 and TGF-β in a cell contact–independent manner (54). Sinha and coworkers identified a subset of CD8+CD28– T cells with potential Treg function that were present in newly remittent PV patients but not in active or fully remittent PV patients (55). These findings suggest that different subsets of Tregs may be involved in the maintenance of peripheral tolerance to Dsg3 in healthy individuals and in the restoration of tolerance against Dsg3 in patients with remittent PV (Figure 5).

Little is known about the transcriptional regulation of Tr1s in autoimmune disorders in general. Recently Foxp3, which encodes a transcription repressor, has been shown to be expressed by Tregs and to be critical for Treg development. Our group showed that expression of Foxp3 mRNA and protein is an inherent fea-
ture of human Th1s specific for Dsg3 (56). These Dsg3-specific Th1s expressed phenotypic markers of Tregs such as cytotoxic T cell antigen-4, glucocorticoid-induced TNF receptor, and membrane-bound TGF-β and inhibited the activation of autoreactive Th1 and Th2 cells via the secretion of IL-10 and TGF-β (Figure 5). Antisense-driven inactivation of Foxp3 mRNA induced a shift of Dsg3-specific Th1 clones toward a Th2-like phenotype (Figure 5): Foxp3 antisense oligodeoxynucleotide–treated Dsg3-specific Th1 clones secreted IL-2 and the Th2 cytokines IL-5 and IL-10, but no IFN-γ or TNF-α (56). Moreover, they lost their phenotypic Treg markers and inhibitory effect on Th1 and Th2 cells and showed a vigorous proliferative response to Dsg3. This finding suggests that there is a potential relationship between autoreactive Th2 cells and Th1s in PV, which may be derived from a common precursor cell. There is also evidence that Th cells may be converted into Tr1s by bystander mechanisms involving cell-cell contact with naturally occurring CD4+CD25+ Tregs (57).

In vivo, there may be a physiological balance between Dsg3-responsive autoreactive Th2 cells and Dsg3-specific Th1s required for the maintenance of peripheral tolerance, since a high ratio of IL-10+ Th2/Th1 cells was seen in PV patients, while a low IL-10+ Th2/Th1 ratio was commonly found in Dsg3-responsive healthy carriers of PV-associated HLA class II alleles (Figure 5) (54). These findings in PV provide a sound explanation as to why B cell tolerance against Dsg3 exists in healthy individuals who carry autoreactive T cells with epitope specificity identical to that of patients.

Conclusions
There is increasing evidence for a critical role of autoreactive T cells in the regulation of the production of pathogenic autoantibodies in 2 prototypes of autoimmune bullous disorders — pemphigus and pemphigoid. Both disorders are presumably associated with a dysregulation of Th1 and Th2 responses against cutaneous autoantigens, since Th2 responses are primarily associated with disease and Th1 responses are found in healthy individuals. In addition, PV may be the consequence of an imbalance between Dsg3-responsive Th2 cells and Tregs specialized in counter-regulating the devastating T cell autoimmune response. Thus autoreactive Tregs may represent an ideal tool to specifically restore immune tolerance in autoimmune bullous skin disorders.

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