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Mucosal pemphigus vulgaris anti-Dsg3 IgG are pathogenic to the oral mucosa of humanized Dsg3 mice

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Abstract

There are two major clinical subsets of pemphigus vulgaris (PV), mucosal PV (mPV) and mucocutaneous PV (mcPV). The mPV subset exhibits anti-human desmoglein (Dsg) 3 autoantibodies that fail to recognize murine Dsg3; thus, passive transfer experiments of mPV IgG into WT mice have been unsuccessful at inducing disease. We therefore generated a fully humanized Dsg3 (*hDSG3*) murine model utilizing a human Dsg3 transgenic animal crossed to the murine Dsg3 knockout line. Expression of hDsg3 in the mucosa rescues the murine Dsg3 knockout phenotype. Well characterized mPV sera bind mucosal epithelia from the hDsg3 mice, but not mucosal tissues from WT mice by as detected by indirect immunofluorescence. The majority of mPV sera preferentially recognize hDsg3 compared to mDsg3 by immunoprecipitation as well. Passive transfer of mPV IgG into adult hDsg3 mice, but not WT mice, induces suprabasilar acantholysis in mucosal tissues, thus confirming pathogenicity of mPV anti-hDsg3 IgG in vivo. Human anti-hDsg3 antibodies are detected in perilesional mucosa as well as in sera of recipient mice by immunofluorescence. These findings suggest that the Dsg3 epitopes targeted by pathogenic mPV IgG are human specific. This hDsg3 mouse model will be invaluable in studying the clinical transition from mPV to mcPV.

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CONFLICT OF INTEREST

D. Cowley is employed by, has equity ownership in and serves on the board of directors of TransViragen, the company which has been contracted by UNC-Chapel Hill to manage its Animal Models Core Facility.

INTRODUCTION

Pemphigus vulgaris (PV) is an autoimmune blistering disease affecting the skin and mucosa (Lever, 1965). Autoantibody binding to keratinocyte adhesion proteins desmoglein (Dsg) 1 and Dsg3 leads to acantholysis with intraepidermal clefting histologically, and blister formation clinically. Two distinct clinical variants of PV have been described, mucosal predominant PV (mPV) and mucocutaneous PV (mcPV) (Ding *et al.*, 1997). Patients with mPV present with disease localized to the mucosal tissues and typically harbor autoantibodies to Dsg3. Patients with mcPV have disease affecting both the mucosa and skin and typically harbor autoantibodies to both Dsg3 and Dsg1 (Amagai *et al.*, 1999b; Ding *et al.*, 1997). Interestingly, the clinical course of most PV patients begins with mucosal lesions (Eversole *et al.*, 1972; Herrero-Gonzalez *et al.*, 2010; Lever, 1965; Meurer *et al.*, 1977). Following a variable period of time, most patients will have disease progress to involve not only the mucosa, but the skin as well. While mPV patients have autoantibodies to Dsg3 alone, the transition from mPV to mcPV is marked the additional development of autoantibodies to Dsg1 (Amagai *et al.*, 1999b; Ding *et al.*, 1997; Ishii *et al.*, 1997; Miyagawa *et al.*, 1999). The factors that precipitate this progression to mcPV in some patients are not known. Indeed, not all mPV patients progress to mcPV as approximately 40% of patients remain with disease limited to the mucosa (Scully *et al.*, 1999).

Aside from the clinical distinction between mPV and mcPV, recent studies suggest a difference in disease course between mPV and mcPV. While early reports suggested that initial mucosal involvement was associated with a poor prognosis, newer findings show that the presence of initial mucosal involvement is a prognostic factor for achieving complete remission off treatment (Almugairen *et al.*, 2013; Mimouni *et al.*, 2010). In addition, mPV patients have a lower mortality compared to patients with mcPV (Mourellou *et al.*, 1995; Wolf *et al.*, 1995), suggesting that mPV patients have an overall better prognosis than mcPV patients. Despite the fact that mPV may be associated with a better outcome than mcPV, mucosal lesions can be recalcitrant in mcPV patients and often persist after cutaneous disease has remitted (Scully *et al.*, 1999). Therefore, exploring the factors involved in the transition from mPV to mcPV and the differences in the anti-Dsg3 autoantibodies from mPV and mcPV patients could have important clinical implications.

Significant progress has been made in defining the pathogenicity of autoantibodies from mcPV patients using the passive transfer model, whereby purified IgG from mcPV sera induces acantholysis and blister formation upon transfer to neonatal mice (Ding *et al.*, 1997; Ding *et al.*, 1999). Unfortunately, similar studies using mPV IgG have been hampered as autoantibodies from mPV patients fail to recognize mucosal or cutaneous tissues in WT mice, and thus, fail to induce disease in the passive transfer model (Ding *et al.*, 1997; Mahoney *et al.*, 1999). To further characterize the pathogenicity of mPV autoantibodies in an in vivo system, we have generated a fully humanized Dsg3 murine model utilizing a human Dsg3 transgenic animal crossed to the murine Dsg3 knockout line. Human Dsg3 is expressed predominantly in the mucosal tissues, similar to that of murine Dsg3 in WT mice. We show that the majority of sera from well characterized mPV patients preferentially recognize hDsg3 by indirect immunofluorescence on hDsg3Tg murine mucosal tissues as well as by immunoprecipitation. Furthermore, passive transfer of mPV IgG leads to IgG

deposition in the intercellular spaces (ICS) of mucosal epithelium, mucosal erosions, and acantholysis with suprabasilar clefting on histology in the hDsg3Tg animals, but not WT animals. These findings suggest that the pathogenic epitopes recognized in mPV anti-Dsg3 autoantibodies are human specific.

RESULTS

Generation of fully humanized Dsg3 Tg mice

The humanized Dsg3 Tg line was generated via standard pronuclear microinjection techniques using the human Dsg3 BAC (Figure 1a). Offspring carrying the BAC were crossed to murine Dsg3 (mDsg3) KO heterozygote mice. Following a complex breeding strategy (Supplementary Figure 1), crossing of resultant $hDsg3^{Tg}mDsg3^{WT/KO}$ and $hDsg3^{Neg}mDsg3^{WT/KO}$ animals gave offspring of multiple genotypes on the B6 background including $hDsg3^{Tg}mDsg3^{KO/KO}$ (fully humanized Dsg3, here forward termed hDsg3Tg), $hDsg3^{Neg}mDsg3^{WT/WT}$ (WT), and $hDsg3^{Neg}mDsg3^{KO/KO}$ (mDsg3KO) mice. Genotyping was performed on genomic DNA from tail snips using hDsg3 BAC specific primers as well as mDsg3 WT and KO allele specific primers (Figure 1b).

Expression of hDsg3 in hDsg3Tg mice

To confirm the expression of the hDsg3 transgene in the hDsg3Tg mice, palatal tissue was harvested from hDsg3Tg mice and mDsg3KO and WT littermate controls. Immunofluorescence (IF) was then performed using antibodies to murine Dsg3 (clones AK18 and AK23) and human Dsg3 (clone 5G11). While AK23 is known to have significant crossreactivity to human Dsg3, AK18 has been reported to have less human Dsg3 crossreactivity and was therefore included in this analysis. As shown in Figure 2a, anti-mDsg3 AK18 recognizes the ICS of WT palate with some recognition of the ICS of hDsg3Tg palate, while anti-mDsg3 AK23 recognized the ICS of both WT and hDsg3Tg palate. Anti-hDsg3 5G11 recognizes the ICS only in the palate of the hDsg3Tg mice, confirming specific expression of hDsg3 in the hDsg3Tg palate. Expression of hDsg3 was also noted in the buccal mucosa, tongue, esophagus, basal layer of the skin, and hair follicles in the hDsg3Tg animals in a similar pattern to that of mDsg3 in WT mice (data not shown). There was no expression in the stomach, small and large intestine, lung, heart, kidney and liver (data not shown).

Immunoblotting of mucosal extracts from mDsg3KO, WT, and hDsg3Tg mice was performed as a secondary method to confirm expression. Anti-mDsg3 AK23 recognizes conformational epitopes, and was not included in this analysis (Tsunoda *et al.*, 2003). The anti-mDsg3 AK18 detects a band of approximately 130kDa in the WT mucosal extracts, while the anti-hDsg3 5G11 detects a band of similar size in the hDsg3Tg mucosal and human extracts (Figure 2b). There is no detection of mDsg3 or hDsg3 in mDsg3KO mucosal extracts as expected.

Thus, hDsg3 is expressed in hDsg3Tg mice in a similar pattern to that of mDsg3 in WT mice. Furthermore, expression of endogenous Dsg1 and desmocollin 3 (Dsc3) is similar in

WT and hDsg3Tg skin and mucosa, suggesting that introduction of hDsg3 does not alter Dsg1 or Dsc3 expression in the hDsg3Tg mice (Supplementary Figure 2 and Figure 4a).

Expression of hDsg3 rescues the mDsg3KO phenotype

As previously described, mDsg3KO mice show runting and mucosal acantholysis with suprabasilar clefting histologically (Koch *et al.*, 1997). To investigate whether hDsg3 could compensate for the loss of mDsg3, we examined littermates clinically and obtained weights at 21 days of age. Fully humanized mice (hDsg3Tg) are similar in size (Figure 3a) and weight (Figure 3b) to WT littermates. As noted previously, mDsg3KO mice show a more severe phenotype on the B6 background (Hata *et al.*, 2011). Indeed, the majority of littermates with the mDsg3KO genotype on the B6 background die prior to weaning (data not shown). The lifespan of hDsg3Tg mice is similar to that of WT littermates.

Mucosal tissue was also harvested from mDsg3KO, WT, and hDsg3Tg littermates for routine histology. While the mDsg3KO buccal mucosa shows suprabasilar clefting with acantholysis, hDsg3Tg mucosa shows normal histology similar to that of the WT littermates, confirming that expression of hDsg3 is sufficient to rescue the mDsg3KO phenotype (Figure 3c).

IgG from mPV sera preferentially recognize hDsg3

The sera from well-known mPV (n=10) and mcPV (n=10) patients were characterized in terms of IgG and IgG4 indirect IF titers using monkey esophagus substrate. The sera were also tested for IgG and IgG4 reactivity to recombinant human Dsg3 and Dsg1 by ELISA. All sera tested showed reactivity to Dsg3 by ELISA. As expected, mPV patients IgG and IgG4 recognize Dsg3 without a high degree of reactivity to Dsg1 (Table 1). One mcPV cohort (mcPV11-15) includes patients with mcPV that had autoantibodies to Dsg3 in the absence of autoantibodies to Dsg1 at the time of the blood draw. The second mcPV cohort (mcPV16-20) includes patients with strong recognition of IgG and IgG4 to both Dsg3 and Dsg1 (Table 1).

Using hDsg3Tg and WT palate as substrate, the sera of these patients were tested by indirect IF. As IgG4 is known to be pathogenic in PV, an anti-human IgG4 secondary antibody was used for detection. As shown in Figure 4a, mPV patient sera preferentially bind to the ICS of the hDsg3Tg palate compared to the WT palate. Similarly, mcPV patients with autoantibodies to Dsg3, but without autoantibodies to Dsg1 preferentially recognize hDsg3 as well (mcPV11 shown).

Differential recognition of hDsg3 and mDsg3 by the sera from mcPV patients with anti-Dsg3 and anti-Dsg1 is not possible by indirect IF due to the additional presence of autoantibodies to Dsg1 which are also detected by this method. Therefore, immunoprecipitation was used as a secondary method to confirm preferential recognition of mPV IgG to hDsg3 and to assess differential recognition of hDsg3 and mDsg3 by mcPV IgG. Sera from all mPV patients and mcPV with ant-Dsg3 alone (mcPV11-15) were able to immunoprecipitate hDsg3, while only some sera were able to precipitate mDsg3 (Figure 4b). Sera from mcPV patients with both anti-Dsg3 and anti-Dsg1 (mcPV16-20) were able to

immunoprecipitate both hDsg3 and mDsg3, though two patients (mcPV17 and mcPV20) also preferentially recognize hDsg3.

IgG from mPV patients are pathogenic in hDsg3Tg mice

As mPV sera preferentially recognize hDsg3 expressed in the mucosa of hDsg3Tg mice, we sought to assess pathogenicity of mPV IgG by passive transfer. IgG purified from mPV patients (mPV2 and 4) as well as mcPV11 was passively transferred via submucosal injection to WT and hDsg3Tg mice at amounts ranging from 1–8mg of IgG. These sera were chosen as they had high titers of autoantibodies by indirect IF and large volumes available. At 18–20 hours post transfer, the sera of both WT and hDsg3Tg recipients both show detectable circulating human IgG by indirect IF on monkey esophagus substrate with equivalent titers (1:40–1:320) confirming successful and equivalent passive transfer of IgG (Figure 5d). However, only hDsg3Tg recipients showed clinical mucosal erosions (Figure 5a), positive DIF with ICS deposition of human IgG4 in mucosal tissues (Figure 5b), and acantholysis with suprabasilar clefting on histology (Figure 5c). WT recipients had normal appearing mucosa and no evidence of acantholysis or suprabasilar clefting on histologic examination. DIF was negative in WT recipients despite the presence of circulating human autoantibodies in the sera. Clinical and histologic features of PV following passive transfer were dose dependent with more severe disease being induced when recipient mouse sera reached higher indirect IF titers. ICS deposition of human IgG was detected in the basal layer and hair follicles of recipient mice, but no cutaneous erosions nor hair loss were noted on examination. Histology of the skin of recipient mice showed no evidence of acantholysis or clefting (data not shown). The data are summarized in Supplementary Table 1.

DISCUSSION

The clinical distinction between mPV and mcPV has long been noted and described by clinicians (Lever, 1965). Autoantibody profiles against Dsg1 and Dsg3 have revealed that mPV patients have autoantibodies to Dsg3, while mcPV patients have autoantibodies to both Dsg3 and Dsg1 (Amagai *et al.*, 1999b; Ding *et al.*, 1997; Ishii *et al.*, 1997). Indeed, progression from mPV to mcPV is accompanied by the acquisition of autoantibodies to Dsg1 (Amagai *et al.*, 1999b; Ding *et al.*, 1997; Ishii *et al.*, 1997; Miyagawa *et al.*, 1999). Early studies confirmed that affinity purified anti-Dsg1 and anti-Dsg3 autoantibodies from mcPV patients are independently pathogenic upon passive transfer to neonatal mice (Ding *et al.*, 1999). However, IgG from mPV patients that harbor antibodies to Dsg3 alone are nonpathogenic in the neonatal passive transfer model due to lack of mDsg3 reactivity (Ding *et al.*, 1997). Mahoney et al. described similar findings and attribute the lack of mPV IgG pathogenicity to differential expression patterns of Dsg1 and Dsg3 in the skin and oral mucosa of humans and mice (Mahoney *et al.*, 1999). Even at very high doses (up to 26mg IgG/pup) IgG from PV sera with anti-Dsg3 autoantibodies alone are not able to induce blister formation in murine neonatal skin or mucosa upon passive transfer (Mahoney *et al.*, 1999). Little progress has been made in the understanding of mPV IgG pathogenicity and the immunological basis of the transition of mPV to mcPV since these initial studies over 15 years ago.

We show here that mPV IgG with autoantibodies to Dsg3 are pathogenic in vivo, but require the expression of human Dsg3. These findings confirm that the pathogenic epitopes recognized by most mPV anti-Dsg3 autoantibodies are specific to hDsg3. Transfer of fairly low doses (1–8mg/mouse) of mPV IgG into adult submucosa is sufficient to induce classic IgG ICS deposition and suprabasilar acantholysis and clefting in hDsg3Tg, but not WT mucosa. Clinical and histologic features of PV following passive transfer are dose dependent with more severe disease induced when higher titers are reached. Dsg1 and Dsc3 expression patterns are similar in hDsg3Tg and WT tissues, further validating this animal model. Studies to demonstrate similar desmocollin 1 (Dsc1) expression have not been performed in this animal model, and therefore it is possible that hDsg3 insertion influences Dsc1 expression, which could have an impact on epidermal adhesion.

Though mPV IgG was able to efficiently induce mucosal lesions in the hDsg3Tg animals, there was no evidence of skin lesions in the recipient mice. As mentioned previously, affinity purified anti-Dsg3 autoantibodies from mcPV patients do recognize murine Dsg3 and are able to induce skin lesions upon neonatal passive transfer (Ding *et al.*, 1999), though neonatal mucosal tissues were not examined in the study. These findings suggest that while most mPV IgG preferentially recognize hDsg3 specific epitopes, mcPV anti-Dsg3 autoantibodies are able to recognize pathogenic epitopes shared by mDsg3 and hDsg3. This idea is further supported by our data, whereby mcPV patients with both anti-Dsg3 and anti-Dsg1 autoantibodies are able to immunoprecipitate mDsg3. We speculate that PV patients with initial mucosal disease may harbor autoantibodies that preferentially recognize hDsg3 specific epitopes and that through intramolecular epitope spread, upon transition to mcPV patients develop autoantibodies to additional Dsg3 epitopes that are shared by hDsg3 and mDsg3 in addition to developing anti-Dsg1 autoantibodies. It is interesting that two mPV patients (mPV6 and mPV8) recognize hDsg3 and mDsg3 to a similar degree by immunoprecipitation, suggesting they may be in the earliest stages of transition to mcPV. Similarly, two mcPV patients (mcPV17 and mcPV20) preferentially recognize hDsg3. Clinically, mcPV17 had recalcitrant mucosal lesions throughout the course of disease. We speculate that differential epitope recognition over the disease course may play a role in clinical phenotypes. Additional studies are required to explore this intriguing possibility. There is a high degree of homology between hDsg3 and mDsg3, particularly in the extracellular (EC) domains 1 and 2 which are felt to harbor pathogenic epitopes (Futei *et al.*, 2000). The implication of these data is that there are relatively few amino acid residues responsible for the preferential binding of mPV to hDsg3.

As recent studies have shown that initial mucosal only involvement is a prognostic factor for achieving complete remission off therapy, understanding and potentially halting the progression of mPV to mcPV could have important clinical implications. This humanized Dsg3 murine model will be of great value in further exploring autoantibodies in mPV and the transition from mPV to mcPV.

MATERIALS AND METHODS

Generation of the hDsg3 mice

BAC clone RP11-1123B14 was obtained from Children's Hospital Oakland Research Institute. The human Dsg3 BAC includes the entire human Dsg3 gene sequence with approximately 57kb 5′ and 30kb 3′ of the Dsg3 sequence. The hDsg3 BAC was microinjected into B6D2 embryos following standard pronuclear microinjection techniques (Nagy *et al.*, 2003). Resulting animals were genotyped with 6 primer sets specific for human DNA regions spaced approximately every 20kb across the BAC. Founders that included the entire BAC were each bred to a WT (B6 background) animal. Resulting offspring were screened for presence of the BAC using genomic DNA from tail snips and the aforementioned primers. Offspring carrying the BAC ($hDsg3^{Tg}mDsg3^{WT/WT}$) were crossed to murine Dsg3 (mDsg3) knockout heterozygotes ($hDsg3^{Neg}mDsg3^{WT/KO}$) obtained from Jackson Laboratories (Bar Harbor, ME). The F1 generation yielded $hDsg3^{Tg}mDsg3^{WT/KO}$ which were backcrossed to B6 for three additional generations (total four backcrosses to B6). Crossing of the resultant $hDsg3^{Tg}mDsg3^{WT/KO}$ and $hDsg3^{Neg}mDsg3^{WT/KO}$ produced littermates of multiple genotypes including $hDsg3^{Tg}mDsg3^{KO/KO}$ (fully humanized Dsg3, termed hDsg3Tg), $hDsg3^{Neg}mDsg3^{WT/WT}$ (WT), and $hDsg3^{Neg}mDsg3^{KO/KO}$ (Dsg3 KO) mice. The breeding strategy is shown in Supplementary Figure 1. Genotyping was performed on genomic DNA from tail snips using hDsg3 BAC specific as well as mDsg3 WT and KO allele specific primers. Primers are listed in Supplementary Table 2. All animal experiments were approved by the University of North Carolina Institutional Animal Care and Use Committee.

Histology and immunofluorescence studies

Tissues were harvested from adult mice 6–8 weeks of age. Formalin fixed, paraffin embedded sections were processed by H&E staining for histologic evaluation by a board certified dermatopathologist blinded to the specimen genotype.

For immunofluorescence studies, tissue was embedded, frozen in OCT and cryosections prepared. Anti-mouse Dsg3 (clones AK18 and AK23, MBL, Woburn, MA) and anti-human Dsg3 (clone 5G11, AbD serotec, Kidlington, Oxford, UK) antibodies diluted in Tris-buffered-saline 5mM Ca^{++} (TBS/Ca^{++}) were applied to the sections in a humidity chamber at RT for 30 minutes. Following three 10 minute washes, polyclonal goat anti-mouse IgG-H +L-FITC (abcam, Cambridge, MA) was used as a secondary antibody before evaluation under a fluorescence scope. Anti-mouse Dsc3 (gp2280, the kind gift of Dr. Peter Koch) was used to detect Dsc3 as above, but with a primary overnight incubation at 4°C followed by goat anti-guinea pig Alexa 488 (Life Technologies, Grand Island, NY). Similar studies were performed using patient sera followed by mouse anti-human IgG4 FITC (clone HP-6025, Sigma, St. Louis, MO) as a secondary antibody. For direct immunofluorescence studies, cryosections were directly stained with anti-human IgG4 FITC (clone HP-6025, Sigma, St. Louis, MO).

Protein extraction and western blotting

Whole tissue extracts were prepared by homogenizing murine mucosal tissues in 2% SDS/beta-mercaptoethanol lysis buffer on ice. Extracted protein was quantified and equal amounts were subjected to SDS-PAGE and transferred to PVDF membrane. Membranes were blocked for 1h in 5% fat-free-milk, 0.1% Tween-20-TBS. Following three 10 min washes in 0.1% Tween-20-TBS, membranes were incubated overnight with anti-mouse-Dsg3 antibody (clone AK18, MBL, Woburn, MA) and anti-human-Dsg3 antibody (clone 5G11, AbD serotec, Kidlington, UK). The membranes were washed, incubated for 1h with goat anti-mouse-IgG-HRP (R&D Systems, Minneapolis, MN), and developed using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL).

Sources of sera

Sera were collected from patients at the autoimmune blistering disorder clinic with disease confirmed by clinical, histological, and immunofluorescent findings. Control sera were obtained from the University of North Carolina Blood Bank. The study was approved by the University of North Carolina Institutional Review Board, and conducted according to the Declaration of Helsinki Principles. Participants gave their written informed consent.

Construction and production of recombinant human Dsg1, human Dsg3 and murine Dsg3

We have previously constructed and expressed the entire extracellular domains of human Dsg1 and Dsg3 using the baculovirus system (Ding *et al.*, 1997; Ding *et al.*, 1999; Flores *et al.*, 2012). The murine Dsg3 DNA plasmid pEVmod-murineDsg3-His (kind gift of Dr. Masayuki Amagai, Keio University, Tokyo, Japan) was used to generate the mDsg3 recombinant baculovirus using the BacPAK Baculovirus Expression System (Clontech, Mountain View, CA). Soluble ectodomains of hDsg1, hDsg3, and mDsg3 were produced in the baculovirus system and purified by nickel chromatography as described (Flores *et al.*, 2012).

ELISA

Immunomicrotiter plates (Costar, Cambridge, MA) were coated with purified human Dsg3 or Dsg1 at 4 °C overnight. IgG and IgG4 ELISA was then performed as described (Flores *et al.*, 2012; Qaqish *et al.*, 2009). Results were expressed as index values (Amagai *et al.*, 1999a; Diaz *et al.*, 2008; Flores *et al.*, 2012).

Immunoprecipitation

Initial chessboard titration experiments were performed to determine the optimal amount of hDsg3 and mDsg3 supernatant with equivalent his-tagged recombinant protein levels to be incubated with 2ul of known positive and negative control sera. The sera and culture supernatant containing hDsg3 or mDsg3 were incubated overnight and immunoprecipitated as described (Li *et al.*, 2003). The precipitated protein was then subjected to SDS-PAGE, transferred, probed with anti-His-HRP (Penta-his HRP, Qiagen) and developed using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL).

Passive transfer of mPV IgG

IgG from mPV sera was purified using HiTrap Protein G HP columns (GE Healthcare Life Sciences) and concentrated using Amicon Ultra Centrifugal Filters (Millipore). Purified, concentrated IgG was quantified and transferred submucosally to 6 week old hDsg3Tg or WT mice. Doses ranged from 1–8 mg of total IgG/mouse. Mice were sacrificed and tissues harvested at 18–20 hours and processed for routine histology and direct immunofluorescence using mouse anti-human-IgG4 FITC (clone HP-6025, Sigma, St. Louis, MO). Sera of recipient mice were also harvested for indirect immunofluorescence studies as described above.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Dsg3	desmoglein 3
hDsg3	human desmoglein 3
mDsg3	murine desmoglein 3
Dsg1	desmoglein 1
Dsc3	desmocollin 3
PV	pemphigus vulgaris
mPV	mucosa pemphigus vulgaris
mcPV	mucocutaneous pemphigus vulgaris
ICS	intercellular spaces

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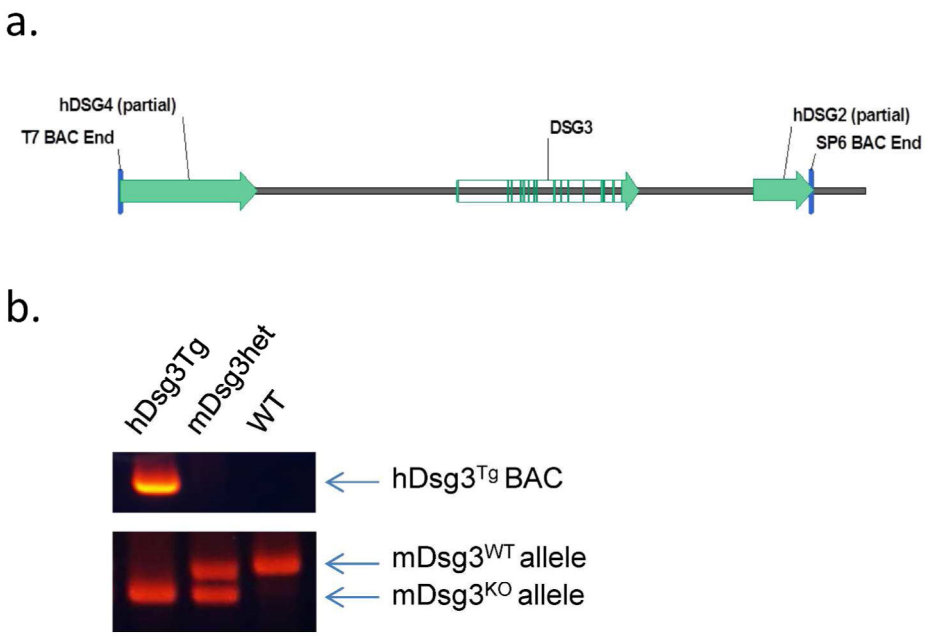

Figure 1.

Generation of fully humanized Dsg3 Tg mice. (**a**) The human Dsg3 BAC clone RP11-1123B14 (hDsg3 BAC) was used to create the hDsg3Tg line by standard pronuclear injection technique. (**b**) Following a series of crosses to the mDsg3KO line and subsequently B6 background, genotyping of the F1and F2 offspring was performed using hDsg3BAC as well as mDsg3WT and mDsg3KO allele specific primers.

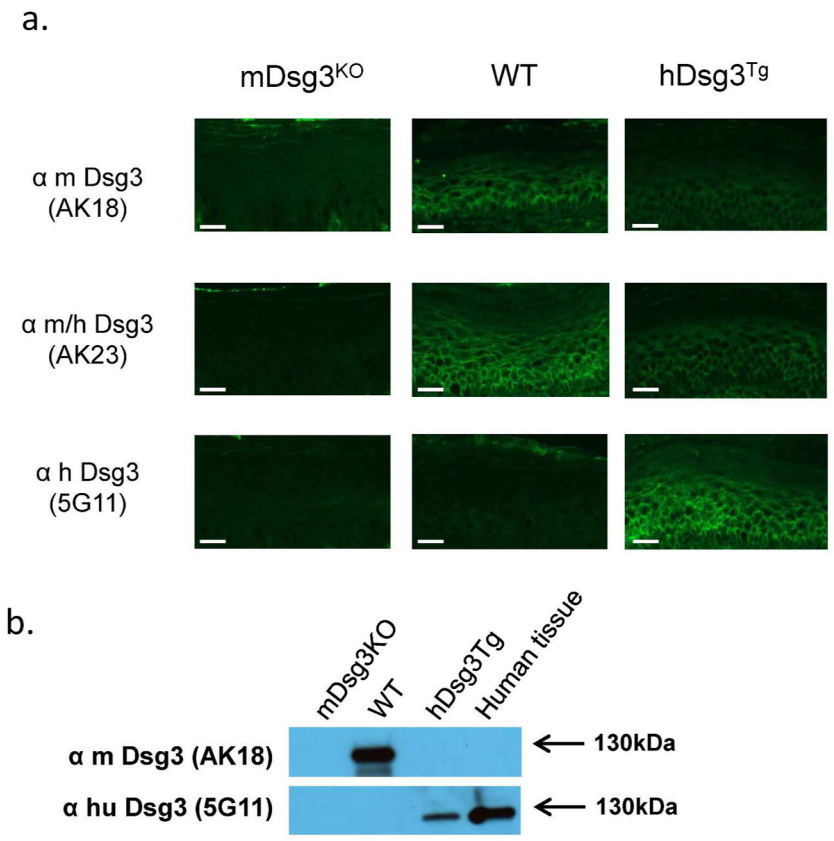

Figure 2.

hDsg3Tg mice express hDsg3 in mucosal tissues. (**a**) Indirect immunofluorescence using mDsg3KO, WT and hDsg3Tg oral mucosal tissue (palate) was performed with anti-mDsg3 mAb AK18, anti-mDsg3 mAb AK23, and anti-hDsg3 mAb 5G11 and detected by goat anti-mIgG FITC. Bar = 25um. (**b**) Western blot of mucosal tissue extracts from mDsg3KO, WT and hDsg3Tg mice was performed with anti-mDsg3 mAb AK18 and anti-hDsg3 mAb 5G11. Human tissue extract is shown as a control.

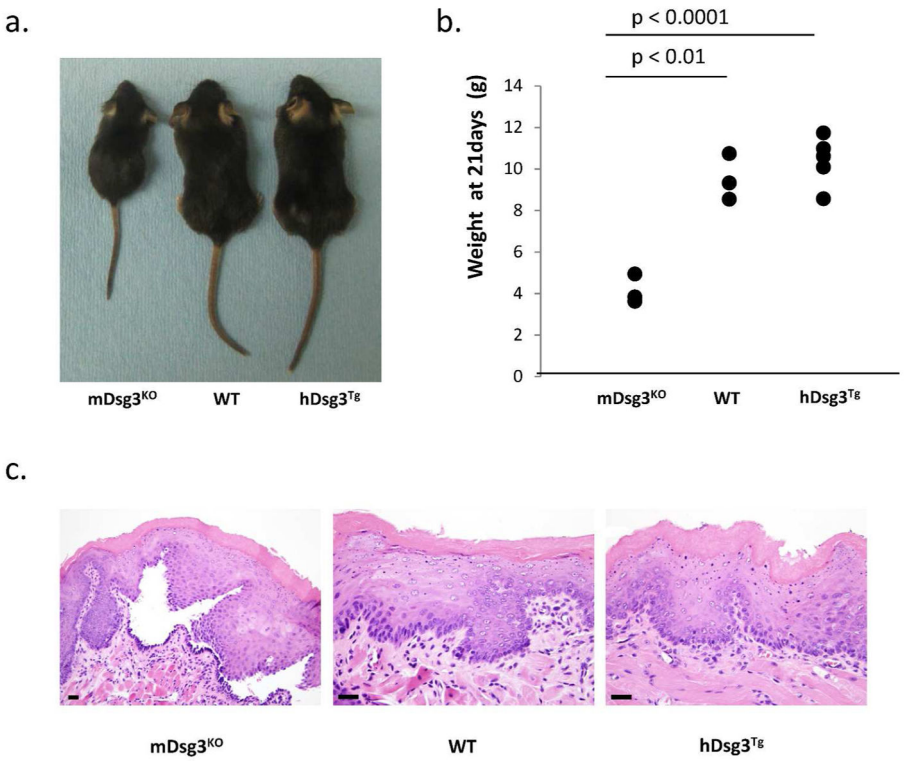

Figure 3.

Expression of hDsg3 rescues the mDsg3KO phenotype. (**a**) Clinical phenotype and (**b**) weights (in grams) of mDsg3KO, WT and hDsg3Tg mice are shown at 21 days of age. (**c**) Histology of the mDsg3KO, WT and hDsg3Tg buccal mucosa are shown (x200). Bar = 100um.

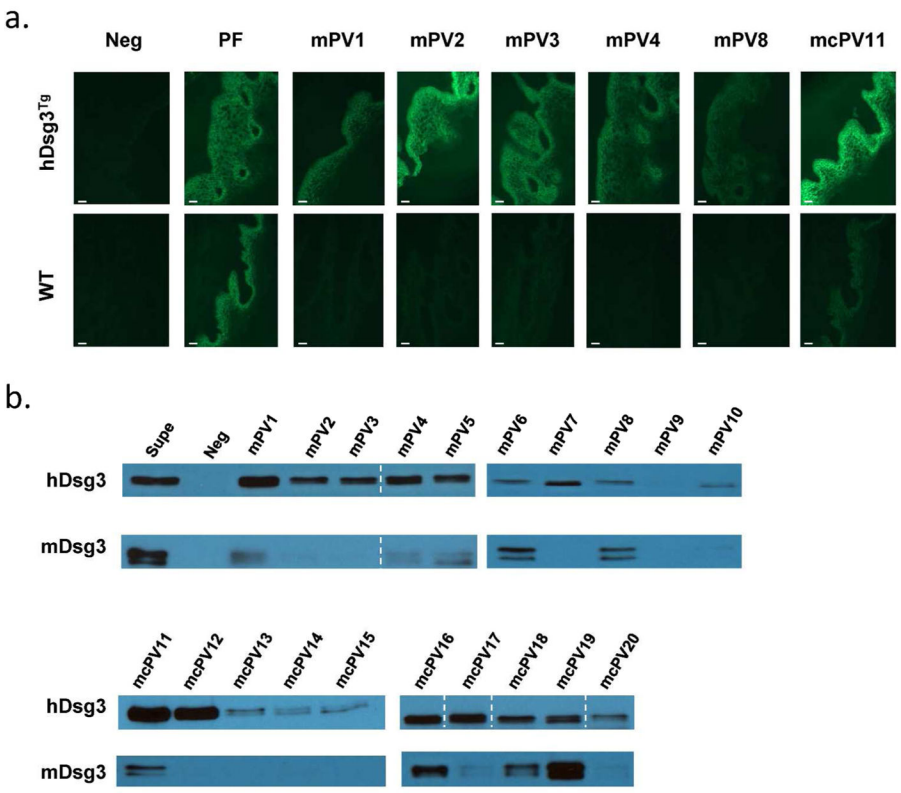

Figure 4.

Sera from mPV patients preferentially recognize hDsg3. Sera from well characterized mPV and mcPV patients were tested by indirect IF (**a**) using WT and hDsg3Tg mucosa with anti-human IgG4 FITC as the secondary antibody. Healthy control sera (Neg) and pemphigus foliaceus (PF) sera are included as negative and positive controls, respectively. Bar = 25um. (**b**) Immunoprecipitation was performed using either recombinant his tagged hDsg3 or mDsg3 and patient sera. Immunoprecipitated hDsg3 or mDsg3 was detected by anti-His HRP. All detected bands ran at the expected size of approximately 77kDa. Noncontiguous lanes run on the same gel are indicated by a white dashed line.

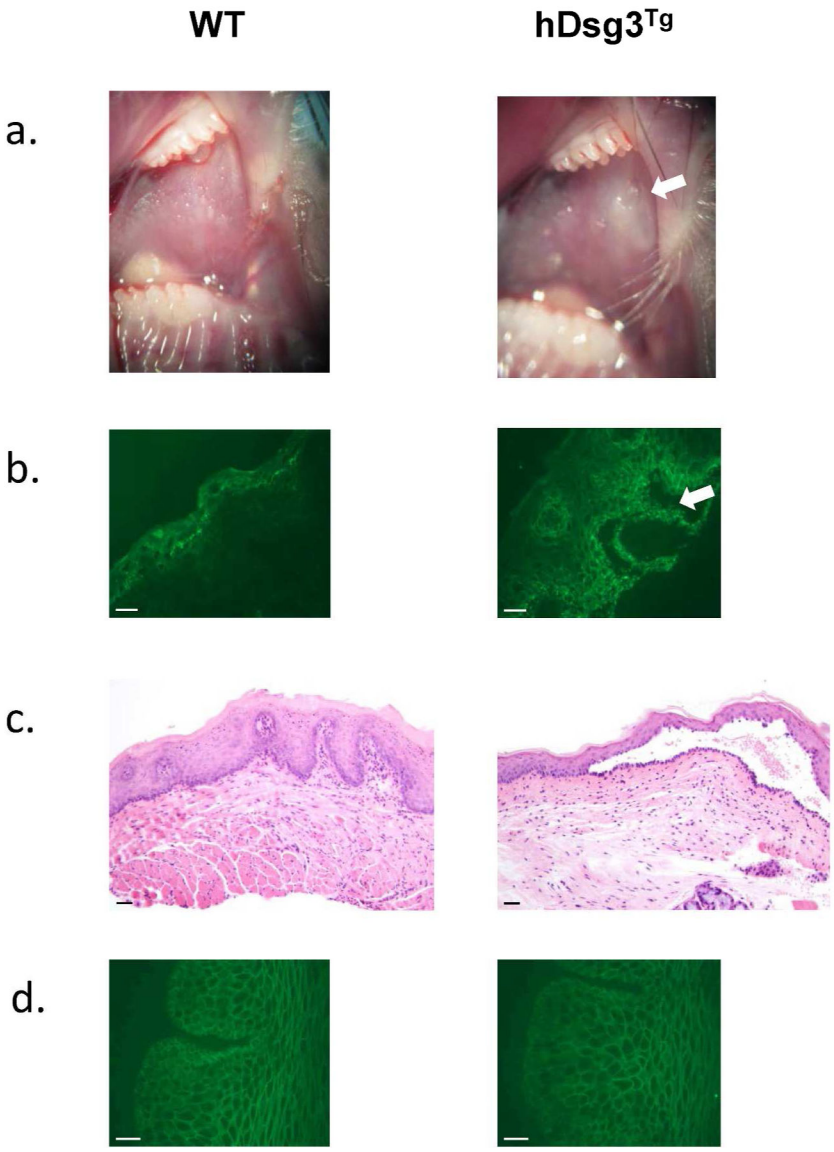

Figure 5.

IgG from mPV patients induce acantholysis in hDsg3Tg mucosa upon passive transfer. Mucosal tissues from hDsg3Tg mice show clinical erosions (**a**), positive direct IF with anti-human IgG4 FITC (**b,** Bar = 25um), and histology with suprabasilar clefting and acantholysis (x100) (**c**, Bar = 100um) as compared to WT mucosal tissues. Both WT and hDsg3Tg sera show positive indirect IF (**d**, Bar = 25um) with equivalent titers (1:80) on the monkey esophagus substrate confirming successful passive transfer.

Table 1

Characteristics of PV patient sera.

Clinical Phenotype[1]	IIF titers[2]		ELISA Index Values[3]			
			Dsg3		Dsg1	
	IgG	IgG4	IgG	IgG4	IgG	IgG4
mPV						
mPV-1	1:640	1:320	124.6	72.3	8.8	0.8
mPV-2	1:640	1:320	122.9	157.6	3.0	0.2
mPV-3	1:640	1:320	108.4	125.9	6.9	0.8
mPV-4	1:320	1:320	100.0	100.0	3.8	0.5
mPV-5	1:640	1:40	58.3	31.7	2.6	2.0
mPV-6	1:160	1:40	67.9	50.8	6.2	0.9
mPV-7	1:320	1:160	43.7	38.0	5.6	0.3
mPV-8	1:160	1:80	40.8	50.7	2.9	0.9
mPV-9	1:160	1:40	41.0	43.2	2.6	1.3
mPV-10	1:320	1:40	13.9	4.8	1.9	0.2
mcPV with antibodies to Dsg3 only						
mcPV-11	1:640	1:640	130.7	193.0	1.6	0.3
mcPV-12	1:1280	1:640	82.8	71.8	3.4	0.1
mcPV-13	1:160	1:160	43.6	72.3	3.7	1.1
mcPV-14	1:320	1:160	14.3	2.8	5.4	0.2
mcPV-15	1:320	1:40	28.6	20.9	1.3	0.6
mcPV with antibodies to Dsg3 and Dsg1						
mcPV-16	1:1280	1:640	139.7	259.9	114.0	144.9
mcPV-17	1:640	1:160	111.2	97.3	30.5	32.7
mcPV-18	1:640	1:160	96.0	90.5	31.8	15.7
mcPV-19	1:320	1:320	81.2	117.6	50.6	66.8
mcPV-20	1:640	1:160	42.7	34.6	21.7	10.4

[1] Patients were selected based on their clinical phenotype and divided into three cohorts: mPV, mcPV with anti-Dsg3 antibodies, but lacking anti-Dsg1 antibodies, and mcPV with both anti-Dsg3 and anti-Dsg1 antibodies

[2] Indirect IF titers as tested on monkey esophagus substrate

[3] ELISA results are reported as index values