3. Allen E, Yu QC, Fuchs E: Mice expressing a mutant desmosomal cadherin exhibit abnormalities in desmosomes, proliferation, and epidermal differentiation. J Cell Biol 1996, 133:1367–1382
4. Aoki-Ota M, Tsunoda K, Ota T, Iwasaki T, Koyasu S, Amagai M, Nishikawa T: A mouse model of pemphigus vulgaris by adoptive transfer of naive splenocytes from desmoglein 3 knockout mice. Br J Dermatol 2004, 151:346–354
5. Nishimura H, Strominger JL: Involvement of a tissue-specific autoantibody in skin disorders of murine systemic lupus erythematosus and autoinflammatory diseases. Proc Natl Acad Sci U S A 2006, 103:3292–3297
6. Nguyen VT, Lee TX, Ndoye A, Shultz LD, Pittelkow MR, Dahl MV, Lynch PJ, Grando SA: The pathophysiological significance of non-desmoglein targets of pemphigus autoimmunity. Pemphigus vulgaris and foliaceus patients develop antibodies against keratinocyte cholinergic receptors. Arch Dermatol 1998, 134:971–980
7. Kalantari-Dehaghi M, Anhalt GJ, Camilleri MJ, Chernyavsky AI, Chun S, Felgner PL, Jasinskas A, Leiferman KM, Liang L, Marchenko S, Nakajima-Sasaki R, Pittelkow MR, Zone JJ, Grando SA: Pemphigus vulgaris autoantibody profiling by proteomic technique. PLoS One 2013, 8:e57587
8. Dmochowski M, Hashimoto T, Chidgey MAJ, Yue KKM, Wilkinson RW, Nishikawa T, Garrod DR: Demonstration of antibodies to bovine desmocollin isoforms in certain pemphigus sera. Br J Dermatol 1995, 133:519–525
9. Chen J, Den Z, Koch PJ: Loss of desmocollin 3 in mice leads to epidermal blistering. J Cell Sci 2008, 121:2844–2849
10. Saleh MA, Hashimoto R, Kase Y, Amagai M, Yamagami J: Low pathogenicity of anti-desmoglein 3 immunoglobulin G autoantibodies contributes to the atypical clinical phenotypes in pemphigus. J Dermatol 2015, 42:685–689
11. Grando SA: Pemphigus autoimmunity: hypotheses and realities. Autoimmunity 2012, 45:7–35
12. Amagai M, Hashimoto T, Shimizu N, Nishikawa T: Absorption of pathogenic autoantibodies by the extracellular domain of pemphigus vulgaris antigen (Dsg3) produced by baculovirus. J Clin Invest 1994, 94:59–67
13. Nguyen VT, Ndoye A, Shultz LD, Pittelkow MR, Grando SA: Antibodies against keratinocyte antigens other than desmogleins 1 and 3 can induce pemphigus vulgaris-like lesions. J Clin Invest 2000, 106:1467–1479

http://dx.doi.org/10.1016/j.ajpath.2015.06.023

Authors' Reply

Evgueni Kountikov, Yasuhiro Fujisawa, and Thomas F. Tedder

From the Department of Immunology, Duke University Medical Center, Durham, North Carolina

We are grateful that Drs. Grando and Pittelkow[1] 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

Disclosures: None declared.

Address correspondence to Thomas F. Tedder, Ph.D., Box 3010, Department of Immunology, Room 353, Jones Building, 207 Research Dr, Duke University Medical Center, Durham, NC 27710. E-mail: thomas.tedder@duke.edu.

importance of Dsg3 autoantibodies and pemphigus in our publication.

We agree that the development of animal models of pemphigus is an important area of research. However, the pathology of pemphigus is strongly, if not exclusively, associated with the development of anti-cadherin autoantibodies that block keratinocyte intercellular adhesions. Thus, it should be expected that mice genetically deficient in cadherins would not faithfully recapitulate the manifestations of pemphigus autoimmune disease in the absence of pathogenic autoantibody formation, as this is not the root cause of the disease in humans. Instead, genetic alterations in cadherin structure, expression, and function will likely result in diverse manifestations as is documented in our publication and within the published literature. Therefore, the development of animal models of pemphigus must unquestionably require the study of pathogenic autoantibodies, not the study of congenital genetic alterations of their target adhesion molecules. The future study of autoantibodies of differing target molecule specificities in mouse pemphigus models will determine whether antibody binding to cadherins other than Dsg3 alone or in combination can induce pathology that echoes the manifestations of pemphigus in patients as hypothesized by Grando and Pittelkow.[1]

Acknowledgments

We thank Laura Ostendorff for editorial assistance and Drs. Masayuki Amagai and Russell Hall for valuable comments and input.

References

1. Grando SA, Pittelkow MR: Pseudo pemphigus phenotypes in mice with inactivated desmoglein 3: further insight to the complexity of pemphigus pathophysiology. Am J Pathol 2015, [Epub ahead of print], doi:10.1016/j.ajpath.2015.06.023
2. Kountikov EI, Poe JC, Maclver NJ, Rathmell JC, Tedder TF: A spontaneous deletion within the desmoglein 3 extracellular domain of mice results in hypomorphic protein expression, immunodeficiency, and a wasting disease phenotype. Am J Pathol 2015, 185:617−630
3. Koch PJ, Mahoney MG, Ishikawa H, Pulkkinen L, Uitto J, Shultz L, Murphy GF, Whitaker-Menezes D, Stanley JR: Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. J Cell Biol 1997, 137:1091−1102
4. Veraitch O, Ohyama M, Yamagami J, Amagai M: Alopecia as a rare but distinct manifestation of pemphigus vulgaris. J Eur Acad Dermatol Venerol 2013, 27:86−91
5. Amagai M, Klaus-Kovtun V, Stanley JR: Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. Cell 1991, 67:869−877
6. Kershenovich R, Hodak E, Mimouni D: Diagnosis and classification of pemphigus and bullous pemphigoid. Autoimmun Rev 2014, 13:477−481
7. Hertl M, Jedlickova H, Karpati S, Marinovic B, Uzun S, Yayli S, Mimouni D, Borradori L, Feliciani C, Ioannides D, Joly P, Kowalewski C, Zambruno G, Zillikens D, Jonkman MF: Pemphigus. S2 Guideline for diagnosis and treatment−guided by the European Dermatology Forum (EDF) in cooperation with the European Academy of Dermatology and Venereology (EADV). J Eur Acad Dermatol Venerol 2015, 29:405−414
8. Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA: Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. N Engl J Med 1982, 306:1189−1196
9. Stanley JR, Nishikawa T, Diaz LA, Amagai M: Pemphigus: is there another half of the story? J Invest Dermatol 2001, 116:489−490

http://dx.doi.org/10.1016/j.ajpath.2015.09.002