Patients with pemphigus foliaceus (PF) have blisters on skin, but not mucous membranes, whereas patients with pemphigus vulgaris (PV) develop blisters on mucous membranes and/or skin. PF and PV blisters are due to loss of keratinocyte cell–cell adhesion in the superficial and deep epidermis, respectively. PF autoantibodies are directed against desmoglein (Dsg) 1; PV autoantibodies bind Dsg3 or both Dsg3 and Dsg1. In this study, we test the hypothesis that coexpression of Dsg1 and Dsg3 in keratinocytes protects against pathology due to antibody-induced dysfunction of either one alone. Using passive transfer of pemphigus IgG to normal and DSG3<sup>−/−</sup> neonatal mice, we show that in the areas of epidermis and mucous membrane that coexpress Dsg1 and Dsg3, antibodies against either desmoglein alone do not cause spontaneous blisters, but antibodies against both do. In areas (such as superficial epidermis of normal mice) where Dsg1 without Dsg3 is expressed, anti-Dsg1 antibodies alone can cause blisters. Thus, the anti-desmoglein antibody profiles in pemphigus sera and the normal tissue distributions of Dsg1 and Dsg3 determine the sites of blister formation. These studies suggest that pemphigus autoantibodies inhibit the adhesive function of desmoglein proteins, and demonstrate that either Dsg1 or Dsg3 alone is sufficient to maintain keratinocyte adhesion.
Explanations for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris

Mý G. Mahoney,1 Zhihong Wang,1 Kyle Rothenberger,1 Peter J. Koch,1 Masayuki Amagai,2 and John R. Stanley1

1Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA
2Department of Dermatology, Keio University, Tokyo 160, Japan

Address correspondence to: John R. Stanley, 211 Clinical Research Building, 415 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA. Phone: (215) 898-3240; Fax: (215) 573-2033; E-mail: jrstan@mail.med.upenn.edu

Received for publication September 16, 1998, and accepted in revised form December 22, 1998.

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Introduction
Pemphigus is a life-threatening blistering skin disease in which patients’ autoantibodies are directed against desmosomal glycoproteins, resulting in the loss of keratinocyte cell–cell adhesion (1). The two classic forms of pemphigus are pemphigus foliaceus (PF) and pemphigus vulgaris (PV) (2). In PF, patients develop skin erosions that result from blisters within the granular layers of the superficial epidermis. Patients with PF, however, do not develop blisters or erosions of the mucous membranes. Two-thirds of patients with PV develop mucous membrane blisters early in the course of their disease, and as the disease progresses, they develop skin blisters as well (3). Histologically, PV blisters occur deep in the epidermis between the basal and most immediate suprabasal keratinocytes, as well as between the basal cells themselves.

Pemphigus autoantibodies recognize cell-surface antigens of keratinocytes (4). These antigens have been identified as desmogleins (Dsg’s), transmembrane desmosomal glycoproteins belonging to the cadherin supergene family of calcium-dependent adhesion molecules (5–7). Dsg1 is the autoantigen recognized by PF antibodies, whereas Dsg3 is specifically recognized by PV autoantibodies (8–13). However, about one-half to two-thirds of PV sera also contain antibodies against Dsg1 (10, 14–16). Most patients with early PV and only mucous membrane lesions have only anti-Dsg3 antibodies, whereas most patients with later disease, involving the skin, have both anti-Dsg3 and anti-Dsg1 antibodies (13, 16).

There is compelling evidence for the pathogenicity of these autoantibodies in pemphigus. One example is the development of skin blisters in neonatal mice when injected with pemphigus IgG (17, 18). Similar to the pathology of human pemphigus, PF IgG induces blisters in the superficial epidermis, and PV IgG induces deep suprabasilar blisters in neonatal mice. Immunoabsorption and affinity chromatography of pemphigus sera have confirmed that the anti-desmoglein antibodies are pathogenic in pemphigus. PF sera that are immunoabsorbed with the extracellular domain of Dsg1 are no longer pathogenic in neonatal mice, whereas IgG that has been affinity purified from PF sera on Dsg1 causes superficial skin blisters (19). Similarly, PV sera immunoabsorbed with the extracellular domain of Dsg3 lose pathogenic activity (20, 21).

Although these anti-desmoglein antibodies have been shown to cause the blister in pemphigus, the pathophysiological mechanism by which they do so has been controversial. Some studies have suggested that pemphigus antibody binding mediates protease release that, in turn, causes loss of cell adhesion (22–25). However, we have postulated a more direct effect in which antibodies block the function of the Dsg’s in stabilizing cell adhesion in desmosomes (7). Lending credence to this theory has been

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the characterization of DSG3null mice that are essentially equivalent to mice whose Dsg3 function has been completely blocked (26). These mice have a similar phenotype to patients with PV, with oral mucous membrane lesions in neonates due to suckling and skin erosions at sites of trauma. Both types of lesions show histology typical of PV.

It has been suggested (27–30) that the distribution and expression levels of Dsg1 and Dsg3 might account for the characteristic distribution of lesions. For example, Dsg3 is expressed throughout the oral mucosa, whereas it is only expressed in the basal and immediate suprabasal layer of the epidermis (29, 30). Conversely, Dsg1 is expressed throughout the epidermis and oral mucosa, but more intensely superficially, and very weakly in the deep epidermis. These types of observations have led to the hypothesis, first advanced by Shirakata et al. (30), that where Dsg3 and Dsg1 are coexpressed, antibodies against either one alone are not efficient at causing spontaneous blistering (e.g., anti-Dsg1 in the oral mucous membrane where Dsg3 is highly expressed throughout). On the other hand, in areas where Dsg1 is expressed without concomitant Dsg3, antibodies against Dsg1 alone are efficient at causing spontaneous blister formation (e.g., in the superficial epidermis of patients with PF).

In this report, we use passive transfer of pemphigus IgG to normal and DSG3null neonatal mice to test this hypothesis. We will show that if both Dsg1 and Dsg3 are present concomitantly at a tissue site, antibodies against either alone are inefficient at causing a spontaneous blister. In contrast, antibodies against both Dsg’s are highly efficient at blister formation if both are concomitantly expressed in the tissue, or antibodies against one alone are efficient if the other is not coexpressed. These findings are consistent with an explanation for blister formation in which autoantibodies against either Dsg1 or Dsg3 specifically block only its function, in contrast to causing release of proteases that nonspecifically cause loss of cell adhesion. Finally, by knowing the anti-desmoglein specificity of pemphigus sera and the Dsg distributions in stratified squamous epithelia, the localization of blister formation in PV and PF can be explained.

Methods

Serum IgG purification and characterization. Ten human sera were used: one normal human serum (NHS3019), sera from two patients with PF (PF982 and PF1239), and sera from seven patients with PV (JPV21, NIH2054, PV1236, PV1581, PV3014, PV3015, and PV3024). The IgG fractions of the sera were isolated by chromatography using DEAE Affi-Gel Blue according to the manufacturer’s protocol (Bio-Rad Laboratories Inc., Richmond, California, USA). For each purification, serum (20 ml) was dialyzed overnight at 4°C against 20 mM KH2PO4 (pH 8.0) and 0.02% NaN3 and loaded on a 100-ml column bed. The eluted IgG was concentrated using Centriprep 10 (Amicon, Lexington, Massachusetts, USA) and dialyzed against PBS (pH 7.4; GIBCO BRL, Grand Island, New York, USA) overnight at 4°C. The protein concentration was then determined using Bio-Rad Protein Assay (Bio-Rad Laboratories Inc.). The final IgG concentrates were sterile-filtered through 0.22-μm membranes (Millipore Corp., Bedford, Massachusetts, USA) and stored at −20°C.

Before affinity purification, all sera were tested by indirect immunofluorescence for cell-surface staining on monkey esophagus cryosections (Scimedx Corp., Denville, New Jersey, USA). The titers were greater than 320 for all PV and PF sera.

Dsg1 and Dsg3 ELISA. Procedures for ELISA using baculovirus-expressed recombinant Dsg1 and Dsg3 have been described previously (13). The methods were slightly modified, and index values were used for ELISA scores instead of reaction units. Briefly,
all sera were diluted 200-fold and incubated for 1 h at room temperature on the recombinant Dsg-coated 96-well ELISA plates. After washing, the plate was incubated with peroxidase-conjugated mouse monoclonal anti-human IgG antibody (MBL, Nagoya, Japan) for 1 h at room temperature. After washing again, the color was developed with a tetramethylbenzidine solution for 30 min, and the reaction was stopped with 2N H2SO4.

The absorbance was measured at 450 nm by ELISA reader (Bio-Rad Laboratories Inc.). Index values were obtained from the following formula: Index = [(OD of tested serum – OD of negative control) / (OD of positive control serum – OD of negative control)] × 100. Positive controls for Dsg1 and Dsg3 ELISAs were diluted standard PF and PV sera, respectively. Negative control was a diluted serum obtained from a normal individual.

IgG injection into neonatal mice. C57Bl/6f mice were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). The DSG3+/- mice were obtained by DSG3+/– heterozygote intercrosses (26). Newborn mice (1–2 days old) weighing between 1.3 and 1.8 g were injected subcutaneously between the shoulder blades using a 1-cc insulin syringe (Becton Dickinson, Franklin Lakes, New Jersey, USA). Each animal received between 1 and 26 mg of the purified IgGs and was sacrificed 16 h later for evaluation. The mice were injected with 100 units of heparin (Lyphomed, Deerfield, Illinois, USA) and decapitated 10 min later. Skin and head sections were fixed in 10% PBS-buffered formalin (Sigma Chemical Co., St. Louis, Missouri, USA) for histology. Tails were collected for DNA extraction and genotyping.

After immunoadsorption with Dsg1, the ELISA readings for anti-Dsg3 antibodies decreased by 17% (from 0.561 to 0.468) with JPV21 serum and by 21% (from 0.894 to 0.704) with NIH2054 serum. To compensate for the decrease of anti-Dsg3 antibodies, we increased the amount of IgG injected per pup: from 4.3 mg/pup of unadsorbed JPV21 to 5.2 mg/pup of Dsg1-adsorbed JPV21 and from 9.4 mg/pup of unadsorbed NIH2054 to 12 mg/pup of Dsg1-adsorbed NIH2054.

To score the animals or histological tissues based on the degree of blistering, we have devised the following protocols: We scored gross blisters on animals before sacrifice on a scale from 1+ to 4+, where 1+ denotes blisters only when handled; 2+, localized blisters 30% (of tissue); 3+, extensive blisters >30%; and 4+, very extensive blisters >80%. Animals without any blisters received a (–) mark. Histological blisters were scored from 0.5+ to 4+, where 0.5+ denotes very minor blisters; 1+, blisters at the edge; 2+, localized blisters >50%; 3+, extensive blisters >50%; and 4+, very extensive blisters >75%. Tissues that did not show any blisters received a (–) mark.

PCR and DSG3 genotyping. The DSG3 exon 1 (200 bps) was amplified with primers 5’- GGAGGAACAGACTAACAGGC and 5’- ACCATCAGGAGGGCCAGAGA, and the neomycin DNA fragment (300 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- ACCATCAGGAGGGCCAGAGA, and the neomycin DNA fragment (300 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- GGAGGAACAGACTAACAGGC and 5’- ACCATCAGGAGGGCCAGAGA, and the neomycin DNA fragment (300 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC. The DSG3 exon 1 (200 bps) was amplified with primers 5’- CGGTGGTTG- GAGAGGCTATTC and 5’- AGGTGAGATTACAGGAGATC.
sylvania, USA) or fixed in 10% formalin (Sigma Chemical Co.). With frozen OCT tissue, sections (4 µm) were fixed either by air drying or 5 min in methanol/acetone (–20°C) and then washed in PBS (GIBCO BRL) for 15 min. The tissue sections were then blocked for 1 h in blocking buffer (1% BSA; fraction V; Sigma Chemical Co.) and incubated with PF982 or PF1239 sera in blocking buffer overnight at 4°C. After a 15-min wash in PBS, the tissue sections were incubated with FITC-conjugated goat anti-human IgG (1:80 in blocking buffer, Cappel Laboratories, Cochranville, Pennsylvania, USA) for 1 h at room temperature. The tissue sections were again washed for 15 min in PBS, blocked for 15 min in blocking buffer, and incubated with the sera or IgG fractions suspended in blocking buffer for 1 hr at room temperature. The remaining steps were as described earlier here.

To stain for Dsg3, AP904, an affinity purified rabbit anti-mouse Dsg3 antibody, was used. This antibody was shown by immunoblot to recognize a 130-kDa protein in DSG3+/+ but not DSG3 null mouse skin extracts (31). Formalin-fixed paraffin-embedded tissues (4 µm) were deparaffinized for 5 min each in xylene (2 times), 100% ethanol (2 times), 95% ethanol, 75% ethanol, and 50% ethanol. After a brief rinse in PBS, the tissues were microwaved at 900 W for 4.5 min in an antigen-retrieving medium (TUF; Signet, Dedham, Massachusetts, USA). The tissues were then treated at 41°C for 7 min each with 0.1% trypsin and then 3% hydrogen peroxide. After a 7-min incubation in 20% ethanol in PBS, the tissues were incubated in blocking buffer for 1 h at room temperature and then with AP904 (1:100) in blocking buffer overnight at 4°C for immunofluorescence.
ELISA. Levels of anti-Dsg1 and anti-Dsg3 antibodies in PV sera measured by ELISA. Dsg1 ads. indicates that the serum was adsorbed with the extracellular domain of Dsg1 produced in baculovirus. The ELISA index is defined in Methods.
were more susceptible to gross blister formation by anti-Dsg1 antibodies in PF sera.

Similar to humans, neonatal mice express Dsg3 throughout the oral epithelium. Therefore, we hypothesized that this expression should protect against blister formation from the anti-Dsg1 antibodies in PF sera. PF (PF982 and PF1239) IgG (10 mg/pup) was injected into C57Bl/6j (n = 12), DSG3−/− (n = 6), and DSG3+/− (n = 12) neonatal mice within one to two days after birth. Within 16 hours after the IgG transfer, all 30 animals had gross blisters over 80% of their skin. Histologically, the blisters were observed in the superficial epidermis between cells within the granular layers (Fig. 3a). In spite of these extensive skin blisters, none of the animals developed blisters in the oral mucosa (Fig. 3b). These results show that in both the deep epidermis and throughout the epiderelia of oral mucous membrane, expression of Dsg3 protects against blister formation by anti-Dsg1 antibodies.

If, indeed, Dsg3 protects against blistering from anti-Dsg1 antibodies, then in DSG3null mice injected with PF antibodies, we would expect to see loss of cell adhesion extending to the deep epidermis and throughout the mucous membranes, sites protected by Dsg3 expression in normal mice. To test this prediction, six DSG3null neonatal mice were injected with 10 mg IgG from two different PF sera (PF982 and PF1239). All six animals developed extensive gross blisters. The histology of the skin mostly showed acantholytic blisters deep in the epidermis (Fig. 3c); however, in some areas, extensive acantholysis was observed throughout the entire epidermis, from the suprabasal to the granular layers (Fig. 3d). We assayed these mice for mucous membrane lesions by histological analysis of the anterior palate, a region that never shows blistering in un.injected DSG3null neonates. All PF IgG–injected DSG3null mice developed extensive suprabasilar blisters on the anterior palate (Fig. 3e). Thus, in DSG3null mice, IgG IgG causes blistering in areas normally protected from pathology by coexpressed Dsg3 in wild-type mice.

Anti-Dsg1 antibodies in PV sera are pathogenic. All PV sera contain antibodies against Dsg3, whereas one-half to two-thirds also contain antibodies against Dsg1 (10, 14–16). To determine whether the anti-Dsg1 antibodies in PV sera are pathogenic, we injected six DSG3null animals from three different litters with IgG from three different PV sera (PV3014, JPV21, and NIH2054) that contained high levels of anti-Dsg1 antibodies, as determined by enzyme-linked immunosorbent assay (ELISA) (Fig. 4). All six animals developed extensive gross skin blisters (example shown in Fig. 2d). By histology, the blisters were localized to the deep suprabasal layer of the epithelium (Tables 1 and 2; Fig. 3f). To demonstrate that it is the anti-Dsg1 antibodies that are pathogenic in these mice, we adsorbed two PV sera (JPV21 and NIH2054) with the extracellular domain of Dsg1 produced in baculovirus. ELISA assay confirmed that these sera no longer contained anti-Dsg1 antibodies (Fig. 4). IgG from these adsorbed sera no longer caused blisters in DSG3null mice (Table 2). These data show that anti-Dsg1 antibodies from these PV sera result in the same pathology in DSG3null mice as those from PF sera (Fig. 3e).

Both anti-Dsg3 and anti-Dsg1 antibodies are necessary for efficient blister formation by PV sera. To determine whether anti-Dsg1 antibodies increase the pathogenicity of PV sera, we injected neonatal wild-type mice with different PV sera, all containing anti-Dsg3 antibodies, but with varying amounts of anti-Dsg1 antibodies, as determined by ELISA (Fig. 4 and Table 1). Mice injected with IgG from PV 1236 (10 mg/pup) or high-dose IgG from PV3024 (26 mg/pup), both containing insignificant anti-Dsg1 antibodies, did not develop gross or significant histological blisters (Figs. 2 and 3g). In contrast, PV sera with increasing amounts of anti-Dsg1 antibodies caused increasingly severe blistering, even when injected at lower doses (10 mg/pup). In pups injected with PV3015, which by ELISA contained a low level of anti-Dsg1 antibodies, no gross blisters were observed, but suprabasilar blisters were detected by histology in most animals. The two PV sera with the highest level of anti-Dsg1 antibodies, PV1581 and PV3014, caused gross skin blisters in all pups and oral blisters in 30%, all suprabasilar by histology (Fig. 3, h and i). These observations demonstrate that PV sera containing anti-Dsg3 alone are inefficient at causing blister formation and that PV sera that contain both anti-Dsg3 and anti-Dsg1 are much more pathogenic.

To demonstrate further the importance of anti-Dsg1 antibodies in the pathogenesis of PV, we adsorbed these antibodies from pathogenic PV sera (JPV21 and NIH2054), leaving only the anti-Dsg3 antibodies, which

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>C57Bl/6j or DSG3+/−</th>
<th>DSG3−/−</th>
<th>DSG3null</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV3024</td>
<td>Skin</td>
<td>0.5+D</td>
<td>4+D</td>
</tr>
<tr>
<td>(26 mg IgG/pup)</td>
<td>Palate</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PV1236</td>
<td>Skin</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(10 mg IgG/pup)</td>
<td>Palate</td>
<td>1+D</td>
<td>–</td>
</tr>
<tr>
<td>PV3015</td>
<td>Skin</td>
<td>1+D</td>
<td>–</td>
</tr>
<tr>
<td>(10 mg IgG/pup)</td>
<td>Palate</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PV1581</td>
<td>Skin</td>
<td>2+D</td>
<td>2+D</td>
</tr>
<tr>
<td>(10 mg IgG/pup)</td>
<td>Palate</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PV3014</td>
<td>Skin</td>
<td>4+D</td>
<td>4+D</td>
</tr>
<tr>
<td>(10 mg IgG/pup)</td>
<td>Palate</td>
<td>3+D</td>
<td>3+D</td>
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See Methods for definition of scale. Minus sign represents no blisters; D represents blisters deep in the epithelium. PV, pemphigus vulgaris.
were then tested for their ability to cause skin blisters in neonatal mice (Table 2). ELISA assay demonstrated that before adsorption both sera contained anti-Dsg3 and anti-Dsg1 antibodies, but after adsorption with the extracellular domain of Dsg1, essentially only anti-Dsg3 antibodies were detectable (Fig. 4). The unadsorbed sera induced extensive gross blisters (Fig. 2f) that were histologically in the deep epidermis. In contrast, after adsorption, even though higher amounts of IgG were injected to compensate for the slight decrease in anti-Dsg3 antibody titers, mice did not develop gross (Fig. 2g) or histological blisters. These results confirm that the anti-Dsg1 antibodies in PV sera are critical for efficient pathogenicity.

Final proof that anti-Dsg1 antibodies are critical to PV sera pathogenicity was provided by taking nonpathogenic PV sera that contained only anti-Dsg3 antibodies and adding small amounts of anti-Dsg1 antibodies from PF sera. Even when injected at a dose of 26 mg per neonatal mouse, PV3024, which contains only anti-Dsg3 antibodies (Fig. 4), was extremely inefficient at causing blisters (Table 1). However, 10 mg of IgG from this PV serum plus 1 mg of PF IgG produced extensive suprabasilar blisters in wild-type mice (Figs. 2h and 3j).

These data demonstrate the importance of both the anti-Dsg3 and anti-Dsg1 antibodies in the pathophysiology of PV blister formation and support the hypothesis that it is necessary to interfere with the function of both Dsg's in areas of skin in which they overlap to produce blisters efficiently. According to this hypothesis, the reason that anti-Dsg3 antibodies alone are inefficient at causing loss of cell adhesion deep in the epidermis might be weaker than that in the superficial epidermis at least down to the apical membrane of the basal cells (Fig. 1, e and f) and can compensate for lost Dsg3 function. However, in adult humans, as opposed to mice, there is much less Dsg1 in oral mucosa than in skin; therefore, in the deep mucosa, where the intensity of Dsg1 expression falls off dramatically, there may be little, if any, Dsg1 (30). Therefore, in humans, anti-Dsg3 alone may be efficient at causing oral mucous membrane blisters in early disease. In later disease, patients with PV develop anti-Dsg1 antibodies in addition to the anti-Dsg3 antibodies. We propose that in later disease, mice and humans develop deep epidermal blisters because the PV anti-Dsg3 and anti-Dsg1 antibodies interfere with the function of both Dsg’s and penetrate from the dermis into the deep epidermis, where cell–cell adhesion may be weakest, as already discussed.

The results discussed here do not support the theory that antibodies in pemphigus indirectly cause loss of cell adhesion through mediation of protease release from keratinocytes. If antibody binding caused protease release, which in turn caused blisters, then in areas where Dsg3 and Dsg1 are coexpressed, antibody binding to one of the two antigens would be expected to produce blisters. However, we show here that these areas are actually protected from blister formation.

Our results strongly suggest that antibodies in pemphigus interfere with the function of Dsg’s and that if either Dsg1 or Dsg3 is still functional, there is limited spontaneous blistering, but that if neither is functional, then extensive blistering results. These observations also underscore the importance of Dsg’s in maintaining cell adhesion in stratified squamous epithelia.

A logical extension of our findings is that it would be reasonable to screen for pharmacologic agents that increase the expression of Dsg’s that are not bound by antibodies in particular types of pemphigus, thus protecting against blister formation. It is our hope that an understanding of the pathophysiology of pemphigus at this level may lead to innovative therapy for these life-threatening diseases.

Acknowledgments
We thank Dorothy Campbell and Valerie Johnson of the Department of Dermatology at the University of Pennsylvania for the tissue histology processing. This work was supported by grants from the National Institutes of Health (K08-AR-41557; R01-DK-39872).

Table 2
Effects of adsorption of anti-Dsg1 antibodies from PV sera on their ability to cause skin blisters in neonatal mice

<table>
<thead>
<tr>
<th>Sera</th>
<th>DSG3+/+</th>
<th>DSG3+/–</th>
<th>DSG3null</th>
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<tbody>
<tr>
<td>JPV21 (4.3 mg IgG/pup)</td>
<td>2+D</td>
<td>3+D</td>
<td>1+D</td>
</tr>
<tr>
<td>JPV21-Dsg1 adsorbed</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(5.2 mg IgG/pup)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIH2054 (9.4 mg IgG/pup)</td>
<td>4+D</td>
<td>4+D</td>
<td>4+D</td>
</tr>
<tr>
<td>NIH2054-Dsg1 adsorbed (12.0 mg IgG/pup)</td>
<td>–</td>
<td>1+D</td>
<td>–</td>
</tr>
</tbody>
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*See Table 1 and Methods for definition of histological assessment.*
from the National Institutes of Health. M.G. Mahoney was a recipient of a Dermatology Foundation Research Fellowship.


