The Anti-Desmoglein 1 Autoantibodies in Pemphigus Vulgaris Sera are Pathogenic

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Pemphigus vulgaris and pemphigus foliaceus are two closely related, but clinically and histologically distinct, autoimmune skin diseases. The autoantigens for pemphigus vulgaris and pemphigus foliaceus are desmoglein 3 and desmoglein 1, respectively. The anti-desmoglein 1 antibodies in pemphigus foliaceus and anti-desmoglein 3 antibodies in pemphigus vulgaris are pathogenic as determined by immunoglobulin G passive transfer animal models. More than 50% of pemphigus vulgaris sera also contain anti-desmoglein 1 autoantibodies; however, the pathogenicity of the anti-desmoglein 1 autoantibodies in pemphigus vulgaris remains unknown. In this study, we used soluble recombinant extracellular domains of desmoglein 1 and desmoglein 3 to obtain affinity-purified anti-desmoglein 1 and anti-desmoglein 3 autoantibodies from pemphigus vulgaris sera and examined the pathogenicity of each fraction separately using the passive transfer mouse model. By immunoprecipitation, the purified anti-desmoglein 1 and anti-desmoglein 3 showed no cross-reactivity. The anti-desmoglein 1 autoantibodies in pemphigus vulgaris induced typical pemphigus foliaceus lesions in neonatal mice, whereas the anti-desmoglein 3 fraction induced pemphigus vulgaris-like lesions. In addition, the pathogenic anti-desmoglein 1 and anti-desmoglein 3 autoantibodies in pemphigus vulgaris had predominant IgG4 subclass specificity. These findings suggest that the anti-desmoglein 1 antibodies in pemphigus vulgaris are pathogenic. ***Key words: acantholysis/autoimmunity/cell adhesion/desmosome. J Invest Dermatol 112:739–743, 1999***

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are the two major subtypes of pemphigus, a group of autoimmune blistering diseases affecting the skin and mucous membranes (Lever, 1953; Beutner and Jordon, 1964). Although both PV and PF involve acantholysis (the loss of cell-to-cell adhesion) and blister formation, they are clinically and histologically distinguishable. PV targets the skin and mucosal epithelia, whereas PF affects only the epidermis (Rivitti *et al*, 1994). In PV, the acantholysis is observed just above the basal cell layer (the suprabasilar layer), whereas in PF the acantholysis occurs in the upper part of the epidermis, at the level of the granular layer (Lever, 1965). PV and PF patients have circulating autoantibodies directed against cell surface antigens of epidermal keratinocytes. These autoantibodies are pathogenic as determined by the passive transfer studies in which IgG isolated from sera of pemphigus patients were injected into neonatal mice and induced acantholysis with the classic immunohistologic features seen in human patients (Anhalt *et al*, 1982, 1986; Roscoe *et al*, 1985; Jones *et al*, 1988; Futamura *et al*, 1989). The pathogenic PF and PV autoantibodies are predominantly IgG4 (Rock *et al*, 1989; Ding *et al*, 1997).

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Abbreviations: Dsg, desmoglein; IF, immunofluorescence; PF, pemphigus foliaceus; PV, pemphigus vulgaris.

PV and PF autoantibodies recognize a 130 kDa and a 160 kDa desmosomal glycoprotein, respectively (Stanley *et al*, 1982, 1984; Eyre and Stanley, 1987; Calvanico *et al*, 1991). Both these autoantigens belong to the desmoglein subfamily of the cadherin superfamily, a class of molecules that play an important part in mediating cell-to-cell adhesion (Amagai *et al*, 1991; Ringwald *et al*, 1987; Takeichi, 1987; Goodwin *et al*, 1990; Wheeler *et al*, 1991; Stanley, 1993). The PF antigen is designated desmoglein 1 (Dsg1) and the PV antigen is designated desmoglein 3 (Dsg3). By indirect immunofluorescence (IF), Dsg1-specific autoantibodies stain keratinocyte cell surfaces predominantly in the upper part of the epidermis, whereas Dsg3-specific autoantibodies predominantly stain the basal and immediate suprabasal layers of the epidermis (Arnemann *et al*, 1993; Amagai *et al*, 1996; Koch *et al*, 1997; Shirakata *et al*, 1998). Anti-Dsg3 autoantibodies in PV and anti-Dsg1 autoantibodies in PF have been shown to be pathogenic (Amagai *et al*, 1992, 1994, 1995; Memar *et al*, 1996). The expression of Dsg1 and Dsg3 in the epidermis corresponds precisely to the site where blisters appear in PF and PV, respectively. Interestingly, over 50% of PV sera recognize both Dsg3 and Dsg1, whereas no PF sera have been found to cross-react with Dsg3 (Eyre and Stanley, 1987; Hashimoto *et al*, 1990; Dmochowski *et al*, 1992; Amagai *et al*, 1995; Emery *et al*, 1995; Ding *et al*, 1997). In this study we determined whether the anti-Dsg1 autoantibodies in PV are pathogenic. Anti-Dsg1 and anti-Dsg3 autoantibodies in PV sera were affinity-purified using the full length of extracellular domains of Dsg1 and Dsg3 expressed in the baculovirus system. Each IgG fraction was then characterized by immunoblotting and immunoprecipitation and assayed for pathogenicity by passive transfer experiments.

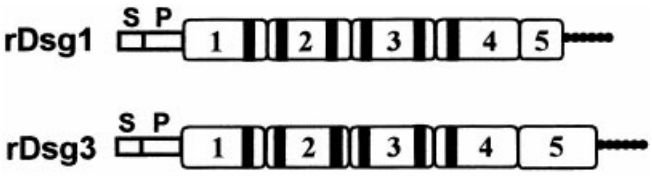

Figure 1. Schematic diagram of the recombinant extracellular domain of Dsg1.His (rDsg1.His) and Dsg3.His (rDsg3.His). rDsg1.His and rDsg3.His were constructed and expressed in a baculovirus expression system. Both proteins contain a signal sequence (S), a propeptide (P), and the entire extracellular domain (EC1–5) linked to a six-histidine tail. The black strips represent the Ca^{2+}-binding sites.

MATERIALS AND METHODS

Source of sera Two PV sera (PV1 and PV2) containing both anti-Dsg1 and anti-Dsg3 antibodies were used in this study. One PV serum (Lef) containing only Dsg3 antibodies with generalized disease and one PF serum (Celio) were used as positive controls. Both PV1 and PV2 had the characteristic clinical and histologic diagnosis of PV. Both had generalized disease involving both the skin and the mucous membranes. By indirect IF both patients had titers >1:160 against human skin and mouse skin. In PV1 the relative titers of the anti-Dsg1 and anti-Dsg3 were 1 : 80 and 1:320, respectively. In PV2 the relative titers of the anti-Dsg1 and anti-Dsg3 were 1:160 and 1:320, respectively. Celio had clinical and histologic diagnosis of PF and exhibited an indirect IF titer of 1:320 against human and mouse skin. A rabbit anti-human Dsg1 anti-serum (corresponding to amino acids 76–158) and rabbit anti-human Dsg3 (corresponding to amino acids 145–192) were also used.

The expression of the extracellular domains of Dsg1 and Dsg3 using the baculovirus expression system The expression of the entire extracellular domain of Dsg3 using the baculovirus system was described previously (Ding *et al*, 1997). The recombinant protein is soluble and fully conformational (Ding *et al*, 1997). In order to affinity-purify anti-Dsg3 autoantibodies, the expression vector was re-engineered by adding a stretch of six-histidine codons (5′-GAT CTG AGC ACC ATC ATC ACC ATC ACG CGC GCT AG-3′) immediately downstream of the Dsg3 extracellular domain (see **Fig 1**). Similarly, the expression vector for the entire extracellular domain of Dsg1 with a histidine tag was also constructed. The junction regions and inserts in the vector PVL1393 were sequenced to insure sequence integrity. The recombinant proteins were expressed following the same procedure as previously described (Ding *et al*, 1997).

Affinity-purification of anti-Dsg1 and anti-Dsg3 autoantibodies from PV sera The affinity columns were generated according to the previously described procedure (Gu *et al*, 1994). In brief, 100 ml of cell culture supernatant containing about 10 mg of the recombinant Dsg1 or Dsg3 protein was incubated with 4 ml Ni-NTA agarose (QIAGEN, Chatsworth, CA) at room temperature for 1 h with gentle shaking. The mixture was packed into a column, washed and equilibrated in Tris-buffered saline/Ca^{2+} buffer (10 mM Tris, 145 mM NaCl, 5 mM $CaCl_2$, pH 7.5). Three milliliters total IgG isolated from PV sera by 50% ammonium sulfate precipitation was loaded on to the column. The column was washed with 10 bed volumes of Tris-buffered saline/Ca^{2+}, followed by 10 bed volumes of low salt washing buffer (150 mM NaCl, 50 mM Tris, pH 7.5) and the same volume of high salt washing buffer (1 M NaCl, 50 mM Tris, pH 7.5). The bound anti-Dsg1 or anti-Dsg3 antibodies were then eluted with 4 M $MgCl_2$ buffer. The affinity-purified IgG was concentrated by a Centricon-30 concentrator (Amicon, Beverly, MA).

Immunologic characterization of the anti-Dsg1 and anti-Dsg3 antibodies The affinity-purified anti-Dsg1 and anti-Dsg3 antibodies were analyzed by immunoblotting, indirect IF and immunoprecipitation. For immunoblotting (Ding *et al*, 1997), the baculovirus expressed rDsg1 and rDsg3 extracellular domains were resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and subsequently transferred to a nitrocellulose membrane. The membrane was incubated with a rabbit anti-human Dsg1 anti-serum, rabbit anti-human Dsg3 anti-serum, purified anti-Dsg1 autoantibodies, purified anti-Dsg3 autoantibodies or controls. For indirect IF, the purified autoantibodies were applied to human skin cryosections and then stained by fluorescein isothiocyanate-conjugated goat anti-human IgG as previously described (Matis *et al*, 1987). The IgG subclass was determined using human IgG subclass-specific monoclonal antibodies (ATCC, Rockville, MD) (Rock *et al*, 1989). The immunoprecipitation experiments were performed as previously reported (Calvanico *et al*, 1991; Olague-Alcala *et al*, 1994; Ding *et al*, 1997). Briefly, recombinant Dsg1 or Dsg3 was iodinated using chloramine-T and incubated with anti-Dsg1 or anti-Dsg3 antibodies. The soluble immune complexes were mixed with *Staphylococcus aureus* cells containing staphylococcal protein A (Sigma, St. Louis, MO). The bound IgG and antigen were then eluted with 1 × SDS sample buffer and analyzed by 10% SDS–PAGE gels under denatured and reducing conditions. The gels were dried and the bands were visualized by autoradiography.

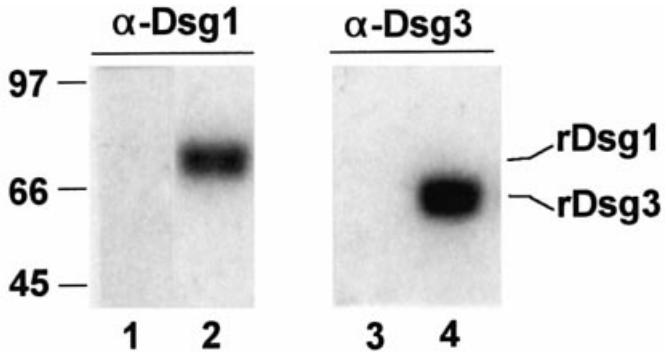

Figure 2. Immunoblotting analysis of recombinant extracellular domains of Dsg1 and Dsg3. rDsg1 and rDsg3 were electrophoresed by 10% SDS–PAGE, transferred to a nitrocellular filter and blotted with a polyclonal rabbit anti-Dsg1 (*lanes 1* and *2*) or rabbit anti-Dsg3 antibody (*lanes 3* and *4*). *Lanes 1* and *3*, Hi-5 cell culture medium; *lane 2*, medium from Hi-5 cells infected with rDsg1.His baculovirus; *lane 4*, medium from Hi-5 cells infected with rDsg3.His baculovirus.

Laboratory animals Breeding pairs of BALB/C mice were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained at the Medical College of Wisconsin Animal Resource Center. Neonatal mice (24–36 h old with body weights between 1.4 and 1.6 g) were used for passive transfer experiments.

Induction of experimental pemphigus and animal evaluation A 100 μl aliquot of affinity-purified anti-Dsg1 or anti-Dsg3 antibodies (0.56–0.8 mg per g body weight) in PBS was administered to neonatal mice by intradermal (i.d.) injection. The injection techniques have been described elsewhere (Liu *et al*, 1997). The skin of neonatal mice from the test and control groups was examined 20 h after the IgG injection. The animals were then killed and the following specimens were obtained. Skin sections were taken for light microscopy (hematoxylin and eosin staining) and for direct IF analysis to detect *in vivo* bound human IgG. Sera of injected animals were obtained for indirect IF to determine the circulating titers of anti-Dsg1 and anti-Dsg3 IgG. Direct and indirect IF analyses were performed as previously described (Hashimoto *et al*, 1990). Monospecific fluorescein isothiocyanate-conjugated-conjugated goat anti-human IgG was obtained commercially (Kirkeggard & Perry Laboratories, Gaithersburg, MD).

RESULTS

The production of recombinant Dsg1 and Dsg3 We have previously reported the expression of the extracellular domain of Dsg3 in the baculovirus system and demonstrated that the recombinant Dsg3 extracellular domain is soluble and exhibits conformational epitopes by immunoprecipitation (Ding *et al*, 1997). In this study, we re-engineered the expression construct and added a six histidine-tag (His-tag) to the C-terminus of the protein to facilitate affinity purification of the recombinant Dsg3 (rDsg3-His) and Dsg3-specific autoantibodies (**Fig 1**). Similarly, we also expressed the entire extracellular domain of Dsg1 with a C-terminal His-tag (rDsg1-His) (**Fig 1**). Immunoblot analysis of these baculovirus expressed recombinant proteins using rabbit antisera directed against human Dsg1 and Dsg3 is shown in **Fig 2**. The rabbit anti-Dsg1 antibodies reacted with the 70 kDa rDsg1-His in culture medium of Hi-5 cells infected with rDsg1-His baculovirus (**Fig 2**, *lane 2*). The rabbit anti-Dsg3 antibodies specifically recognized the 67 kDa rDsg3-His (**Fig 2**, *lane 4*). No bands were shown in control media (**Fig 2**, *lanes 1* and *3*). The same immunoblotting results were obtained with PF and PV sera and purified anti-Dsg1 and anti-

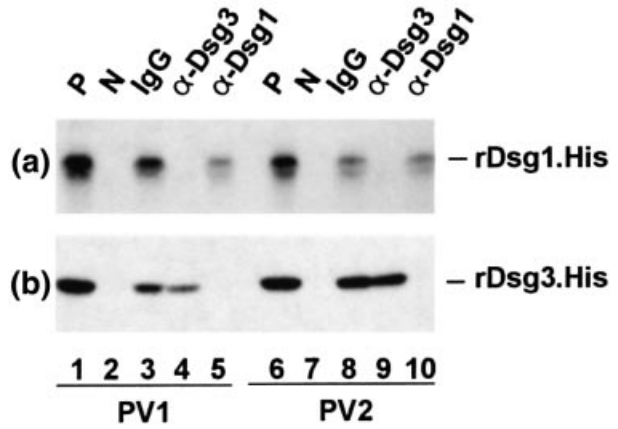

Figure 3. The immune specificity of affinity purified anti-Dsg1 and anti-Dsg3 from PV patients. Anti-Dsg1 and anti-Dsg3 antibodies were purified from two PV patients by using a rDsg1 or rDsg3 affinity column, respectively. The purified anti-Dsg1 or anti-Dsg3 antibodies were used to immunoprecipitate ^{125}I-labeled rDsg1.His (*a*) and rDsg3.His (*b*). The precipitates were fractionated on a 10% SDS–PAGE and visualized by autoradiography. *Lanes 1* and *6*, positive control sera (P) (*a*, PF serum; *b*, PV serum); *lanes 2* and *7*, normal control sera (N); *lanes 3* and *8*, total IgG from PV patients; *lanes 4 and 9*, affinity purified anti-Dsg3 antibodies; *lanes 5* and *10*, affinity purified anti-Dsg1 antibodies.

Dsg3 autoantibodies (data not shown). These findings show that the baculovirus expressed rDsg1 and rDsg3 extracellular domains are soluble and reactive with anti-Dsg1 and anti-Dsg3 antibodies, respectively.

Anti-Dsg1 and anti-Dsg3 autoantibodies in PV sera are not cross-reactive To determine whether anti-Dsg1 and anti-Dsg3 antibodies have cross-reactivity, Dsg1 and Dsg3-specific antibodies in PV sera (PV1 and PV2) were separated using rDsg1-His and rDsg3-His affinity columns. The anti-Dsg1 antibodies from a PF serum (Celio) and anti-Dsg3 antibodies from a PV serum (Lef), which lacks anti-Dsg1 activity, were also purified using the same affinity columns and used as positive controls. The purified IgG preparations were then analyzed by immunoprecipitation. As shown in **Fig 3**, the rDsg1-His was recognized by control anti-Dsg1 IgG from the PF serum Celio (**Fig 3*a***, *lanes 1* and *6*), total IgG from PV1 and PV2 (**Fig 3*a***, *lanes 3* and *8*), and anti-Dsg1 IgG from PV1 and PV2 (**Fig 3*a***, *lanes 5* and *10*). The rDsg1-His, however, was not recognized by anti-Dsg3 IgG from PV1 and PV2 (**Fig 3*a***, *lanes 4* and *9*), nor by normal control human IgG (**Fig 3*a***, *lanes 2* and *7*). Similarly, the rDsg3-His reacted with control anti-Dsg3 IgG from the PV serum Lef (**Fig 3*b***, *lanes 1* and *6*), total IgG from PV1 and PV2 (**Fig 3*b***, *lanes 3* and *8*), as well as anti-Dsg3 IgG from PV1 and PV2 (**Fig 3*b***, *lanes 4* and *9*). In contrast, the rDsg3-His was not reactive with anti-Dsg1 IgG from PV1 and PV2 (**Fig 3*b***, *lanes 5* and *10*), nor with normal control human IgG (**Fig 3*b***, *lanes 2* and *7*). These results demonstrate that the anti-Dsg1 autoantibodies in PV sera do not cross-react with Dsg3 and the anti-Dsg3 autoantibodies do not cross-react with Dsg1.

Anti-Dsg1 autoantibodies in PV sera induce PF-like acantholysis in mice To test whether anti-Dsg1 antibodies in PV sera are pathogenic, neonatal BALB/C mice were injected intradermally with the affinity-purified anti-Dsg1 IgG from PV sera and from the PF serum Celio. Mice (n = 6) injected with anti-Dsg1 IgG from PV sera developed clinical blisters (**Fig 4*A***, also see **Table I**). Direct IF of the skin of the injected animals showed binding of anti-Dsg1 antibodies to the intercellular space of subcorneal keratinocytes (**Fig 4*B***). Hematoxylin and eosin examination of the lesional skin revealed subcorneal acantholysis (**Fig 4*C***). These immunohistologic features are identical to those found in mice (n = 5) injected with anti-Dsg1 IgG from PF sera (**Table I**). As expected, mice (n = 6) injected i.d. with the affinity-purified anti-Dsg3 IgG from the PV sera developed blisters (**Fig 4*D***, also see

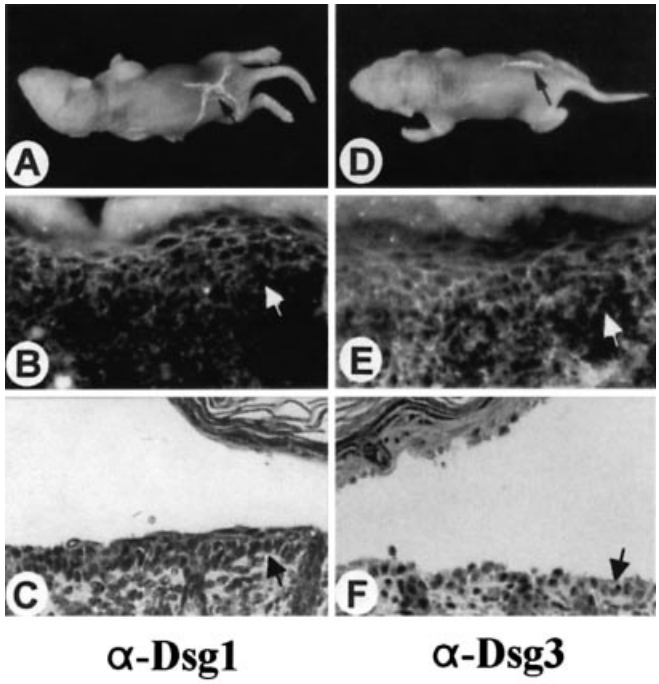

Figure 4. Pathogenicity of affinity purified anti-Dsg1 and anti-Dsg3. Neonatal BALB/C mice were injected i.d. with anti-Dsg1 (*A–C*) or anti-Dsg3 (*D–F*) (0.56–0.8 mg per g body weight), respectively. The clinical, immunologic, and histologic examinations were performed 20 h post IgG injection. Nikolskey sign was seen in mice injected with both anti-Dsg1 (*A*) and anti-Dsg3 antibodies (*D*). Direct IF showed the binding of anti-Dsg1 antibodies to the intercellular spaces of subcorneal keratinocytes (*B*), whereas the binding of anti-Dsg3 were shown to locate to the suprabasal layer of the murine epidermis (*E*). Hematoxylin and eosin examination demonstrated that anti-Dsg1 antibodies induce subcorneal acantholysis (*C*), whereas anti-Dsg3 antibodies produce suprabasilar acantholysis (*F*) in neonatal mice. Site of basal keratinocytes (*arrow* in *B–F*). Original magnification, ×200.

Table I). Direct IF of the skin of the mice showed the deposition of anti-Dsg3 antibodies at the suprabasal layer of the epidermis (**Fig 4*E***) and hematoxylin and eosin staining of the lesion skin revealed suprabasal acantholysis (**Fig 4*F***). Affinity-purified anti-Dsg3 IgG from the positive control PV serum Lef induced a similar phenotype in neonatal mice (n = 5, **Table I**). These results demonstrate that, like anti-Dsg1 autoantibodies in PF, anti-Dsg1 IgG in PV are indeed pathogenic and induce typical PF lesions in mice.

The pathogenic anti-Dsg1 autoantibodies in PV belong to the IgG4 subclass The subclass of anti-Dsg1 IgG in PV was determined by indirect IF using human and mouse skin as substrates. The predominant IgG subclass of the anti-Dsg1 antibodies in PV was IgG4 with minimal other IgG subclasses (**Table I**). The anti-Dsg3 antibodies in these PV sera were also IgG4 (**Table I**). These results demonstrate that, like anti-Dsg1 antibodies in PF, the pathogenic anti-Dsg1 and anti-Dsg3 IgG in PV are also predominantly of the IgG4 subclass.

DISCUSSION

It is well documented that in addition to anti-Dsg3 antibodies, more than 50% of PV sera also contain anti-Dsg1 activity (Eyre *et al*, 1987; Hashimoto *et al*, 1990; Dmochowski *et al*, 1992; Amagai *et al*, 1995; Emery *et al*, 1995; Ding *et al*, 1997; Ishii *et al*, 1997). In this study, we provide direct evidence that Dsg1-specific autoantibodies in PV are pathogenic. The affinity-purified anti-Dsg1 antibodies from PV sera that contain both anti-Dsg1 and anti-Dsg3 IgG were able to induce cutaneous lesions in neonatal mice. Interestingly, the clinical and immunohistologic features of these mice are identical to the mice injected with pathogenic anti-

Table I. Pathogenicity and IgG subclass of affinity-purified anti-Dsg1 and anti-Dsg3 antibodies from PV sera

IgG injected[a]	IgG subclass[b]	Number of mice	Disease activity[c]	Lesional site (H/E)	Direct IF[d]
PV1:αDsg1	IgG4	6	3+	granular	+
PV1:αDsg3	IgG4	6	2+	suprabasilar	+
PV2:αDsg1	IgG4	6	3+	granular	+
PV2:αDsg3	IgG4	6	3+	suprabasilar	+
Celio:αDsg1	IgG4	5	3+	granular	+
Lef:αDsg3	IgG4	5	3+	suprabasilar	+

[a]Neonatal BALB/C mice were injected intradermally with 100 μl of affinity-purified anti-desmoglein 1 (Dsg1) or anti-Dsg3 autoantibodies. PV1 and PV2 sera contained both anti-Dsg1 and anti-Dsg3 activities. Celio was PF serum containing only anti-Dsg1 antibodies, whereas Lef was PV serum containing only anti-Dsg3 antibodies.

[b]The IgG subclass was determined by indirect IF using human and mouse skin as substrates and human IgG subclass-specific monoclonal antibodies.

[c]Injected animals were examined clinically 20 h after IgG injection. Disease activity is scored on a scale of "–" to "3+": –, no detectable lesion clinically and histologically; 1+, means means no evidence of clinical blister but acantholysis involving less than 25% of the epidermis at the injection site; 2+, means clinical blister with acantholysis involving 25%–50% of the epidermis at the injection site; 3+, means intense blister with acantholysis involving >50% of the epidermis at the injection site. See *Materials and Methods* for details.

[d]For direct IF, skin sections were stained with monospecific fluorescein isothiocyanate-conjugated-conjugated goat anti-human IgG.

Dsg1 autoantibodies from PF sera. In addition, like the anti-Dsg1 antibodies in PF, the Dsg1-specific IgG in these PV sera belong to the IgG4 subclass.

Dsg1 and Dsg3 are 46% identical at the amino acid sequence level. It has been speculated that the presence of anti-Dsg1 reactivity in PV sera is due to cross-reactivity of certain anti-Dsg3 antibodies with Dsg1. The alternative is that these PV sera contain two distinct and non-cross-reacting populations of antibodies-one against Dsg1 and the other against Dsg3. In order to discriminate between these two possibilities, we affinity purified PV autoantibodies using recombinant forms of Dsg1 and Dsg3 expressed in the baculovirus system. Using these highly purified reagents, we found no cross-reactivity between autoantibodies directed against Dsg1 and Dsg3 extracellular domains as determined by immunoblotting and immunoprecipitation. These findings were supported by our *in vivo* direct IF experiments. Dsg1-specific antibodies in PV bind mainly to the subcorneal keratinocytes, whereas Dsg3-specific IgG in PV predominantly deposit on the suprabasal keratinocytes. These results are consistent with the previous findings reported (Ishii *et al*, 1997). In this study, it was shown that the immune reactivity of PV sera against Dsg3 by ELISA was abolished by preadsorption with recombinant Dsg3 but not Dsg1. And conversely, reactivity of PV sera with Dsg1 was blocked by preadsorption with recombinant Dsg1 but not Dsg3.

Knowing that the anti-Dsg1 and anti-Dsg3 antibodies in PV are not cross-reactive, we next directly assessed the pathogenicity of the anti-Dsg1 and anti-Dsg3 antibodies in PV. The anti-Dsg3 antibodies from the PV sera caused suprabasilar acantholysis, the characteristic feature of PV. Previously, it was shown that Dsg3 ectodomain expressed in the baculovirus system was able to block blisters in mice induced by autoantibodies from PV sera (Amagai *et al*, 1994). The authors of the report, however, did not test directly for the pathogenic activity of the anti-Dsg1 component of the sera, whereas we have shown that the anti-Dsg1 antibodies in these PV sera are indeed pathogenic. Interestingly, the anti-Dsg1 antibodies in PV induced PF-like lesions in neonatal mice, even though the patients from whom the sera were drawn had typical PV blisters. The discrepancy between our mouse model results and clinical observations is most likely due to the difference in anti-Dsg1 antibody titers in those PV patients and in the injected mice which received a high dose of the purified IgG.

It has been reported that certain patients progress from PV to PF (Hashimoto *et al*, 1991, 1995; Iwatsuki *et al*, 1991; Kawana *et al*, 1994), and this conversion is associated with a change of autoantibody profiles from predominant anti-Dsg3 to anti-Dsg1. One interpretation of this phenomenon is that the original autoimmune response is directed against a pathogenic epitope on Dsg3 and later the response spreads to other epitope(s) that cross-react with Dsg1. Our data, however, suggest an "intermolecular epitope spreading" rather than an "intramolecular epitope spreading" mechanism, as anti-Dsg1 and anti-Dsg3 antibodies in PV sera are not cross-reactive. Further investigation will be needed to address this important issue.

In summary, our study demonstrated that the anti-Dsg1 autoantibodies in PV are pathogenic and have IgG4 subclass specificity. These findings might be helpful for further dissecting the immunopathogenesis of pemphigus and developing more effective therapeutic strategies to manage these human autoimmune diseases.

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