Desmoglein 3 anchors telogen hair in the follicle

Peter J. Koch, M. G. Mahoney, George Cotsarelis, Kyle Rothenberger, Robert M. Lavker and John R. Stanley*

Department of Dermatology, University of Pennsylvania School of Medicine, 211 CRB, 415 Curie Blvd, Philadelphia, PA 19104, USA
*Author for correspondence (e-mail: jrstan@mail.med.upenn.edu)

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SUMMARY

Little is known about the function of desmosomes in the normal structure and function of hair. Therefore, it was surprising that mice without desmoglein 3 (the autoantigen in pemphigus vulgaris) not only developed mucous membrane and skin lesions like pemphigus patients, but also developed hair loss. Analysis of this phenotype indicated that hair was normal through the first growth phase (‘follicular neogenesis’). Around day 20, however, when the hair follicles entered the resting phase of the hair growth cycle (telogen), mice with a targeted disruption of the desmoglein 3 gene (DSG3−/−) lost hair in a wave-like pattern from the head to the tail. Hair then regrew and was lost again in the same pattern with the next synchronous hair cycle. In adults, hair was lost in patches. Gentle hair pulls with adhesive tape showed that anagen (growing) hairs were firmly anchored in DSG3+/+ mice, but telogen hairs came out in clumps compared to that of DSG3+−/− and +/+ littermates in which telogen hairs were firmly anchored. Histology of bald skin areas in DSG3−/− mice showed cystic telogen hair follicles without hair shafts. Histology of hair follicles in early telogen, just before clinical hair loss occurred, showed loss of cell adhesion (acantholysis) between the cells surrounding the telogen club and the basal layer of the outer root sheath epithelium. Electron microscopy revealed ‘half-desmosomes’ at the plasma membranes of acantholytic cells. Similar acantholytic histology and ultrastructural findings have been previously reported in skin and mucous membrane lesions of DSG3−/− mice and pemphigus vulgaris patients. Immunoperoxidase staining with an antibody raised against mouse desmoglein 3 showed intense staining on the cell surface of keratinocytes surrounding the telogen hair club in normal mice. Similar staining was seen in human telogen hair with an anti-human desmoglein 3 antibody. Finally, a scalp biopsy from a pemphigus vulgaris patient showed empty telogen hair follicles. These data demonstrate that desmoglein 3 is not only critical for cell adhesion in the deep stratified squamous epithelium, but also for anchoring the telogen hair to the outer root sheath of the follicle and underscore the importance of desmosomes in maintaining the normal structure and function of hair.

Key words: Desmoglein, Pemphigus, Desmosome, Hair

INTRODUCTION

Desmoglein 3 (Dsg3) is a desmosomal transmembrane glycoprotein that belongs to the cadherin superfamily of cell adhesion receptors (Amagai et al., 1991; Koch and Franke, 1994). It is expressed and assembled into desmosomes in certain strata of stratified squamous epithelia (Franke et al., 1994; Karpati et al., 1993; Schwarz et al., 1990; Arnemann et al., 1993; Schafer et al., 1994). In skin, Dsg3 is expressed in keratinocytes of the basal and immediate suprabasal cell layers (e.g. Amagai et al., 1996).

Even though desmosomes are multiprotein complexes thought to be important in maintaining cell-to-cell adhesion, there have been few in vivo models that confirm their actual function. One exception has been the study of the pathobiology of the autoantibody-mediated pemphigus diseases, which has provided strong evidence for the importance of desmogleins in providing cell adhesion between keratinocytes.

There are two major types of pemphigus, pemphigus vulgaris (PV) and pemphigus foliaceous (PF) (reviewed by Stanley, 1993). In both diseases patients develop autoantibodies against desmogleins; in PV against Dsg3 and in PF against desmoglein 1 (Dsg1). It has been shown convincingly that these desmoglein-specific antibodies are pathogenic, that is they bind to the cell surface of desmoglein-expressing keratinocytes in vivo and induce loss of cell adhesion leading to the formation of blisters (Amagai et al., 1992, 1994, 1995). PF blisters occur in the superficial epidermis where Dsg1 is expressed, whereas PV blisters occur in the deep epidermis and stratified squamous epithelia of mucous membranes where Dsg3 is expressed. This histologic finding in PV is called suprabasilar acantholysis.

Evidence that antibodies in PV interfere directly with an adhesive function of Dsg3 in desmosomes was obtained from analyzing mice that we recently generated with a targeted disruption of the DSG3 gene (DSG3−/−) (Koch et al., 1997).
The phenotype of these mice strikingly resembled that of PV patients. These DSG3−/− mice developed oral and vaginal mucous membrane lesions with the typical histology of PV. At sites of trauma, gross and histologic PV-like lesions of the skin were seen as well. These findings showed that a loss of Dsg3 function is sufficient to cause a PV-like phenotype, thus supporting the idea that PV autoantibodies directly interfere with Dsg3-dependent cell-cell adhesion.

Surprisingly, around day 20 after birth, DSG3−/− mice displayed another prominent phenotype, hair loss. Because little is known about the role of desmosomes and their components in the structure and function of hair, we analyzed the balding phenotype of these knockout mice to learn how Dsg3 may function in the hair follicle.

In this study we demonstrate defective anchorage of telogen hairs to the follicular epithelium due to splitting of desmosomes between the keratinocytes surrounding the hair club and the basal layer of the outer root sheath (ORS). This same area of the hair follicle, in both mice and humans, shows intense staining for Dsg3. These data are the first to show a structural function for a desmosomal cadherin in the hair follicle.

MATERIALS AND METHODS

Mice

The DSG3−/− mice were obtained from matings of DSG3+/− heterozygotes (Koch et al., 1997).

Generation of a mouse Dsg3 fusion protein

Total RNA was isolated from the skin of the back of a two day old DSG3+/− mouse with the RNAzol B reagent (Tel-Test Inc., Friendswood, TX). Subsequently, RNA was converted into single-stranded cDNA using the ‘Superscript Preamplification System’ (Gibco-BRL, Grand Island, NY). A cDNA fragment of 317 base pairs encoding the amino acid sequence of the extracellular domain 5 (EC5) of mouse Dsg3 (for domain designation see Amagai et al., 1991) was amplified by PCR using primers MDSG3F (5′-GCGGATCCATCTTCAACCTTGCATG-3′) and MDSG3R (5′-GCGAAGTCTTCCATATGTTGGCCT-3′; cloning sites are underlined). In the PCR, various amounts of cDNA were used as a template in the presence of 20 pmol of each primer, 200 μM dNTP, 2 U AmpliTaq and 1× PCR buffer (enzyme and buffer supplied by Perkin Elmer, Norwalk, CT) in a total volume of 100 μl. The amplification conditions were: 1 minute 94°C, 2 minutes 54°C and 2 minutes 72°C followed by 30 cycles with 45 seconds 94°C, 1 minute 54°C, 1 minute 72°C and a final incubation for 10 minutes at 72°C.

The PCR product was purified with the ‘QIAquick PCR Purification Kit’ (Qiagen, Santa Clarita, CA), digested with the appropriate restriction enzymes, and then cloned into the BamHI and HindIII sites of the bacterial expression vector pQE30 (Qiagen) following standard procedures. A fusion protein of approximately 12 kDa consisting of the mouse Dsg3 EC5 domain and six amino-terminal histidine residues was expressed in JM109 bacteria (Promega, Madison, WI) and purified using Ni-NTA Agarose (Qiagen) essentially as described (Holzinger et al., 1996).

Antibody production and affinity purification of antibodies

Two rabbits were immunized with the mouse Dsg3 EC5 fusion protein (Tanaka et al., 1990).

For the affinity purification of Dsg3-specific antibodies, the Dsg3 EC5 fusion protein was coupled to ‘Affi-Gel 10 Gel’ (Bio-Rad Laboratories, Hercules, CA) following the protocol for aqueous coupling provided by Bio-Rad. The affinity matrix was then washed with 10 column volumes each of buffer 1 (10 mM Tris-HCl, pH 7.5), buffer 2 (10 mM Tris-HCl, pH 7.5/0.5 M NaCl) and buffer 3 (100 mM glycine/10 mM Tris-HCl/0.5 M NaCl, pH 2.5). After equilibration of the column in buffer 1, the heat inactivated serum (20 minutes, 56°C), diluted 1:4 in buffer 1, was loaded onto the affinity matrix. The loading step was repeated three more times. The affinity matrix was then washed with 20 volumes each of buffers 1 and 2. Antibodies were eluted with 10 column volumes of buffer 3. Neutralization of approximately 18 ml of eluate with 2 ml of 1 M Tris-HCl, pH 8, was followed by dialysis against phosphate-buffered saline (PBS). Antibodies were then concentrated with Centriprep Concentrators (Amicon, Beverly, MA). Bovine serum albumin (BSA) was added (final concentration 0.1%) and the affinity-purified antibodies were stored at −20°C.

Western blotting

Whole skin extracts from newborn mice were prepared by pulverizing skin in liquid nitrogen then incubating in 2× Laemmli buffer (Bio-Rad Laboratories) for 10 minutes at 100°C. Immunoblotting was performed as previously described (Koch et al., 1997). The following primary antibodies were used: mouse monoclonal anti-desmoglein antibody (DG3.10, Biodesign, Kennebunk, ME; specific for Dsg1 and Dsg2) (Koch et al., 1990, 1991) and rabbit anti-Dsg3 (immunoaffinity-purified, see above).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was sectioned (4 μm thick) and tissue sections were subsequently deparaffinized by incubations in xylene and in ethanol (2× 5 minutes in each solvent). The tissue was then microwaved in ‘target unmasking fluid’ (TUF; Signet, Dedham, MA) for 4.5 minutes at 900 W. After several PBS washes, the tissue was incubated in 0.1% trypsin/PBS for 10 minutes at 41°C and then again washed in PBS. After an incubation of 7 minutes in 3% hydrogen peroxide solution and washing with 20% ethanol, tissues were incubated for 5 minutes in blocking buffer (1% normal goat serum/1% BSA/PBS). The first antibody was diluted in blocking buffer and incubated with the tissues for 2 hours at room temperature or overnight at 4°C. After three washes in PBS (5 minutes each), biotinylated goat anti-rabbit IgG or biotinylated goat anti-mouse IgG (Vector Laboratories, Burlingame, CA; diluted 1:200 in blocking buffer) was added and the sections were incubated for 1 hour. After three PBS washes (5 minutes each), the sections were incubated with ‘Vectastain’ (Vector Laboratories) for 30 minutes and then washed with PBS (3× 5 minutes). Antibody binding was detected with ‘Stable DAB’ (Research Genetics, Huntsville, AL). The DAB reactions were stopped by rinsing the slides several times in water. After counterstaining with hematoxylin (Gill’s Formulation #3, 1:10 diluted in water; Fisher Scientific, Pittsburgh, PA), sections were dehydrated in ethanol and, finally, coverslips were applied with Elvanol. The following primary antibodies were used: rabbit anti-mouse Dsg3 (3.9 μg/ml, immunoaffinity-purified, see above) and mouse monoclonal anti-human Dsg3 antibody 5G11 (generous gift of Dr Margaret Wheelock, University of Toledo, OH).

Histology and electron microscopy

Histology and electron microscopy were performed as described (Lavker et al., 1991).

RESULTS

Analysis of timing and pattern of clinical hair loss in DSG3 knockout mice suggests loss of telogen hair

In both DSG3−/− mice and their littermates, hair appeared at the same time after birth and at day 15 the coats were indistinguishable, indicating that the initial hair growth
Desmoglein 3 in telogen hair

(‘follicular neogenesis’) in DSG3−/− mice was clinically normal (Fig. 1A). As hairs are synchronized in anagen in this initial hair growth, this finding suggested that anagen hairs might be normal in DSG3 knockout mice. However, at around day 20 after birth, DSG3−/− mice started losing their coat. This hair loss occurred whether the animals were housed individually or in groups. While most animals showed signs of hair loss 23 to 25 days after birth, some mutants lost hair as early as 16-18 days after birth. The hair loss started on the forehead and proceeded towards the back of the animals (Fig. 1B,C). Previous analysis of the hair cycle in mice has shown that the early hair cycles are synchronized along the dorsal body axis, in that hair goes from the first anagen into telogen at about 18-20 days and that this transition proceeds from the head to the back (Dry, 1926; Chase, 1954). The timing and pattern of hair loss in DSG3−/− mice, therefore, suggested some defect in the telogen hair anchorage. Although the DSG3−/− mice were also losing hair on the ventral site, this process did not seem to proceed along the body axis, perhaps because the head to tail synchronization of hair growth is more apparent dorsally.

Most telogen hairs of normal mice are initially retained and remain firmly anchored when new hair growth (anagen) is initiated (for a detailed discussion see Dry, 1926). It is therefore not unusual to see telogen club hairs retained in a follicle together with a growing anagen hair (see example in Fig. 2B). This retention and anchorage of telogen hair prevents bald skin areas due to loss of telogen hair before anagen hair has a chance to replace it. Conversely, premature loss of telogen hair, as in these mutant mice, will result in bald areas.

One week after the hair loss was first visible, most of the head and at least part of the back were bald (Fig. 1B,C). This finding clearly indicated that all truncal hair types were affected by the DSG3−/− mutation. (For a description of the different types of mouse hairs see (Dry, 1926; Sundberg and Hogan, 1994).) One to two weeks later, hair regrowth was visible on the head. Individual variations of this pattern were observed. For example, hair regrowth on the head was sometimes seen before total hair loss occurred on the back (Fig. 2D). Three to four weeks after the initial hair loss, most animals showed a normal coat (data not shown).

The DSG3 knockout mice went through at least two complete cycles of hair loss and regrowth in a dorsal to ventral pattern. In older animals, hair was lost and regrown in a mosaic (patchy) pattern, i.e. the head to tail pattern was lost (Fig. 1E). This pattern of hair loss correlates with the finding that in older normal mice hair cycles from anagen to telogen in random patches (Dry, 1926; Chase, 1954). Thus, these bald patches in the DSG3−/− mice are consistent with premature loss of hair in those patches in telogen before the anagen hair has replaced it.

To confirm loose anchorage of telogen hair in these knockout mice we devised a ‘hair pull’ test. A small (approx. 1 cm) piece of adhesive tape was placed on the hair of the back, just distal.

Fig. 1. Different stages of hair loss in DSG3−/− mice. (A) 15 day old DSG3−/− mouse with a normal coat. (B) About one week after the initial hair loss, the head and the neck are bald (31 day old mouse). (C,D) Two to three weeks after the initial hair loss, a new wave of hair regrowth is visible (C, 36 day old mouse; D, 37 day old mouse). The dark backskin in C indicates that the hair follicles are in anagen. Short agouti hair are seen on the back of the mouse in C as well. Note the pronounced hair growth on the forehead of D. As exemplified by C and D, individual variations in the timing and extent of the initial hairloss and the regrowth of hair were observed. (E) Older mice (3 month old mouse) lose and regrow hair in patches. (F,G) By applying adhesive tape to the back followed by a gentle pull in the direction of hair growth, lose anchoring of hair in mutant mice can easily be demonstrated (F, 23 day old DSG3−/− mouse; G, 20 day old DSG3+/+ control littermate; both mice were in telogen as confirmed by histology). (H) The hair on the adhesive tape shown in F is telogen club hair. Note that the hair club (cl) is surrounded by at least one cell layer derived from the club sheath.
to the neck, and peeled off in the direction of hair growth (i.e. front to back). Few or no hairs were found on tape from either DSG3−/− animals or their DSG3+/− and +/+ littermates in the first anagen hair growth cycle (13 day old mice), showing that anagen hairs were well anchored. In contrast, starting on day 18, when hair follicles on the dorsum had just gone into telogen (as confirmed by histology), clumps of hair came out in DSG3−/− mice but few, if any, hairs came out in their +/+ and +/+ littermates (Fig. 1F,G). This result in normal mice is expected because, as discussed above, mice must retain telogen hairs until anagen hairs replace them to prevent bald areas from forming. Microscopic examination of the hair pulled from DSG3−/− mice showed telogen hairs with characteristic clubs (Fig. 1H).

**Histology and ultrastructure of hair loss in DSG3−/− mice show loss of telogen hairs due to an adhesion defect around the hair clubs**

We next evaluated the skin histology of DSG3−/− and control mice (DSG3+/−, DSG3+/+) at various stages of the first hair cycle. We did not observe any consistent differences between knockout and control mouse hair follicles in the anagen phase of the hair growth cycle (Fig. 2B). However, in early telogen, hair follicles of the DSG3−/− mice showed separation between the cells surrounding the telogen hair club and the ORS, i.e. within the ‘club sheath’ (Dry, 1926) (Fig. 2A,C,F). This histology was reminiscent of the PV-like lesions seen in mucous membranes and traumatized skin of DSG3−/− mice, where clefts were initially formed between the basal keratinocyte cell layer and the first suprabasal cell layer (Koch et al., 1997). These clefts in the telogen hair follicles were never observed in control mice (Fig. 2E). Histology of bald skin areas revealed empty, dilated telogen hair follicles, consistent with the loss of the telogen hairs. However, the ORS epithelium was preserved (Fig. 2D). The histopathology described above was also observed in older mice that underwent phases of hair loss.

To determine whether abnormal desmosome function was associated with the loss of telogen hairs in DSG3 knockout mice, we performed electron microscopy of hair follicles in early telogen. Cells from the base and roof of lesions within the club sheath showed separation at their plasma membranes (Fig. 3A). In some cases, ‘half-desmosomes’ with attached cytokeratin filaments were seen within the cytoplasm,
suggesive of internalization after the desmosomes split (Fig. 3B,C). As reported previously, these half-desmosomes were also found in suprabasilar lesions in mucous membranes of DSG3−/− mice (Koch et al., 1997). We also noted a reduction in the number of desmosomes that were formed between the epithelial cell layers surrounding the DSG3−/− telogen hair clubs when compared to wild-type controls (Fig. 4). This observation is consistent with the previous observation that the basal layer of DSG3−/− mucous membranes may have fewer desmosomes (Koch et al., 1997). In addition to a reduction in desmosomes, the cell surface of epithelial cells surrounding the DSG3−/− telogen club hair was organized into numerous microvillus extensions that protruded into the enlarged intercellular spaces. Telogen follicles from control mice consisted of round to cuboidal keratinocytes, closely apposed to each other with a normal complement of desmosomes. Taken together these ultrastructural findings are consistent with a defect in cell-cell adhesion within the telogen club sheath.

Finally, sequential skin histology of DSG3−/− and control littermate skin indicated that the initiation of the second anagen (anagen II), which usually occurred at around day 22 to 23 after birth in the skin of the back of control mice, was delayed in DSG3−/− mice (Table 1, compare Fig. 2A and B). In some animals, anagen was delayed up to 8 days.

**Localization of Dsg3 in mouse telogen hair follicles**

Because Dsg3 seemed to have an important function in the telogen hair follicle (especially the club), we wanted to determine its localization. As no antibody was available that was specific for mouse Dsg3, we raised rabbit antisera against a His-fusion protein containing the mouse Dsg3 fifth extracellular domain (EC5) (Amagai et al., 1991), an area that...
shows little homology among desmogleins. The antisera was affinity purified on the recombinant fusion protein that was used for immunization (see Materials and Methods). Specificity of this antibody was demonstrated on immunoblotting of newborn mouse skin extracts. The antibodies recognized the 130 kDa Dsg3 from DSG3+/+ but not DSG3−/− skin, whereas they did not recognize Dsg1 or Dsg2 from either skin extract (Fig. 5A).

The anti-Dsg3 antibody showed intense immunoperoxidase staining of the cell surface of keratinocytes surrounding the mouse telogen hair club (Fig. 5B). The basal layer of the ORS at the hair club showed cell surface staining, most intensely between the apex of the cells and the cells surrounding the club (exactly where the split occurs, arrow in Fig. 5B). There was less intense staining between the lateral margins of the ORS basal cells (arrowhead in Fig. 5B) and no staining at the basement membrane. DSG3−/− mice showed no staining at all (data not shown). These data correlate the localization and function of Dsg3 in the telogen hair follicle.

Dsg3 in human telogen hair follicles and loss of hair in pemphigus vulgaris
Immunoperoxidase staining of human telogen hair follicles with a monoclonal antibody against human Dsg3 showed similar staining to that in the mouse, described above (Fig. 6A). The keratinocytes surrounding the hair club were intensely stained. The lateral margin of the basal cells of the ORS was less intensely stained, however, their apices showed a strong reaction.

Because PV is a blistering disease mediated by autoantibodies against Dsg3 in which the mucous membrane and skin phenotype is similar to DSG3−/− mice, we speculated that there might be telogen hair loss in these patients. As opposed to mice in which essentially all hair in a patch are synchronized in the hair cycle, adult human hair growth is asynchronous with only a small fraction of the total scalp hair follicles in telogen. Therefore, telogen hair loss may not be an obvious clinical phenotype in pemphigus patients. In addition
there are not many scalp biopsies from pemphigus patients. Nevertheless, we did find histological evidence for telogen hair loss in one patient. In this case acantholysis extended from the epidermis down the entire ORS of mostly empty dilated hair follicles that contained only small residual unanchored hair shafts and necrotic detached cells (Fig. 6B). Similar findings of acantholysis extending down the ORS of hair follicles have been reported, although hair loss has not been commented upon (Ackerman, 1978). Therefore, even though hair loss is not a major phenotype in pemphigus patients, there are abnormalities in the hair follicles, consistent with a role for Dsg3 in the structure and function of human hair.

**DISCUSSION**

The data in this report demonstrate the importance of Dsg3, a desmosomal cadherin, in the structure and function of hair.

On the dorsum of the mouse, hair follicles in any particular area cycle in a synchronous fashion and enter telogen at approximately the same time (Dry, 1926; Chase, 1954). It is therefore not surprising that the telogen defect in our DSG3−/− mice resulted in a dramatic loss of hair in a large area of the coat following a defined pattern.

In the absence of Dsg3, as soon as hair follicles enter telogen, a defect in adhesion between the cells surrounding the club sheath of telogen hair follicles in DSG3−/− mice. 1 One DSG3+/+, one DSG3+/−; 2 two DSG3+/+, one DSG3+/−; 4 two DSG3+/−; 5 one DSG3+/−; mt, mutant (DSG3−/−); ct, control (DSG3+/+, DSG3+/−).
mouse hair club and the basal cells of the ORS, i.e. within the club sheath, causes loss of anchorage of the club hair to its follicle. Electron microscopy of the affected area demonstrates ‘half-desmosomes’ and widened intercellular spaces, suggesting that although desmosomes form, their adhesive function is abnormal. Furthermore, the number of desmosomes within the telogen club sheaths of DSG3−/− mice is reduced, contributing to the fragility of this tissue. We have previously reported analogous histologic and ultrastructural findings in studies of the anancylosis that occurs in the stratified squamous epithelium of epidermis, oral and vaginal mucous membrane of these DSG3 knockout mice (Koch et al., 1997).

Consistent with these findings suggesting a role for Dsg3 in anchoring the telogen hair club to the ORS, we demonstrate intense immunoperoxidase staining for Dsg3 in normal mice in this area, analogous to the localization of Dsg3 in the basal and immediate suprabasal layers of epidermis, where suprabasilar acantholysis occurs in DSG3−/− mice.

In spite of this dramatic telogen hair loss in DSG3−/− mice, the first anagen hair cycle is normal in both growth and anchorage, demonstrating that the absence of Dsg3 is, in principle, compatible with hair growth and that anagen hair has alternative or additional anchorage mechanisms compared to telogen hair. Although subsequent hair cycles may be delayed, the bald skin areas are still capable of growing new hair, thus showing that the stem cells and follicular papillae, which are required for hair growth, retain their functions (Cotsarelis et al., 1990). We did not detect any defects in anagen hairs in any subsequent hair cycles either. As we could identify acantholysis only in telogen hair, the defect seems limited to that part of the hair cycle and is not one of loss of anagen hair causing the follicle to enter telogen.

The reason for the delay of the second anagen in DSG3−/− mice is unclear. However, it has been reported that malnutrition can delay or even prevent the initiation of anagen in mice (Chase, 1954) (see also the description of ‘badly grown’ mice can delay or even prevent the initiation of anagen in mice is unclear. However, it has been reported that malnutrition is associated with the alopecia of mice). Malnutrition is generally recognized to cause delays in the initiation of anagen in mice.

Montagutelli and colleagues recently described a mouse mutation (balPAS) with a hair phenotype similar to that of DSG3 knockout mice (Montagutelli et al., 1997). Although these authors did not determine the defect in these mice at the molecular level, they did state that this mutation is coallelic with the balding mutation (bal); Davison et al., 1994; Montagutelli et al., 1997; Sundberg, 1994). We have recently shown that the latter mutation (bal) represents a spontaneous DSG3−/− mutation (Koch et al., 1997). The predicted amino acid sequence encoded by the mutated DSG3 gene of the balPAS mouse lacks most of the cytoplasmic sequences of the wildtype protein due to a nucleotide insertion in exon 14 that causes a frame-shift and premature stop codon. Consequently, no Dsg3 protein was detected on the cell surface of keratinocytes in mice homozygous for the balPAS mutation (Koch et al., 1997). Due to its coallelism with the balPAS mutation, it can be assumed that the balPAS mutation prevents the synthesis of a functional Dsg3. Montagutelli et al. (1997) attributed the hair loss of balPAS mice to a defect in the anagen hair follicle, in particular to cleft formation between the outer and inner root sheath cell layers. These findings are fundamentally different from the ones reported in this paper because the pathology in DSG3−/− mice is seen both at a different stage of the hair cycle and in a different area of the follicle. We did not find any gross or histological indication for lesions in the anagen hair of DSG3−/− mice. If there is indeed a difference with respect to the pathology in balPAS and DSG3−/− mice, this difference could be attributed either to the as yet undefined nature of the balPAS mutation or the genetic background of the two mouse lines. Our mice were on a segregating Sv129 and C57BI/6 background, whereas balPAS was congenic to the 129/SvPas inbred strain.

The finding of lesions within mouse hair follicles due to the absence of Dsg3 prompted us to see whether similar lesions were present in PV patients. First we showed that, as in mice, intense staining for Dsg3 occurs in the cells surrounding the telogen hair club. Based on the hypothesis that PV results from a loss of Dsg3 function caused by Dsg3-specific autoantibodies, one might expect to find hair pathology in patients. However, in contrast to the synchronized hair growth seen in mice, in adult humans hair follicles cycle independently from each other and only about 5-20% of follicles on the scalp are in telogen (Kligman, 1961; Courtois et al., 1996). Therefore any clinical phenotype in PV would be subtle if only telogen hair were affected. In one biopsy from a PV patient, we were able to demonstrate loss of the hair from follicles. However, because we had only one biopsy showing the appropriate hair follicles, more extensive studies will be necessary to determine to what extent hair loss occurs in these patients and which part of the hair growth cycle is affected.

The results presented in this paper are a first step in starting to dissect out the function of desmosomes and their components in the structure and function of mammalian hair.

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Desmoglein 3 in telogen hair


