

# A Subset of Pemphigus Foliaceus Patients Exhibits Pathogenic Autoantibodies Against Both Desmoglein-1 and Desmoglein-3

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**In pemphigus vulgaris the major pathogenic antibody binds desmoglein-3, and mediates mucosal disease. Development of cutaneous disease is associated with acquisition of antibodies to desmoglein-1. In pemphigus foliaceus, and its endemic form, fogo selvagem by contrast, the major pathogenic antibody recognizes desmoglein-1 and mediates cutaneous disease only. In this study, we sought to determine the prevalence of antibodies to desmoglein-3 in patients with pemphigus foliaceus and fogo selvagem. We produced recombinant desmoglein-1 and desmoglein-3, and used them in highly sensitive and specific enzyme-linked immunosorbent assays, as well as immunoprecipitation assays. We detected antibodies to desmoglein-3 in 19 of 276 patients with pemphigus foliaceus and fogo selvagem, who had cutaneous disease only. We showed that these antibodies to desmoglein-3 could be absorbed in a con-**

**centration-dependent manner by desmoglein-3 but not by desmoglein-1. Also antibodies to desmoglein-1 could be absorbed in a concentration-dependent manner by desmoglein-1 but not desmoglein-3. This suggests that two separate species of antibody are present rather than one antibody capable of cross-reacting with both desmoglein-1 and desmoglein-3. Finally, it was shown that affinity-purified antibodies to desmoglein-3 from patients with pemphigus foliaceus and fogo selvagem induced a pemphigus vulgaris-like skin disease in mice by passive transfer. These results suggest that a subset of patients with pemphigus foliaceus and fogo selvagem have antibodies to desmoglein-3 that may be involved in the pathogenesis of their cutaneous disease. *Key words: autoantibodies/autoimmunity/desmoglein/fogo selvagem/pemphigus. J Invest Dermatol 118:806-811, 2002***

**P**emphigus vulgaris (PV) is characterized by the gradual onset of blisters and erosions involving not only the skin but also mucosal membranes (Lever, 1965). Approximately one-third of PV patients may have mucosal lesions months before the diagnosis of PV is established. The vesicles are intraepidermal and located above the epidermal basal cell layer (suprabasal blisters). In contrast, pemphigus foliaceus (PF) and fogo selvagem (FS) are characterized by cutaneous blisters and erosions only, without mucosal involvement (Lever, 1965; Diaz *et al*, 1989). The vesicles in PF and FS are located just below the stratum corneum. Blister formation in PV, PF, and FS results from a progressive epidermal cell detachment also known as acantholysis. Electron microscopic examination of lesional epidermis in these patients shows separation of desmosomes with widening of the epidermal intercellular spaces (ICS). Keratinocytes round up and exhibit retraction of intermediate filaments around the nucleus (Lever, 1965). Patients with PV, PF,

and FS produce anti-epidermal autoantibodies, which can be detected in the ICS of perilesional epidermis and circulating in the patients' serum using immunofluorescence (IF) techniques (Beutner and Jordon, 1964; Beutner *et al*, 1968; Diaz *et al*, 1989). The serum titers of autoantibodies by indirect IF roughly correlate with disease activity and extent of the skin involvement. The IgG fractions of PV (Anhalt *et al*, 1982) and PF (Roscoe *et al*, 1985) sera are pathogenic in passive transfer experiments using neonatal mice. The injected animals display the key clinical, histologic, and immunologic features of each disease.

The epidermal autoantigens recognized by antibodies and T cells in PF/FS and PV have been identified as desmoglein-1 (dsg1) and desmoglein-3 (dsg3), respectively (Stanley *et al*, 1984; Labib *et al*, 1990; Amagai *et al*, 1991; Olague-Alcala *et al*, 1994; Emery *et al*, 1995; Wucherpfennig *et al*, 1995; Lin *et al*, 1997a, b, 2000). Dsg1 and dsg3 are transmembrane desmosomal glycoproteins that belong to the cadherin family of proteins (Buxton *et al*, 1993). These molecules exhibit extensive amino acid homology, especially in their extracellular domains (**Fig 1**). Recombinant dsg1 and dsg3, generated in the baculovirus expression system, are capable of absorbing pathogenic antibodies from the sera of PF or PV patients (Amagai *et al*, 1994, 1995).

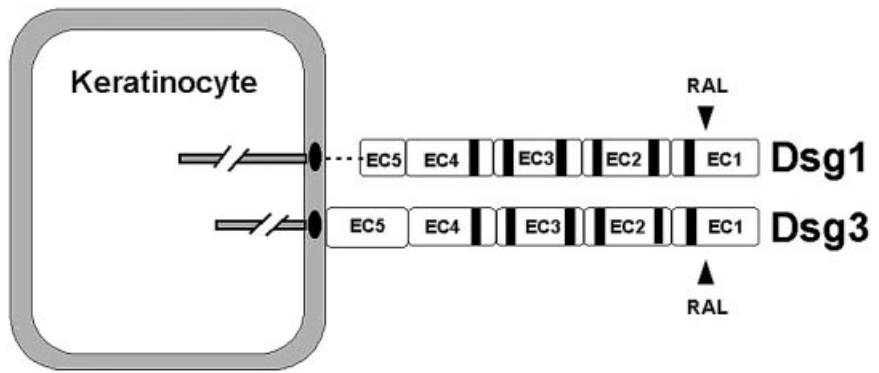
It appears that genetic predisposition is a common feature in patients developing PV or PF, as each disease exhibits unique DR alleles. FS shows a strong association with the HLA-DRB1\*0102, 0404, 1402, 1602 alleles (Cerna *et al*, 1993; Moraes *et al*, 1997),

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Abbreviations: PF, pemphigus foliaceus; PV, pemphigus vulgaris; FS, fogo selvagem; ICS, intercellular spaces; dsg1, desmoglein-1; dsg3, desmoglein-3; OD, optical density; TBS Ca<sup>2+</sup>, Tris-buffered saline with calcium.

**Figure 1. Molecular structure of the extracellular region of *dsg1* and *dsg3*.** *dsg1* and *dsg3* exhibit five major cadherin-like domains (EC-1, EC-2, EC-3, EC-4, and EC-5), stretching from the N-terminus (EC-1) to the C-terminus (transmembrane domain). The six-histidine tag is attached to the C-terminus. The black strips represent  $\text{Ca}^{2+}$  binding motifs. The recombinant ectodomains of *rdsg1* and *rdsg3* are expressed in the baculovirus system as a soluble glycopeptide of approximately 66 kDa.



whereas PV is linked to the HLA-DRB1\*0402 and 1401 alleles (Scharf *et al*, 1988; Sinha *et al*, 1988; Szafer *et al*, 1988; Ahmed *et al*, 1990, 1991).

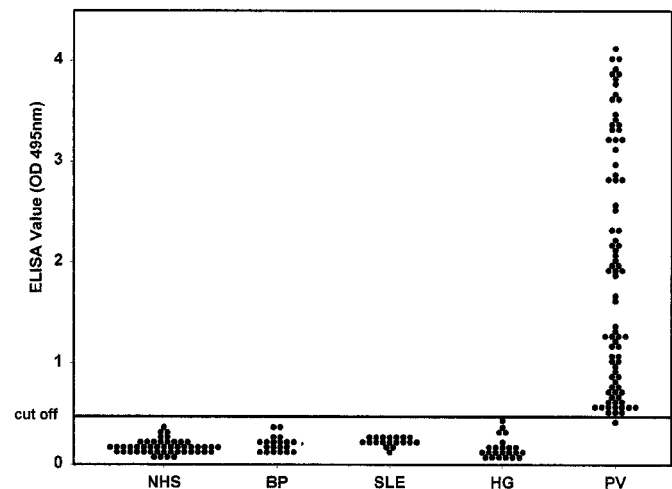
Recent reports have documented the existence of two clinical and serologic variants of PV (Ding *et al*, 1997; Amagai *et al*, 1999; Miyagawa *et al*, 1999; Harman *et al*, 2000a, b). A mucosal variant exhibits a predominantly anti-*dsg3* antibody response and a mucocutaneous form, comprising approximately 50% of the patients, is characterized by a mixed anti-*dsg3* and anti-*dsg1* antibody response and a T cell response to these antigens (Lin *et al*, 1997b). It has been demonstrated that both affinity-purified anti-*dsg3* and anti-*dsg1* autoantibodies from these PV sera are pathogenic in the mouse model (Ding *et al*, 1999). In these studies, each antibody system was shown to reproduce the phenotype of each disease, i.e., antibodies to *dsg3* induced PV-like lesions and antibodies to *dsg1* induced PF-like lesions. There was no immunologic cross-reactivity between these autoantibody systems. Interestingly, it has been reported recently that patients with classic clinical, histologic, and immunologic findings of PV may evolve into PF (Iwatsuki *et al*, 1991; Kawana *et al*, 1994; Chorzelski *et al*, 1995; Chang *et al*, 1997). The evolution of PV into PF is associated with a serologic switch from antibodies against *dsg3*, typical of PV, to antibodies against *dsg1*, which are a marker of PF.

The transition from PF into PV has also been described, however, this progression appears to be extremely rare (Ishii *et al*, 2000). It is well established that the sera of the majority of PF and FS possess a predominantly anti-*dsg1* IgG response (Stanley *et al*, 1986; Warren *et al*, 2000). The frequency of anti-*dsg3* autoantibodies in PF and FS patients, however, is unknown.

In this communication, using specific enzyme linked immunosorbent assays (ELISA) methods, we found anti-*dsg3* autoantibodies in 19 of 276 patients with PF and FS. The reactivity of these autoantibodies with *dsg3* was further confirmed by immunoprecipitation using purified recombinant desmoglein-3 (*rdsg3*). The specific binding of anti-*dsg3* autoantibodies detected by ELISA was inhibited in a concentration-dependent manner by purified *rdsg3*, but not by purified *rdsg1*. Under the same experimental conditions, antibodies to *dsg1* were inhibited by *rdsg1* but not by *rdsg3*. Furthermore, using the passive transfer mouse model, we found that purified anti-*dsg3* antibodies from PF and FS patients are capable of inducing PV-like lesions in neonatal mice. These results suggest that a subset of patients with PF and FS have antibodies to *dsg3* that may be involved in the pathogenesis of their disease.

## MATERIALS AND METHODS

**Source of sera** We tested sera from 35 PF patients (25 from the U.S.A. and 10 from Japan) and 241 FS patients from Brazil (40 from Campo Grande, five from Sao Paulo, 44 from Belho Horizonte, 62 from Goiania, 57 from Brazilia, and 33 from Limao Verde). The diagnosis of PF and FS was established clinically, histologically, and immunologically, as reported previously (Diaz *et al*, 1989). These patients had active disease and none had mucosal lesions. We also tested sera from patients with other cutaneous diseases such as bullous pemphigoid ( $n = 20$ ), lupus



**Figure 2. Sera from PV patients specifically react with *rdsg3* using the *dsg3* ELISA.** The cut-off value for this assay was set at the mean of 50 normal human sera plus 3 SD, equal to 0.48 OD units. All 50 normal sera were negative, as were 20 patients with bullous pemphigoid (BP), 19 with systemic lupus erythematosus (SLE), and 23 with herpes gestationis (HG). Eighty-two of 83 patients with PV were positive. The one PV patient that was negative was also in clinical remission.

erythematosus ( $n = 19$ ), herpes gestationis ( $n = 23$ ), and PV ( $n = 83$ ). Patients with PV included 53 from the U.S.A., seven from Spain, 13 from Brazil, and 10 from Japan. We also tested normal donors from the U.S.A. ( $n = 50$ ) as controls. No pooled sera were used in this study.

**Production and purification of *rdsg1* and *rdsg3*** The expression of the extracellular domain of *dsg3* using the baculovirus system has been described previously (Ding *et al*, 1999). In order to facilitate the purification of this protein, a six histidine stretch was engineered immediately downstream of the *dsg3* extracellular domain. The *rdsg3* was purified using a  $\text{Ni}^{2+}$  NTA column following procedures suggested by the manufacturer (Qiagen, Valencia, CA). The extracellular domain of *dsg1* (*rdsg1*) was produced by similar methods (Ding *et al*, 1999). The purity of each recombinant protein was assured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis.

**Establishment of *dsg1* and *dsg3* ELISA** The *rdsg1* and *rdsg3* ELISA were established following procedures described by Warren *et al* (2000) except that mouse monoclonal anti-human IgG conjugated to horseradish-peroxidase (Zymed Laboratories, San Francisco, CA) was used as a secondary antibody. Each ELISA plate contained three dilutions of a standard positive serum sample that were used to correct for plate-to-plate variability by linear regression analysis. Logarithmic transformation was used to analyze the assay results. The cut-off values were set at the mean plus 3 SD of values of the normal subjects from the U.S.A. This resulted in a cut-off for the *dsg1* ELISA of 0.3 OD units and for the *dsg3* ELISA of 0.48 OD units.

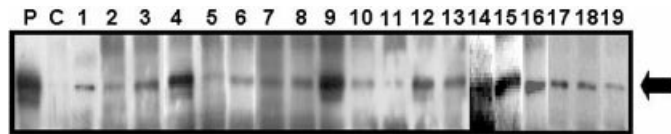
**Table I. Anti-dsg1 and anti-dsg3 autoantibodies in a subset of 19 patients with FS and PF**

Patient code	ELISA <sup>a</sup>		Indirect IF titer
	dsg1 <sup>b</sup>	dsg3 <sup>c</sup>	
BH18	5.683	1.536	1:160
BH26	9.964	2.034	1:2560
BH28	9.974	1.416	1:160
BH30	8.332	2.113	1:320
BH39	9.966	2.603	1:320
Bra19	9.982	5.197	1:320
Bra48	5.900	1.300	1:640
Bra49	2.630	2.083	1:80
Bra 50	1.899	1.495	1:40
Bra52	0.435	3.831	1:320
Bra55	3.078	2.167	1:20
Bra 61	6.956	2.216	1:640
CG14	9.977	9.980	1:1280
CG44	9.859	4.788	1:160
CG50	9.715	1.370	1:160
GO101	9.972	1.330	1:1280
FS12	5.567	2.517	1:80
FS26	4.403	2.492	1:80
PH	4.462	3.246	1:640

<sup>a</sup>The cut-off value of the ELISA was set at the mean of the 50 normal sera plus 3 SD.

<sup>b</sup>The cut-off for the dsg1 ELISA was 0.30 OD units.

<sup>c</sup>The cut-off for the dsg3 ELISA was 0.48 OD units.

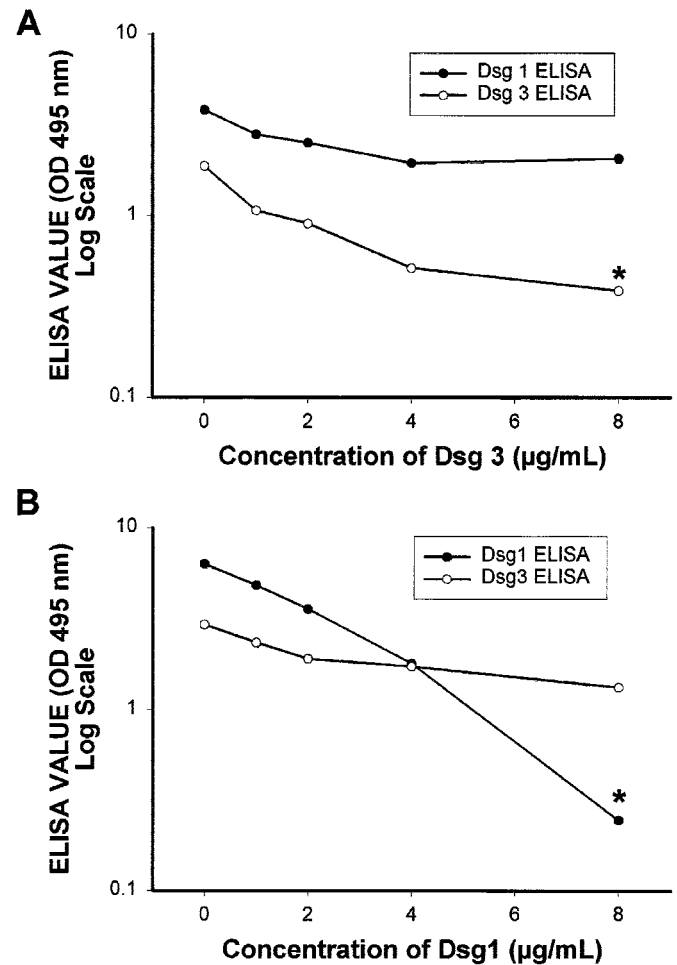


**Figure 3. All 19 patients with antibodies to dsg3 by ELISA were also positive by immunoprecipitation.** The autoradiogram shows immunoprecipitates analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Lane P: PV patient included as a positive dsg3 control. Lane C: Normal human serum included as a negative control. Lanes 1–19: All 19 patients with PF or FS with antibodies to dsg3 by ELISA.

The inhibition of the ELISA assays was performed by incubating a fixed dilution of PF or FS serum in Tris-buffered-saline (TBS), with calcium 5 mM, pH 7.3 (TBS Ca<sup>2+</sup>), with different amounts of rdsg3 or rdsg1 (0–8 µg) at room temperature for 1 h. Following a centrifugation step at 10,000 × g at 4°C for 10 min, the supernatant was tested by rdsg3 ELISA (or rdsg1 ELISA).

**Immunoprecipitation assay** The immunoprecipitation of rdsg3 was carried out as described previously (Ding *et al*, 1999) with the following modifications. Three micrograms of rdsg3 (or rdsg1) was first incubated with 3 µl of serum from PF and FS patients for 1 h at room temperature. Subsequently, 30 µl of a 50% suspension of immobilized recombinant Protein G (Zymed) was added and incubated for 90 min. The protein G immune complexes were washed three times with TBS-Ca<sup>2+</sup>/0.05% Tween buffer (pH 7.3) and then extracted using 30 µl of reducing buffer (4% sodium dodecyl sulfate, 10% 2-mercaptoethanol from Sigma) and boiled for 5 min. The extracts were then fractionated using 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and the protein bands analyzed by western blotting using rabbit anti-dsg3 (or rabbit anti-dsg1) as the primary antibody and horseradish peroxidase-labeled goat anti-rabbit IgG (Zymed) as the second antibody at a dilution of 1:10,000. The specific rdsg3 bands (or dsg1 bands) were visualized by enzymic chemiluminescence western blotting and autoradiography (Amersham Pharmacia Biotech, Buckinghamshire, U.K.).

**Affinity purification of anti-dsg1 and anti-dsg3 autoantibodies from PF/FS sera** The anti-dsg1 and anti-dsg3 autoantibodies from PF



**Figure 4. Competitive inhibition of the dsg1 and dsg3 ELISA by soluble recombinant dsg1 and dsg3.** PF or FS serum was incubated with rdsg1 or rdsg3 (0–8 µg) and then tested in the dsg1 and dsg3 ELISA. (A) Purified rdsg3 was capable of absorbing anti-dsg3 activity, but not anti-dsg1 activity. (B) Purified rdsg1 was capable of absorbing anti-dsg1 activity but not anti-dsg3 activity. The asterisk sign indicates that the ELISA titers of samples are below the cut-offs.

**Table II. Pathogenicity of affinity purified anti-dsg1 and anti-dsg3 autoantibodies from patients with PF and FS**

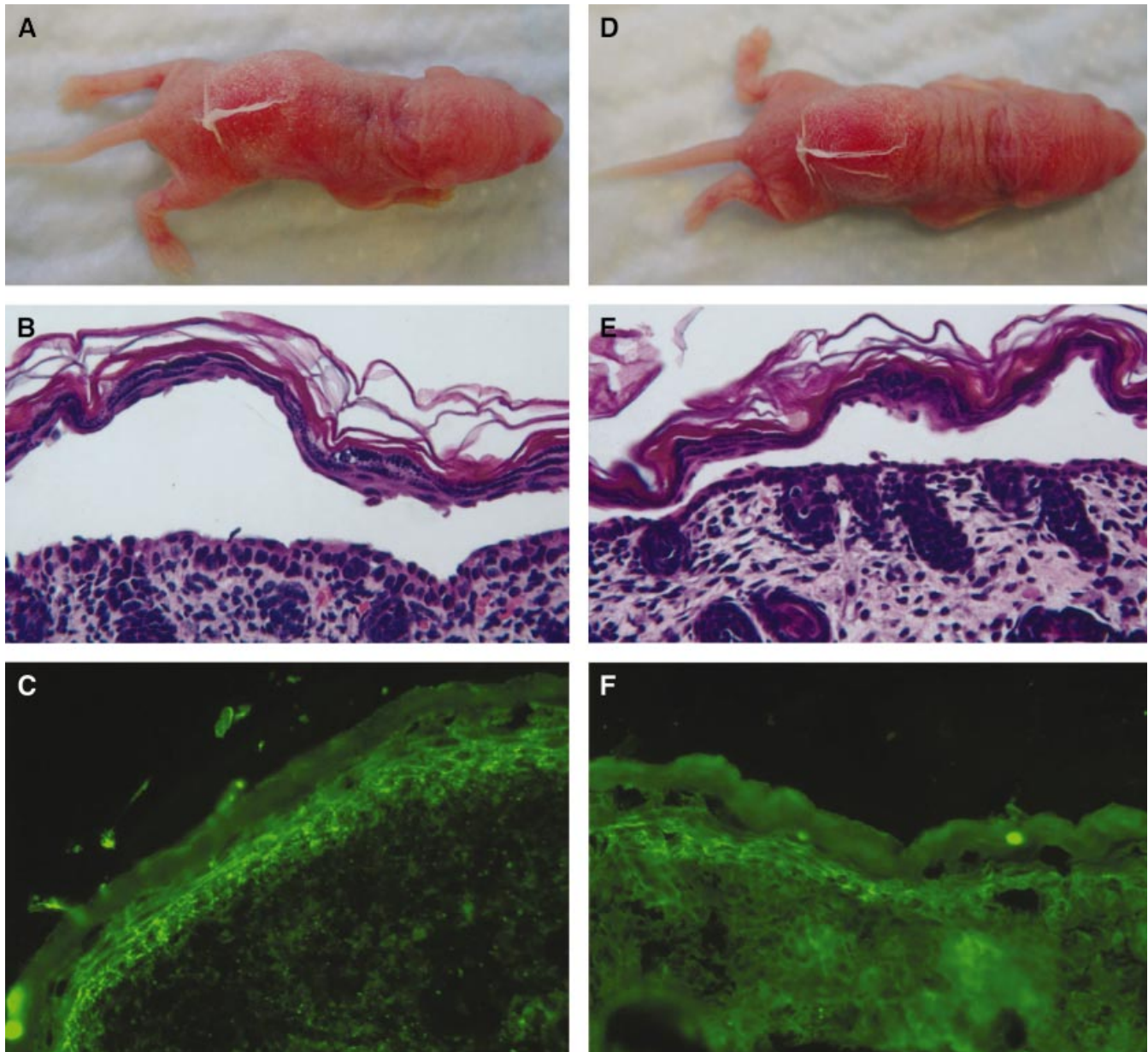
Patient IgG <sup>a</sup>	No. of mice tested	Disease activity <sup>b</sup>	Lesional site (H/E)	Direct IF <sup>c</sup>
Anti-dsg1 (FS12 serum)	5	3 +	subcorneal	(+)
Anti-dsg3 (FS12 serum)	5	3 +	suprabasal	(+)
Anti-dsg1 (FS26 serum)	4	3 +	subcorneal	(+)
Anti-dsg3 (FS26 serum)	4	3 +	suprabasal	(+)
Anti-dsg1 (PH serum)	5	3 +	subcorneal	(+)
Anti-dsg3 (PH serum)	5	3 +	suprabasal	(+)

<sup>a</sup>Affinity purified anti-dsg1 and anti-dsg3 autoantibodies from patients with PF and FS were passively transferred into neonatal mice. The dose of anti-dsg1 IgG was 200 µg per g of body weight, whereas the dose of anti-dsg3 was 130 µg per g of body weight.

<sup>b</sup>Mice were examined clinically 18 h after IgG injection. Disease activity is scored on a scale of no disease (–) to severe skin lesions (+++).

<sup>c</sup>Direct IF is reported as positive (+), if FITC-labeled anti-human IgG antibodies stained the epidermal intercellular spaces.

and FS sera were purified using rdsg1 or rdsg3 Ni-NTA columns as previously described (Ding *et al*, 1999). The affinity-purified autoantibodies were dialyzed against phosphate-buffered saline,



**Figure 5. Anti-dsg1 and anti-dsg3 autoantibody fractions from patients with PF and FS are pathogenic.** Neonatal BALB/C mice were injected intradermally with affinity purified anti-dsg1 autoantibodies (A–C) or anti-dsg3 autoantibodies (D–F), respectively. The clinical, immunologic, and histologic evaluations were performed 18 h post-IgG injection. Mice injected with anti-dsg1 autoantibodies developed blisters (A), which were located in the subcorneal region of the epidermis (B). The anti-dsg1 autoantibodies were detected bound to the epidermal ICS by direct IF (C). Mice injected with anti-dsg3 autoantibodies developed blisters (D), which were located in the suprabasilar region of the epidermis (E). Autoantibodies were detected bound to the epidermal ICS by direct IF (F). The pathogenicity of the anti-dsg3 and anti-dsg1 autoantibodies fractions was blocked by preincubation of these fractions with purified rdsg3 and rdsg1, respectively (data not shown).

concentrated, and filter sterilized. They were then characterized by ELISA assays. These antibodies were then used in passive transfer experiments in neonatal mice as described below.

**Induction of experimental pemphigus by passive transfer experiments** Institutional approval from UNC-Chapel Hill IRB was obtained for the animal studies. Approximately 200  $\mu\text{g}$  per g body weight of affinity-purified anti-dsg1 or anti-dsg3 antibodies diluted in phosphate-buffered saline was administered intradermally to neonatal BALB/C mice following established procedures (Anhalt *et al*, 1982; Roscoe *et al*, 1985; Ding *et al*, 1999). The skin of neonatal mice was examined 18 h after the IgG injection and any signs of skin disease recorded. The animals were then killed. Specimens were obtained from lesional skin for histologic examination by light microscopy. Perilesional skin samples were also obtained for direct IF analysis, which employed

monospecific fluorescein isothiocyanate-conjugated goat anti-human IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

## RESULTS

**Sensitivity and specificity of the dsg3 ELISA** The rdsg3 ELISA was highly sensitive, and detected antibodies to dsg3 in 82 of 83 patients with PV (sensitivity: 98.8%). All 50 normal subjects were negative, as well as all 20 patients with bullous pemphigoid, all 19 patients with lupus erythematosus and all 23 patients with herpes gestationis, giving an overall specificity of 100% (Fig 2).

**A subset of patients with PF and FS produce antibodies to dsg3** Employing the dsg3 ELISA described above we tested a

large number of serum samples from PF and FS patients. These samples were obtained from several regions of Brazil ( $n = 241$ ), Japan ( $n = 25$ ), and the U.S.A. ( $n = 10$ ). We detected antibodies to *dsg3* in 28% of the nonendemic PF samples (one of 35), and in 7.5% of the FS patients (18 of 241). This difference was not statistically significant. (Using Fisher's exact test and one-sided  $p$ -value,  $p = 0.28$ . The two-sided  $p$ -value is 0.48.)

The corrected OD495 readings ranged from 1.3 to 9.98 (Table I). The sera was also assayed by immunoprecipitation (Fig 3). Antibodies to *dsg3* were detected in all 19 patients with PF and FS by immunoprecipitation. Sera of these patients also immunoprecipitated *rdsg1* (not shown). Serum samples from *rdsg3* ELISA negative PF and FS patients, as well as sera from bullous pemphigoid, herpes gestationis, and lupus erythematosus failed to react with *rdsg3* by immunoprecipitation (not shown).

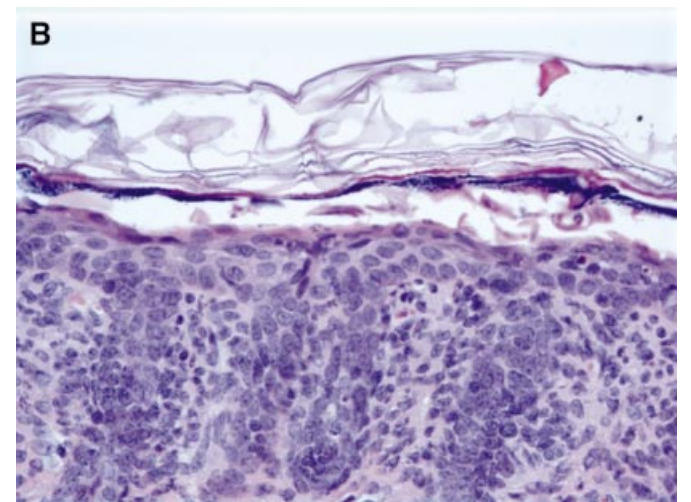
**Preincubation with *rdsg3* inhibits only reactivity with *dsg3*, and preincubation with *rdsg1* inhibits only reactivity with *dsg1*** Using serum from patients with PF and FS who had detectable antibodies to *dsg3*, we added a preincubation step with *rdsg3*, and then tested the sera in the ELISA assay. As shown in Fig 4A preincubation with purified *rdsg3* removes antibodies to *dsg3* in a concentration-dependent manner, but has minimal effect on antibodies to *dsg1*. Pre-incubation with *rdsg1* (Fig 4B) removes antibodies to *dsg1* in a concentration-dependent manner but has minimal effect on antibodies to *dsg3*. These data therefore provide strong evidence that these 19 PF/FS patients exhibit two independent antigen-antibody systems, i.e., anti-*dsg3* and anti-*dsg1*.

**Antibodies to *dsg3* and *dsg1* from PF and FS patients are pathogenic** Antibodies from two patients with FS and one with PF were affinity purified on *dsg1* and *dsg3* affinity columns and the purity of the preparations confirmed by *dsg1* and *dsg3* ELISA. Affinity-purified antibodies were passively transferred into neonatal mice (Table II). Mice injected with antibodies to *dsg1* reproduced the clinical (Fig 5A), histologic (Fig 5B), and immunologic (Fig 5C) features of PF and FS, i.e., subcorneal blisters and antibodies bound to the ICS of lesional skin. Mice that received affinity-purified antibodies to *dsg3* developed clinical skin lesions (Fig 5D) that showed suprabasilar acantholysis on histologic examination (Fig 5E), and positive direct IF staining of the epidermal ICS (Fig 5F). These clinical, histologic, and immunologic features are identical to those seen in mice injected with anti-*dsg3* IgG from PV patients. The IgG fraction of all three sera (one PF and two FS) when injected into neonatal mice induced clinical disease (Fig 6A) and subcorneal acantholysis (Fig 6B) identical to that found previously in other patients with PF.

## DISCUSSION

In PV the presence of mucosal disease alone correlates with antibodies to *dsg3* (Miyagawa *et al*, 1999; Harman *et al*, 2000b). Development of additional cutaneous disease has been correlated with acquisition of antibodies to *dsg1* (Miyagawa *et al*, 1999; Harman *et al*, 2000b). An attractive hypothesis to explain these and other findings has been proposed (Udey and Stanley, 1999). In mucosal epithelia, antibodies to *dsg3* would cause disease due to lack of compensation by *dsg1*. Antibodies to *dsg3* alone would not be able to mediate cutaneous disease because of "compensation" by *dsg1* in the skin. In a PV patient who acquires antibodies to *dsg-1*, compensation by *dsg1* is no longer possible, resulting in cutaneous as well as mucosal disease. In PF by contrast, antibodies to *dsg1* cannot cause mucosal disease as *dsg3* is present at high levels throughout the mucosa. They do cause cutaneous disease, however, which is limited to the upper epidermis where *dsg3* is at very low levels.

We detected antibodies to *dsg3* in 19 of 276 patients with PF and FS, in addition to the previously recognized antibodies to *dsg1*, using highly sensitive and specific ELISA assays. We confirmed our results using immunoprecipitation. Using preincubation assays we



**Figure 6. The purified total IgG fraction from PF and FS patients with antibodies to both *dsg1* and *dsg3* produce clinical and histologic features of PF in the mouse model.** The total IgG fraction from two patients with endemic PF and one with nonendemic PF were purified by ammonium sulfate precipitation and injected intradermally into neonatal mice. All three sera reproduced the clinical (A) and histologic features (B) of PF, namely superficial blistering with subcorneal acantholysis.

showed that reactivity with *dsg3* was depleted by preincubation with *dsg3* but not *dsg1*. This suggests that there may be two separate groups of antibodies in these patients, one group reactive with *dsg1* and one group reactive with *dsg3*. In addition we saw a small drop in reactivity to *dsg1* using preincubation with *dsg3*, and a small drop in reactivity to *dsg3* using preincubation with *dsg1*. As there is a high degree of homology (see Fig 1) in the ectodomains of *dsg1* and *dsg3*, it is likely that a subpopulation of antibodies may cross-react with both molecules. These results would need to be developed using epitope mapping studies. Finally, we showed that the antibodies to *dsg3* from these patients are indeed pathogenic in the mouse model, and reproduce the clinical, histologic, and immunologic features of PV.

All 19 patients with PF and FS who had antibodies to *dsg3* had clinical and histologic features of PF, with no mucosal lesions. It is possible that in these 19 patients with PF and FS, antibodies may contribute to disease pathogenesis by blocking compensation by *dsg3* in the skin. As the total IgG fraction from three patients produced only subcorneal acantholysis in the mouse model, the levels of antibodies to *dsg3* may be too low to cause mucosal disease in these 19. Alternatively these patients may subsequently develop mucosal disease as has been previously reported in nonendemic PF

in Japan (Ishii *et al*, 2000). Additional support for this suggestion comes from the identification of more clear-cut cases of an endemic form of PV in the same regions of Brazil where FS is endemic.<sup>1</sup> These authors collected a small group of young patients with a severe mucocutaneous disease that on histologic and immunologic grounds overlapped with PV. These patients need to be further characterized in terms of antigenic targets.

In conclusion, these results show that the line between PF and PV is becoming somewhat blurred, with the possibility of transition between the two clinical forms existing, presumably by epitope spreading (Lehman *et al*, 1992, 1993; Kaufman *et al*, 1993).

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