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Antibodies to the Desmoglein 1 Precursor Proprotein but Not to the Mature Cell Surface Protein Cloned from Individuals without Pemphigus

Jun Yamagami,* Stephen Kacir,† Ken Ishii,* Aimee S. Payne,* Don L. Siegel,† and John R. Stanley*‡

In pemphigus foliaceus (PF), autoantibodies against desmoglein 1 (Dsg1) cause blisters. Using Ab phage display, we have cloned mAbs from a PF patient. These mAbs, like those from a previous patient, were directed against mature Dsg1 (matDsg1) on the cell surface of keratinocytes and precursor Dsg1 (preDsg1) in the cytoplasm. To determine whether individuals without pemphigus have B cell tolerance to Dsg1, we cloned mAbs from two patients with thrombotic thrombocytopenic purpura and a healthy person. We found mAbs against preDsg1, but not matDsg1. All but 1 of the 23 anti-preDsg1 mAbs from PF patients and those without PF used the VH3-09 (or closely related VH3-20) H chain gene, whereas no PF anti-matDsg1 used these genes. V_{H} cDNA encoding anti-preDsg1 had significantly fewer somatic mutations than did anti-matDsg1 cDNA, consistent with chronic Ag-driven hypermutation of the latter compared with the former. These data indicate that individuals without PF do not have B cell tolerance to preDsg1 and that loss of tolerance to matDsg1 is not due to epitope shifting of anti-preDsg1 B cells (because of different V_{H} gene usage). However, presentation of peptides from Dsg1 by preDsg1-specific B cells may be one step in developing autoimmunity in PF. The Journal of Immunology, 2009, 183: 5615–5621.

Pemphigus foliaceus (PF) is a tissue-specific autoimmune disease in which Abs against the desmosomal cadherin desmoglein 1 (Dsg1) cause loss of keratinocyte adhesion in the superficial epidermis, resulting in skin blisters (1–3). Dsgs are thought to function as cell-cell adhesion molecules in the epidermis and maintain its integrity. Dsg1 is synthesized in the endoplasmic reticulum as an inactive precursor protein with an N-terminal propeptide (precursor Dsg1 [preDsg1]), which is thought to prevent intracellular aggregation with other newly synthesized cadherins within the secretory pathway. The propeptide is cleaved by a Golgi proprotein convertase such as furin to yield a biologically active mature cadherin (mature Dsg1 [matDsg1]) that is assembled into desmosomes on the cell surface (4, 5).

Polyclonal anti-Dsg1 Abs from PF patients have been shown to be pathogenic in organ culture of normal human skin and by passive transfer to neonatal mice. In both models, these autoantibodies cause blisters from loss of cell-cell adhesion with the typical histology of PF (6–8).

Because previous studies of pathogenic PF autoantibodies were performed with polyclonal Abs from sera, we recently isolated mAbs as single-chain variable fragments (scFvs) from a PF patient using phage display to understand the pathogenicity of individual anti-Dsg1 mAbs (9). These scFvs included pathogenic anti-Dsg1 mAbs that bound the keratinocyte cell surface by indirect immunofluorescence (IIF) and induced blisters in the epidermis, as do the sera from PF patients. However, most of the isolated anti-Dsg1 mAbs were nonpathogenic. These nonpathogenic mAbs could be divided into two groups by IIF and immunoprecipitation Ag mapping: one group showing typical keratinocyte cell surface staining and binding of matDsg1, and the other showing no, or very weak intracellular, staining, with binding of preDsg1 (9, 10). The reason such anti-preDsg1 mAbs can be isolated by phage display is that the Ab phage libraries are panned on Dsg1-coated ELISA plates to isolate anti-Dsg1 Abs. Such plates are made from rDsg1 produced by baculovirus in insect cells, and this recombinant protein contains both matDsg1 and preDsg1 (9–11).

The unexpected finding of Abs specific for intracellular preDsg1 in PF patients led us to hypothesize that individuals without PF might also have B cells that express Abs specific for preDsg1 because preDsg1, being intracellular and not normally exposed to the immune system, would not necessarily induce B cell tolerance (and, of course, such intracellular Ags can under certain circumstances, e.g., in lupus erythematosus, induce autoimmunity). In contrast, we hypothesized that only patients with PF would have Abs against extracellular matDsg1, which, being exposed to the immune system, would normally induce tolerance. Stated differently, the specific autoimmune defect in PF would be loss of tolerance to preDsg1. To test these hypotheses experimentally, we cloned anti-Dsg1 mAbs from another PF patient and from three controls: a healthy individual and two patients with another unrelated autoimmune disease, thrombotic thrombocytopenic purpura (TTP). We chose patients with TTP because, like PF, it is an autoimmune disease with a specific autoantigen target...
(ADAMTS13 metalloprotease). We found that mAbs reacting with preDsg1 were isolated not only from this second PF patient, but also from all three controls, whereas mAbs against matDsg1 were isolated only from the PF patients. Our results suggest that lack of tolerance to matDsg1 is specific to PF, whereas autoreactivity to preDsg1 is not limited to PF patients. We discuss a potential role for autoreactivity to preDsg1 in the onset of autoimmunity in PF patients.

Materials and Methods

Construction of phage library

Using previously described methods (12), we constructed separate IgG-κ and IgG-λ phage libraries from 4 × 10^7 mononuclear cells isolated from 30 ml of peripheral blood collected from a PF patient with clinically active disease. Briefly, RT-PCR was used to amplify the Ig V regions of the heavy (VH) and L chains (VL), and the gene fragments were then cloned into the phagemid vector pComb3X (Scripps Institute). The phagemid library was electroporated into XL-1 Blue suppressor strain of Escherichia coli (Stratagene) with superinfection with M13VH helper phage (Stratagene). In this system, filamentous phage particles express scFv Ab fragments (with a C-terminal 6× histidine tag and a hemagglutinin tag) fused to the pH1 bacteriophage coat protein. Recombinant phage were purified from culture supernatants by polyethylene glycol precipitation and resuspended in PBS (pH 7.2/2.5 1% BSA containing 1 mM CaCl2). The library comprised more than 1 × 10^10 independent transformants as determined by titering on E. coli XL-1 Blue. To validate library diversity before selection on Dsg1, we also selected anti-Dsg1 mAbs from previously constructed libraries derived from the PBLS of two patients with TTP and a healthy person donor.

These studies have been approved by the University of Pennsylvania Institutional Review Board for human research.

Panning of phage libraries

ELISA plates coated with rDsg1 (Medical and Biological Laboratories) were used to isolate phage clones that express anti-Dsg1 scFv, as previously described (9, 13). Briefly, four microtiter plate wells were incubated with blocking buffer (PBS with 3% skim milk) at room temperature for 1 h. The phage library was diluted into blocking buffer and was incubated with Dsg1 on the wells for 2 h at room temperature. After 5–10 washes with PBS-Ca containing 0.1% Tween 20, adherent phage were eluted with 76 mM citric acid (pH 2.0), incubated for 10 min at room temperature, and then neutralized with 2 M unbuffered Tris. The eluted phage were amplified in XL-1 Blue E. coli and rescued by superinfection with M13 helper phage. Phage were harvested from bacterial culture supernatant and then repanned on Dsg1 ELISA plates for three additional rounds. Individual phage clones were isolated from each round of panning and analyzed for their binding to Dsg1 by ELISA using HRP-conjugated anti-M13 Ab (GE Healthcare).

Sequence analysis of scFv Abs

Recombinant phagemids were purified with a plasmid preparation system (Qiagen), and the VH and VL inserts were sequenced using pComb3X specific primers previously described (12). The nucleotide sequences were compared with the germline sequences in V Base sequence directory (http://vbase.mrc-cpe.cam.ac.uk/) to determine their germline gene origins and interrelatedness.

Production and purification of soluble scFvs

The Top10 F’ nonsuppressor strain of E. coli (Invitrogen) was infected with monoclonal phage, and soluble scFvs were purified using Fastbreak lysis reagent (Promega) or osmotic lysis and Talon or nickel metal affinity resin (BD Clontech), as previously described (9, 13).

Dsg1 scFv ELISA

The reactivity of scFv against human Dsg1 was measured by Dsg1 ELISA (Medical and Biological Laboratories) using HRP-conjugated anti-HA mAb (clone 3F10, 1/1000 dilution; Roche Diagnostics) as a secondary Ab, as described (9, 13). In some experiments, to increase the ratio of the mature form of Dsg1 on ELISA plates, we pretreated the plates with 10 U/well furin (New England Biolabs) in 20 mM Tris, 500 mM sodium chloride (pH 7.5; TBS), with 1 mM CaCl2 at room temperature overnight.
Table I. **mAbs from PF1 and PF2 libraries**

<table>
<thead>
<tr>
<th>Source</th>
<th>Clone Name</th>
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<th>D Gene</th>
<th>J Gene</th>
<th>VL Gene</th>
<th>Human Skin IIF</th>
<th>Immunoprecipitation</th>
<th>Pathogenicity</th>
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<td>VH4-30</td>
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<td>Cytoplasm</td>
<td>preDsg1</td>
<td>–</td>
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<td>VH3-09</td>
<td>D3-22/D21-9</td>
<td>J1g4d</td>
<td>3j</td>
<td>Cytoplasm</td>
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<tr>
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<td>VH3-30</td>
<td>D6-13/DN1</td>
<td>J1g4d</td>
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<td>preDsg1 and matDsg1</td>
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<td>F23-5</td>
<td>VH1-08</td>
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<td>O12/O2</td>
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<td>matDsg1</td>
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*The information in the parentheses for each Ab from PF1 indicates the names used in the previous study (9).*  

where it is processed to the mature form, which is stabilized and accumulates in desmosomes (5). Finally, mAbs cloned from patient PF1, with similar IIF and immunochromatographic characteristics, have been shown to bind to preDsg1 (9, 10).

To examine the pathogenicity of the cloned mAbs, we injected them into the dermis of normal human skin in organ culture. F24-9 was the only pathogenic mAb cloned from PF2. It showed a cell surface-staining pattern in DIF of the injected skin, and histology showed blister formation from acantholysis in the granular layer of the epidermis (Fig. 2, A and D), typical of the histology of blisters in PF patients. When injected intradermally into normal human skin organ culture, the other three mAbs that bound matDsg1 (F24-2, F24-15, and F24-9) showed the cell surface-staining pattern in DIF of the injected skin, and histology showed typical keratinocyte cell surface staining by DIF. These results demonstrate the three types of Abs cloned from patient PF1, as follows: 1) anti-matDsg1 mAbs that are pathogenic (Fig. 2, A and D); 2) mAbs that are nonpathogenic, but that bind matDsg1 (Fig. 2, B and E); and 3) anti-preDsg1 mAbs that are all nonpathogenic.

**Comparison of Abs cloned from PF2 with previously cloned Abs from patient PF1**

Previous studies have indicated that the immunologic properties of mAbs cloned from pemphigus patients are mainly dependent on the H chain V region, with L chain usage being promiscuous (9, 13, 15). In other words, the same H chain with different L chains had similar Ag-binding characteristics and pathogenicity. Therefore, in comparing mAbs from the two PF patients, we sorted them by their variable H chain usage (Table I). We isolated a total of 17 clones with unique H chains from the two PF patients (Table I). These mAbs could be categorized into three major groups, as follows: 1) anti-matDsg1 mAbs that are pathogenic (Fig. 2, A and D); 2) mAbs that are nonpathogenic, but that bind matDsg1 (Fig. 2, B and E); and 3) anti-preDsg1 mAbs that are all nonpathogenic.

![Figure 1](image1.png)  
**FIGURE 1.** Immunohistological and immunochromatographic analysis of two major types of anti-Dsg1 mAbs isolated from PF patient 2 (PF2). A, B, and IIF on normal human skin with anti-HA of F24-9 and F24-6 mAbs (each cloned with a hemagglutinin tag). F24-9 stains the epidermis (Fig. 2, A and D), typical of the histology of blisters in PF patients. A, B, and IIF stain the epidermis, whereas F24-6 does not stain the epidermis, but, if it stains at all, weakly stains the cytoplasm, suggesting binding of matDsg1 and preDsg1, respectively. C, Immunoblotting with anti-E-tag. Left two lanes, Show immunoblot of an immunoprecipitation of human rDsg1Ehis. F24-36 precipitates a higher m.w. band, preDsg1, whereas F24-9 binds to lower m.w. matDsg1. Right lane, Shows immunoblot of rDsg1Ehis used for the immunoprecipitation, and shows both the high and lower m.w. species.

![Figure 2](image2.png)  
**FIGURE 2.** Pathogenicity and binding of mAbs from patient PF2 as determined by injection into normal human skin in organ culture. A and D, F24-9 causes a blister in the superficial epidermis typical of PF (as shown by histology with routine H&E staining), and shows typical keratinocyte cell surface staining by DIF of the injected skin. B and E, F24-2 does not cause an epidermal blister, but binds the cell surface of keratinocytes. C and F, F24-6 is nonpathogenic and shows no binding by DIF. These results demonstrate the three types of Abs cloned from patient PF2, as follows: pathogenic anti-matDsg1 (F24-9), nonpathogenic anti-matDsg1 (F24-2), and nonpathogenic anti-preDsg1 (F24-6).
(Fig. 2, C and F). Both PF patients had mAbs in each of these three groups.

The properties of these mAbs indicate that pathogenic anti-Dsg1 Abs do not necessarily use the same VH gene in different patients (compare PF1-8-15 and F24-9). Furthermore, pathogenic and nonpathogenic anti-Dsg1 mAbs can use the same VH gene (compare PF1-8-15 and F24-2). These data suggest that simply targeting Abs by virtue of the specific VH gene used for their expression in various patients will not necessarily be a reasonable strategy to pursue for therapy of PF. Interestingly, ELISA studies showed that F24-9 and PF1-8-15 inhibited each other’s binding to Dsg1, whereas a nonpathogenic anti-Dsg1 mAb did not inhibit pathogenic Ab binding (our unpublished observations). Thus, idiotypes and/or epitopes might be shared by pathogenic Abs from different patients.

Remarkably, unlike anti-matDsg1 Abs, almost all anti-preDsg1 Abs use H chain gene VH3-09 (as do other cloned anti-preDsg1 antibodies; see below), indicating marked genetic restriction. Furthermore, anti-matDsg1 mAbs use different VH genes than do anti-preDsg1 Abs, demonstrating that one set is not derived from the other through B cell maturation (also see Discussion).

**Phage cloning of anti-Dsg1 Abs from a healthy person and two patients with TTP identifies only nonpathogenic anti-preDsg1 mAbs**

Our results revealed that PF patients had Abs not only against matDsg1 on the cell surface, but also against preDsg1. PreDsg1 is intracellular, and under nontraumatic conditions may be inaccessible to the immune system, allowing B cells to escape tolerance. If this is the case, then those without pemphigus should also have anti-preDsg1 Abs. However, we did not expect individuals without pemphigus to have anti-matDsg1 because tolerance should normally be developed to a protein exposed to the immune system.

We, therefore, used phage display to clone anti-Dsg1 Abs from patients with TTP (control patients without pemphigus, but with an autoantigen-specific autoantibody-mediated disease) and one healthy person. We isolated anti-Dsg1 mAbs cloned from a healthy person (designated K2; Table II). Ten unique anti-Dsg1 mAbs were isolated from each of the non-PF libraries by panning on Dsg1-coated ELISA plates that contained both matDsg1 and preDsg1 (9, 10). Ten unique anti-Dsg1 mAbs were isolated from each of the two TTP patient libraries (designated TTP2 and TTP3), and four anti-Dsg1 clones were isolated from the library constructed from a healthy person (designated K2; Table II).

Remarkably, all of these mAbs used variable H chain gene VH3-09, except one clone that used the VH3-20 gene, a human V_{H} germline sequence very similar to that of the VH3-09 gene (91% nucleotide identity). Even though all of these mAbs bound to Dsg1 on ELISA, they showed no staining or a very weak cytoplasmic staining pattern by IIF of normal human skin. None showed cell surface staining (Fig. 3A). Furthermore, these mAbs immunoprecipitated a Dsg1 protein from baculovirus-produced rDsg1 with slightly higher m.w. than that precipitated by an anti-matDsg1 mAb from patient PF2 (Fig. 3B). Therefore, these anti-Dsg1 mAbs isolated from normal individuals showed the same immunological characteristics as anti-preDsg1 Abs from PF patients.

To confirm that these mAbs bound preDsg1, we treated Dsg1 ELISA plates with furin proprotein convertase to convert adsorbed preDsg1 Ag to matDsg1. This approach would be expected to decrease, but not completely eliminate, binding of anti-preDsg1 Abs, because the enzyme is probably not 100% efficient on plate-bound substrate and the cleaved propeptide may still remain adsorbed to plate wells. These ELISA experiments showed that the binding of an anti-Dsg1 mAb (K2D14-4) cloned from a healthy individual was, indeed, decreased on a furin-treated plate by 69% compared with a control buffer-treated plate, whereas an anti-matDsg1 mAb from a PF patient (F24-9) increased binding by 222% on furin-treated plates, compared with controls (Fig. 4A). In addition, mAbs cloned from PF and nonpemphigus patients were tested for their ability to immunoprecipitate furin-treated rDsg1. Immunoblotting of baculovirus-produced Dsg1 indicates a higher and a lower m.w. band, preDsg1 and matDsg1, respectively (Fig. 4B, lane 7). After furin treatment of this baculovirus-produced Dsg1, the preDsg1 band is decreased and the matDsg1 band is increased (Fig. 4B, lanes 6 and 7). T2D14-6, a mAb cloned from one of the TTP patients, immunoprecipitates the higher m.w. preDsg1 from the baculovirus-produced Dsg1, but no longer precipitates a band from the furin-treated Dsg1, because the preDsg1 is processed to the mature form (Fig. 4B, lanes 1 and 2). Conversely, F24-9 (anti-matDsg1 mAb from patient PF2) immunoprecipitates the lower m.w. matDsg1 before and after furin treatment of rDsg1, although it precipitated more Dsg1 after furin treatment, presumably because more matDsg1 is present (Fig. 4B, lanes 3 and 4).

Taken together, these results suggest that anti-Dsg1 Abs derived from individuals without pemphigus identified only nonpathogenic anti-preDsg1 mAbs, and interestingly, these anti-preDsg1 mAbs are predominantly encoded by an identical human VH germline gene (VH3-09), as are most anti-preDsg1 mAbs cloned from PF patients.

### Table II. mAbs from TTP and K2 libraries

<table>
<thead>
<tr>
<th>Source</th>
<th>Clone Name</th>
<th>VH Gene</th>
<th>D Gene</th>
<th>J Gene</th>
<th>VL Gene</th>
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### Figure 3. IIF and immunoprecipitation of mAbs cloned from TTP patients (TTP2, TTP3) and a normal individual (K2). A. All of the anti-Dsg1 mAbs isolated from nonpemphigus individuals show either no staining or very weak cytoplasmic staining in IIF of normal human skin. None show cell surface staining. B. These mAbs immunoprecipitate recombinant preDsg1, in contrast to F24-9, a mAb cloned from patient PF2, that binds the keratinocyte cell surface (Fig. 1A) and immunoprecipitates matDsg1.
Analysis of somatic hypermutation indicates greater affinity maturation for anti-matDsg1 than for anti-preDsg1 Abs

When Ag-specific B cells are activated by Ag stimulation, DNA encoding their Ig receptors undergo somatic mutation (16). B cells whose receptors develop higher affinities for an Ag are preferentially selected through a process referred to as affinity maturation (17). In those B cells that undergo affinity maturation, replacement mutations (i.e., mutations that result in a new amino acid) within the variable CDRs are positively selected because these sequences make contact with Ag (18, 19). Thus, B cells that are chronically stimulated by Ag are selected by affinity maturation and show increased replacement to silent mutations compared with their germline-encoded CDRs. Therefore, we predicted that anti-matDsg1 mAbs from PF patients undergo affinity maturation from chronic stimulation by the exposed Ag, and thus, should have more somatic mutations in their V regions with increased replacement ratio in their CDRs compared with anti-preDsg1 mAbs, which are derived from B cells not chronically stimulated by intracellular preDsg1.

To test this hypothesis, we analyzed the rates of somatic mutation of the VH regions of the anti-matDsg1 and anti-preDsg1 mAbs compared with their original VH germline sequences. Fig. 5A shows the average mutation rates of anti-matDsg1 mAbs from PF patients (n = 8) and anti-preDsg1 mAbs using VH3-09 and VH3-20 genes from PF patients and those without pemphigus (n = 22). Rate of mutation in anti-preDsg1 mAbs was 1.8% in the VH framework regions (FRs) and 3.0% in CDRs. Conversely, mutation rates in anti-matDsg1 mAbs were 5.3% in FRs and 13.4% in CDRs (vs CDRs of anti-preDsg1; p < 0.0001), respectively. As expected, the somatic mutations in anti-matDsg1 Abs occurred more often in the CDRs than in the FRs (CDR vs FR of anti-matDsg1; p = 0.0045) because the B cells encoding the former group can be positively selected by increasing Ab affinity, whereas the latter group may be negatively selected by destabilizing the FR (18). Finally, consistent with affinity maturation of anti-matDsg1 Abs, we found an increase in the ratio of mutations causing amino acid replacement to those that are silent in the CDR compared with that in the FR (Fig. 5B). The replacement to silent ratios of anti-matDsg1 mAbs were 1.79 in FRs and 3.23 in CDRs.
Finally, we also compared the rate of somatic mutations in the anti-preDsg1 mAbs from the PF patients and from the individuals without PF, because we thought that in PF there might be some exposure to preDsg1 released from damaged epidermis; therefore, there might be a somewhat higher mutation rate in PF patients due to some Ag stimulation. This was, in fact, the case. Anti-preDsg1 mAbs from PF patients showed higher mutation rates \( (n = 8; 4.7\%) \) than anti-preDsg1 mAbs from non-PF individuals \( (n = 14; 1.9\%) \) in the CDRs \( (p = 0.0158) \), and the former group also showed higher replacement to silent ratios than the latter group.

**Discussion**

In this and a previous study \( (9) \), we cloned a cohort of mAbs from two PF patients (PF1 and PF2) that could be categorized into three groups based on pathogenicity and autoantigen specificity: pathogenic anti-matDsg1 mAbs, nonpathogenic anti-matDsg1 mAbs, and nonpathogenic anti-preDsg1 mAbs.

To determine whether individuals without pemphigus have B cell tolerance to Dsg1, we cloned anti-Dsg1 mAbs from two patients with an unrelated autoimmune disease and a healthy individual. In contrast to anti-Dsg1 mAbs isolated from PF patients, we only found mAbs against the precursor, but not mature form of Dsg1. All but 1 of the 23 anti-preDsg1 mAbs from PF patients and those without PF used the VH domain of VH3-09 (or closely related VH3-20) H chain gene, whereas no PF anti-matDsg1 used these genes. Although no Ab repertoire cloning method, including phage display, may necessarily reflect the exact frequency of VH gene usage in a given individual, the finding of so many independent anti-preDsg1 clones with VH3-09 (or a closely related H chain) in two PF patients and three individuals without PF, and the absence of the use of VH3-09 genes in all anti-matDsg1 Abs, implies the importance of VH3-09 in forming anti-preDsg1 Abs. Furthermore, although methods used to construct phage display libraries may generate H and L chain pairings not present in vivo, previous studies using phage display to study human immune repertoire \( (9, 15, 20, 21) \), and this study, show that the immunological properties of many Ag-specific auto- and alloantibodies are mainly carried by their H chains. That is, the same immunological properties are found when the same H chain is paired with multiple different L chains.

Anti-preDsg1 Abs cloned from pemphigus and nonpemphigus patients suggest lack of B cell tolerance to this intracellular Ag and have similarities to so-called natural autoantibodies (NAA). These NAA have been long recognized as self-reactive Abs in healthy people \( (22, 23) \). The specificity of NAA is mostly carried by the H chain, which has few somatic mutations, and the L chain contributes minimally to the specificity of these Abs \( (22, 24) \). NAA are often directed against nuclear or cytoplasmic Ags, and some of the B cells that express such Abs are thought to survive because the Ags are mostly hidden \( (25–27) \). The mAbs against preDsg1 isolated in this study share many of these features. They are directed against a cytoplasmic Ag; they show restricted VH gene usage, but variable VL gene usage; and their H chains have few somatic mutations. These anti-preDsg1 B cells would not usually be stimulated because preDsg1 is intracellular, and they, therefore, would be expected to be low producers of Abs. In some PF patients, anti-preDsg1 is detected in serum \( (10) \) possibly because epidermal damage releases preDsg1.

One theory of autoimmunity is that B cells with NAA receptors become stimulated either through molecular mimicry or tissue injury with resultant somatic mutation causing pathologic autoantibody production \( (22, 25, 28) \). Such a switch to pathologic Abs resulting from somatic mutation could be from a process called epitope shift or epitope migration in which somatic mutations cause an Ab to bind a different part of the original Ag \( (20, 29, 30) \).

However, in the case of PF autoantibodies against Dsg1, this mechanism does not seem to be operative. The B cells in PF that express anti-matDsg1 Abs are derived from somatic mutation from the B cells expressing anti-preDsg1 Abs, because our clonal analysis indicates that pathologic (and nonpathologic anti-matDsg1) PF autoantibodies are derived from different VH genes than are the anti-preDsg1 B cell receptors.

Although it is possible that the presence of anti-preDsg1 B cells is unrelated to, and independent of, the pathogenicity of PF, anti-preDsg1 B cells could still be involved in the initiation of autoimmunity. For instance, they could present peptides representing parts of the matDsg1, derived from the processing of preDsg1, to T cells that have lost tolerance to peptides from matDsg1, thereby stimulating those cells \( (Fig. 6) \). In the context of tissue destruction, these B cells could take up preDsg1 and then present peptides that are found in matDsg1 in the context of the MHC molecule \( (31) \). In this model, B cells would also have to lose tolerance to matDsg1 as a last step, and they would be stimulated to produce anti-matDsg1 Abs by these T cells. This multiple step model requires many so-called checkpoint failures, and thus, would be consistent with the rarity of PF.

One method that has been postulated for induction of tolerance to self-Ags is L chain receptor editing, in which an autoreactive B cell during its development switches its L chain to become unreactive \( (32) \). Although this particular L chain from the autoreactive B cell could be genetically excised, phage display cloning allows artificial pairing in vitro of H chains with the entire L chain repertoire, which might in turn allow re-expression of these autoimmune receptors that had been deleted in vivo. Our results that we do not find anti-matDsg1 autoantibodies by phage display in individuals without pemphigus suggest that such a L chain receptor editing probably does not take place to induce tolerance to matDsg1. Alternatively, if tolerance is induced by L chain editing, the edited L chain could be essentially unique in the entire L chain repertoire, which seems unlikely, especially because the properties of anti-Dsg1 Abs in PF are H chain dependent, with L chains being...
more promiscuous (i.e., mAbs with different L chains paired with the same H chain have similar immunological properties) (9).

Although the potential physiologic role of anti-preDsg1-expressing B cells is not well understood, the existence of anti-preDsg1 in patient sera is of practical importance when interpreting certain aspects of pemphigus biology. For example, there are pemphigus patients with significant anti-preDsg1 Abs in their sera that give high titer ODs by ELISA, but minimal clinical disease (10). Furthermore, the existence of such B cells is important when interpreting T cell stimulation studies in normals and pemphigus patients in which rDsg, which comprises an admixture of precursor and mature forms, is used (33–35).

The findings of anti-preDsg1, but not anti-matDsg1, in individuals without pemphigus suggest a potential initiating event in the immunopathogenesis of PF and point out the necessity of studying loss of tolerance to matDsg1, and not preDsg1, in studies of the immunology of this disease.

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References