



CORRESPONDENCE

Pseudo Pemphigus Phenotypes in Mice with Inactivated Desmoglein 3

Further Insight to the Complexity of Pemphigus Pathophysiology

To the Editor-in-Chief:



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Results of the study of Kountikov et al¹ mapped the spontaneous mutation squeaky (sqk) to a partial exon deletion in the *Dsg3* gene, which resulted in hypomorphic expression of a truncated desmoglein 3 (Dsg3) protein. The phenotype of *Dsg3*^{sqk/sqk} mice included a runted appearance, cyclic hair loss, obstructed airways, and severe immunodeficiency. The primary mucocutaneous pathology was limited to deep ulcers of the tongue. However, no evidence for acantholysis or separation of the suprabasal epithelium—the histopathological features of the autoimmune blistering disease pemphigus vulgaris (PV)—was observed. The current results, in conjunction with previous animal studies, again failed to substantiate the hypothesis that Dsg3 is the principal desmosomal cadherin that holds keratinocytes together.

Further, there is a lack of convincing evidence in literature supporting the hypothesis that intraepidermal blistering and sloughing of the suprabasal epithelium, leading to the death of untreated PV patients, results solely from inactivation of Dsg3. Nevertheless, Kountikov et al¹ described the mucocutaneous changes in *Dsg3*^{sqk/sqk} mice as representing a pemphigus phenotype. All previous efforts to induce the PV phenotype in mice were driven by the expectation that functional inactivation of Dsg3, due to mutation or antibody action, would result in mucocutaneous blistering. In fact, as evidenced by findings presented by others (Table 1),^{2–5} none of the reported mouse models of Dsg3 inactivation demonstrate overt blistering, which is the indispensable feature of the

PV phenotype. Unfortunately, in most instances, the findings are over interpreted to satisfy expectations, thus erroneously supporting the purported exclusive role of Dsg3.

Development of animal models of PV is an exciting and important area of research requiring detailed phenotype description. In this regard, we direct attention to certain inconsistencies in the report of Kountikov et al.¹ The report states that *Dsg3*^{sqk/sqk} mice developed oral lesions similar to those described in *Dsg3*^{−/−} mice and in humans with PV as well as erosions of the snout, but no supporting data were shown. The authors described the ulcerative tongue lesions in *Dsg3*^{sqk/sqk} mice as resulting from a loss of suprabasilar epithelium characteristic of PV. In marked contrast, Figure 6K¹ shows a banal ulcer lacking the entire epithelium, as in aphthae. Although the authors acknowledged that a cyclical hair loss pattern seen in *Dsg3*^{sqk/sqk} mice does not occur in PV, they erroneously concluded that alopecia is observed frequently in PV patients. The statement that *Dsg3*^{−/−} mice develop widespread blisters is also inaccurate. As evidenced by other original research (Table 1),^{2–5} the spontaneous mucocutaneous blisters in these mice are either absent or small in size and limited in number, and, in the most instances, were induced by trauma by presumed analogy with Nikolskiy sign—a hallmark of PV.

Importantly, it should be clarified that neonatal *Dsg3*^{−/−} mice can develop true PV phenotype featuring widespread blistering only after passive transfer of PVIGs,⁶ thus indicating that certain species of non-Dsg3 developed by PV patients⁷ are also pathogenic. These include anti-desmocollin 3 antibody,⁸ because *Dsc3*^{fl/fl}/K14-Cre mice develop the true PV phenotype despite the presence

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Table 1 The Pseudo Pemphigus Phenotypes in Mice with Inactivated Dsg3

Study	Dsg3 inactivation mouse model	Phenotype
Kountikov et al ¹	<i>Dsg3^{sqk/sqk}</i> mice expressing hypomorphic Dsg3	Distinct squeaking sound, runting, obstructed airways, cyclic hair loss, malnutrition, severe immunodeficiency, and tongue ulcerations. Hyperplasia of the epiglottis resulting in its deformation and thickening, and inflammation and thickening of the epidermis.
Sundberg et al ²	<i>Dsg3^{bal}</i> , <i>Dsg3^{bal-Pas}</i> , and <i>Dsg3^{tm15tan}</i> mice	All three mutated Dsg3 alleles have similar clinical and histological features, such as runting, transient loss of long (pelage) hair on the head and trunk with preserved vibrissae, small inflammatory vesicles with ulceration on footpads and within the oral cavity, and some crusted lesions around the eyes, on the snout, and on the nipples of a nursing mother, resulting presumably from trauma. Dystrophic pelage hair fiber breaking at the cutaneous surface, separation of epithelial layers in hair follicles around the inner layer of the outer root sheath, and short stretches of suprabasal epidermal acantholysis in the tongue, eyelids, and footpads.
Allen et al ³	NH ₂ -terminally truncated Dsg3	Swelling of paws, skin flakiness on the back, and blackening of tail tip. Increased skin thickness, enlarged intercellular spaces in epidermis, and peculiar intercellular junctions with aberrant desmosomes.
Aoki-Ota et al ⁴	C57BL/6J <i>Rag2</i> ^{−/−} mice with adoptively transferred splenocytes from either naive <i>Dsg3</i> ^{−/−} mice or <i>Dsg3</i> ^{−/−} mice immunized with the recombinant extracellular domain of mouse Dsg3	Both models feature patchy hair loss and weight loss. ~30% of mice also develops crusted erosions on the skin around the snout, mouth, and eyes, and on the footpads. Areas of suprabasilar acantholysis are seen in the epithelium lining of hard palate.
Nishimura ⁵	FcγRIIb-deficient mice immunized with human recombinant Dsg3	Tiny erosions on the paw, which generally expand into scab formation on the entire paw. Some mice develop tiny vesicles, pustules, and erosion on the tail. Erosions also occur in the skin of the mammary gland, and a solitary ulcer — on the back. Epidermal thickening and extensive edema beneath epidermis with massive infiltration of inflammatory cells, including lymphocytes, neutrophils, and mast cells, microabscesses, and some areas of ulceration.
Nishimura ⁵	The MRL <i>lpr/lpr</i> mice (model for systemic lupus erythematosus) spontaneously producing anti-Dsg3 antibodies	Skin lesions were described as hair loss and scab formation. Hyperkeratosis, acanthosis, hypergranulosis, liquefaction, vasodilation, and T-cell infiltration into the dermis.

of functional Dsg3.⁹ Most recently, it has been demonstrated that cutaneous blisters in PV patients can be associated with the anti-Dsg3 antibody that cannot induce keratinocyte separation *in vitro*,¹⁰ indicating that non-Dsg antibodies are required to overcome the epidermal cohesion. Unfortunately, as discussed in detail elsewhere,¹¹ recognition of the pathogenic role of non-Dsg antibodies in PV is hampered by interpretation of results of the experiments conducted by Amagai et al.¹² Although the absorption of PVIgGs with the extracellular epitope of Dsg3 did not eliminate the acantholytic activity, chimeric proteins containing the extracellular domain of Dsg3 combined with the Fc-portion of human IgG₁ abrogated acantholytic blistering.¹² However, the Dsg3/Fc-IgG construct was not uniquely specific to the Dsg3 antigen, because eluted PVIgGs reacted with multiple proteins.¹³ Thus, the studies with chimeric Dsg proteins gave rise to the notion of the exclusive role of anti-Dsg antibodies in pemphigus. These findings, however, were flawed as

the pathogenic non-Dsg antibodies were adsorbed out nonspecifically.

In conclusion, after discovery of autoimmunity to Dsg3 in PV approximately 25 years ago, pemphigus research has been dominated by studies focused on the monopathogenic theory of the disease. The fact that mouse models of Dsg3 deficiency repeatedly lack the true pemphigus phenotype provides additional support of the multipathogenic theory and necessitates further investigations.

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Authors' Reply



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We are grateful that Drs. Grando and Pittelkow¹ have chosen to highlight our recent paper in their Letter to the Editor, where they argue that mouse models of desmoglein 3 (Dsg3) deficiency lack the phenotype of human pemphigus, thereby providing support for a multigenic theory of pemphigus. Our

studies focused exclusively on the genetic mapping and phenotype of a spontaneous mouse mutation within the *Dsg3* gene that resulted in a unique squeaky phenotype characterized by immunodeficiency and a wasting disease.² Squeaky mice share phenotypic similarities with Dsg3-deficient mice, but they also exhibit unique characteristics due to their hypomorphic expression of a truncated Dsg3 protein. Nonetheless, pemphigus results from autoantibodies, not congenital genetic alterations in their target molecules.

We appreciate this opportunity to reiterate several key points of our paper to diminish any possible over- or misinterpretation of the data presented. Squeaky mice have a dramatic phenotype that includes severe immunodeficiency and inspiratory stridor (squeaking) due to primary pathological changes within the larynx and airway obstruction. Hallmarks of pathology include significant hyperplasia of the epiglottis that results in its thickening and deformation. This pathology extends to lesions on the back of the tongue as described for Dsg3-deficient mice.³ There is no evidence of acantholysis in the tongue or other sites. Thereby, hypomorphic Dsg3 protein expression may support intercellular adhesion and prevent spontaneous acantholysis in *Dsg3^{sqk/sqk}* mice, but it appears insufficient to prevent epithelial sloughing and lesions resulting from mechanical damage or stresses, such as chewing on solid food or snout abrasions. The histology of these oral lesions and snout erosions was substantiated in Figure 6.² We acknowledge that we incorrectly stated that alopecia is frequently observed in pemphigus patients in our manuscript, which should have read infrequently, as indicated in the cited reference.⁴ These mice have been made available to the research community through the Mutant Mouse Resource and Research Center at the University of North Carolina, Chapel Hill (<http://www.mmrrc.org>).

In their letter, Drs. Grando and Pittelkow claim that our results, in conjunction with previous animal studies, failed to substantiate that Dsg3 is the principal desmosomal cadherin that holds keratinocytes together. Even though this issue was not investigated in our studies, and we made no claim that Dsg3 is the principal cadherin that holds keratinocytes together, it is accepted that the underlying intraepithelial blister formation of pemphigus is caused by IgG autoantibodies against the desmosomal adhesion proteins expressed on epidermal keratinocytes, Dsg3, and/or Dsg1.^{5–7} A pathogenic role for anti-Dsg IgG has also been established by the injection of patients' sera or affinity-purified IgG from pemphigus sera into neonatal mice, which reproduces the immune pathology and clinical symptoms of pemphigus.⁸ Disease activity in most patients is also closely correlated with serum levels of Dsg reactive antibodies.⁹ Although anti-Dsg1 and/or Dsg3 autoantibodies are found in more than 90% of pemphigus patients, autoantibodies reactive with desmocollins, envoplakin, periplakin, A2ML1, desmoplakins I and II, and plectin may also be present in some rare forms of the disease.⁷ Given the strong association between Dsg3 and pemphigus, it is to be expected that we would highlight the

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