Autoimmune blistering diseases are examples of autoantibody-mediated, organ-specific autoimmune disorders. Based on a genetic susceptibility, such as a strong HLA-class II association, as yet unknown triggering factors induce the formation of circulating and tissue-bound autoantibodies that are mainly directed against adhesion structures of the skin and mucous membranes. Compared with other autoimmune diseases, especially systemic disorders, the pathogenicity of autoimmune blistering diseases is relatively well described. Several animal models of autoimmune blistering diseases have been established that helped to uncover the immunological and molecular mechanisms underlying the blistering phenotypes. Each in vivo model focuses on specific aspects of the autoimmune cascade, from loss of immunological tolerance on the level of T and B cells to the pathogenic effects of autoantibodies upon binding to their target autoantigen. We discuss current mouse models of autoimmune blistering diseases, including models of pemphigus vulgaris, bullous pemphigoid, epidermolysis bullosa acquisita, and dermatitis herpetiformis.
disease-specific autoantigens in the human skin, resulting in painful blisters of the skin and/or mucous membranes. Several mouse models of AIBD have been generated, allowing researchers to investigate key pathophysiological mechanisms.

These models are either passive, based on the transfer of previously generated autoantibodies into mice to generate a blistering phenotype in vivo, or active, based on immunization of wild-type or genetically modified mice with the
autoantigen to induce an autoimmune response (see Iwata et al., 2015 for a comprehensive review).

In this article we will describe current active mouse models for AIBD that use immunization of wild-type or genetically modified mice (Figure 1 and Table 1). The models help to show certain key elements of disease pathogenesis as follows: (a) the loss of tolerance to self-antigens leading to the generation of autoreactive immune cells, (b) the T- and B-cell-dependent production of autoantibodies, and (c) autoantibody-dependent tissue damage. Genetic modification of mice can be defined as (i) the introduction of exogenous genes, like human autoantigens, into the genome of mice (gain of function); (ii) the knockout of endogenous genes in mice (loss of function), and (iii) the knockin of modified endogenous genes (change of function). These techniques have been described comprehensively in previous Research Techniques Made Simple articles (Griffin et al., 2015; Günschmann et al., 2014; Scharfenberger et al., 2014; Tellkamp et al., 2014).

MOUSE MODELS FOR PV

In PV, autoantibodies directed against desmogleins (Dsg3 and Dsg1) cause loss of keratinocyte adhesion, resulting in blisters and erosions of the skin and mucous membranes. In most PV patients, autoantibody titers correlate with the clinical activity, indicating a critical role of autoantibodies in disease pathogenesis. Moreover, several in vitro and in vivo studies have clearly shown the pathogenic relevance of Dsg3-reactive IgG autoantibody (Amagai and Stanley, 2012). To study the mechanisms leading to generation of pathogenic autoantibodies in PV, Amagai et al. (2000) developed an active disease model using Dsg3−/− mice (Koch et al., 1997) that lack an established self-tolerance against Dsg3. Isolated splenocytes from Dsg3-immunized or naïve Dsg3−/− mice were transferred into Rag2-immunodeficient knockout mice to generate B cells producing a panel of polyclonal Dsg3-specific antibodies. Mice inoculated with hybridoma cells producing a weakly pathogenic Dsg3-specific antibody (NAK1) did not develop an apparent PV phenotype. In contrast, when mice were inoculated with a combination of several hybridoma cells (NAK1 + NAK2 + NAK7 + NAK11) they developed a phenotype similar to PV including patchy hair loss, IgG deposition in the oral mucosa, and suprabasilar acantholysis in the skin. Scale bar = 50 μm. Adapted from Kawasaki et al. (2006) with permission from Elsevier. Dsg3, desmoglein 3; PV, pemphigus vulgaris.
monoclonal autoantibodies, AK23 could induce blister formation after passive transfer of AK23 into neonatal wild-type mouse or by inoculation of AK23-producing hybridoma cells into Rag-2\(^{-/-}\) recipient mice. AK23 recognizes a calcium-dependent conformational epitope located at the adhesive interface in the N-terminal domain of Dsg3 (Tsunoda et al., 2003), indicating that one highly potent pathogenic autoantibody alone can induce a blistering phenotype in PV. However, in another study by Kawasaki et al. a combination of several weakly pathogenic autoantibodies generated in the same model was shown to also induce a PV phenotype in mice, pointing to potential synergistic effects of polyclonal Dsg3-specific autoantibodies in disease induction (Figure 2) (Kawasaki et al., 2006).

Because PV patients show a high prevalence of distinct HLA-DRB1 alleles such as HLA-DRB1*04:02, DRB1*14:04, and DQB1*05:03, Eming et al. (2014) generated a humanized HLA-class II–transgenic mouse in which antigen presentation...
immunization of mice transgenic for a PV-unrelated HLA-molecule (HLA-DRB1*04:01) did not induce a Dsg3-specific antibody response, indicating that recognition of distinct Dsg3 peptides by CD4⁺ T cells is highly specific for certain HLA molecules that are highly prevalent in PV (Eming et al., 2014). With the same model it was recently shown that CD4⁺CD25⁺FoxP3⁺ T regulatory cells exert a down-regulatory effect on the humoral Dsg3-specific immune response, which supports the hypothesis that the Dsg3-specific CD4⁺ T-cell–dependent immune pathogenesis of PV is modulated by T regulatory cells (Schmidt et al., 2016).

### ANIMAL MODELS FOR BP
BP is a subepidermal blistering disease characterized by autoantibodies against antigens in the epidermal basement membrane, mainly type XVII collagen (COL-17)/BP180 (BP antigen I of 180 kDa) and the intracellular plakin BP230 (BP antigen I of 230 kDa). Autoantibodies from BP patients fail to recognize mouse COL-17 in passive transfer models due to of differences in the amino acid sequences between humans and mice. Therefore, humanized mouse models for BP have been used to study disease mechanisms in vivo (Nishie et al., 2007). Olasz et al. (2007) established an active BP model using transgenic mice expressing human COL-17 in the murine basement membrane. Transgenic mice were generated by crossing COL-17 knockout mice with animals expressing human COL-17 under the control of the human keratin-14 promotor, allowing tissue-specific expression of human COL-17 only in the basement membrane of transgenic mice (Olasz et al., 2007). Skin grafts from COL-17-transgenic mice were then transplanted onto syngeneic wild-type recipients to induce a strong COL-17–specific IgG response with autoantibodies able to induce subepidermal blistering in the skin graft. The model was further developed by Ujiie et al. (2010), who transferred splenocytes from human COL-17–immunized wild-type mice into immunodeficient Rag2⁻/⁻/Col17⁻/⁻ humanized recipients. In this model, the depletion of CD4⁺ T cells from the COL-17–immunized mice suppressed the production of COL-17–specific IgG antibodies, whereas the depletion of CD8⁺ T cells showed no effects, indicating that CD4⁺ T cells, but not CD8⁺ T cells, are essential for the production of antibodies against human COL-17 in the humanized BP model.

### ANIMAL MODELS FOR EBA
In EBA, autoantibodies bind to type VII collagen (COL-7), an anchoring fibril protein of the dermal-epidermal junction, leading to skin fragility and blistering of the skin and mucous membranes. Most EBA sera recognizes the noncollagenous-1 domain of COL-7. Animal models for EBA are mainly based either on the passive transfer of COL-7–specific antibodies (derived from human or other species like rabbits) or on the direct immunization of mice with the autoantigen (see Kasperekwicz et al. [2016] for review). In the active immunization-induced EBA mouse model, wild-type mice are immunized with an immunogenic peptide of the COL-7 epitope from the murine noncollagenous-1 domain. After 4–8 weeks mice start showing a phenotype similar to EBA, with subepidermal blister formation mainly located at the ears.

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**MULTIPLE CHOICE QUESTIONS**

1. Which of the following is not characteristic of all autoimmune blistering diseases?
   A. Blisters on the skin and/or mucous membranes
   B. IgG autoantibodies
   C. Autoantibodies against autoantigens in the skin
   D. Loss of self-tolerance

2. Which knockout mice are immunized with autoantigen in the active disease model of pemphigus vulgaris?
   A. Dsg1⁻/⁻ mice
   B. COL7⁻/⁻ mice
   C. Dsg3⁻/⁻ mice
   D. Dsg2⁻/⁻ mice

3. Which domain of type VII collagen is used for the immunization-induced model for epidermolysis bullosa acquisita?
   A. NC1
   B. NC2
   C. NC3
   D. NC4

4. What is/are the main autoantigen(s) in bullous pemphigoid?
   A. COL17/BP180
   B. BP230
   C. COL17/BP180 and BP230
   D. COL17/BP180 and BP250

5. Which Dsg3-specific antibody can induce a pemphigus vulgaris-resembling phenotype in wild-type mice?
   A. AK7
   B. AK47
   C. AK3
   D. AK23
snout, and around the eyes (Ludwig, 2012; Sitaru et al., 2006). This model is used to study both the initial autoimmune events in loss of self-tolerance leading to development of autoreactive COL-7–specific T and B cells and to study mechanisms of autoantibody-induced tissue damage and inflammation. For instance, Ludwig et al. (2011) showed that the induction and the severity of the EBA-like phenotype strongly depends on the mouse’s MHC haplotype, because mouse strains carrying the H2s haplotype are more prone to develop experimental EBA after COL-7 immunization and show the highest disease severity compared with other inbred mouse strains (Ludwig et al., 2011) (Figure 4).

MOUSE MODELS FOR DH

DH is a blistering skin disease strongly associated with gluten intolerance that is clinically characterized by an intensively pruritic papulosquamous rash on the skin. Immunofluorescence shows IgA deposition at the tips of the papillary dermis, and gluten-induced IgA autoantibodies are directed against transglutaminase-2 and -3 (Kárpáti, 2012). On the basis of the strong genetic association of DH with HLA-DQ2 and HLA-DQ8, Marietta et al. (2004) used autoimmune-prone non-obese diabetic mice lacking the endogenous murine MHC class II (I-As–/–) and introduced the human HLA-DQ8 transgene to establish a transgenic model in which antigen presentation to CD4+ T cells is restricted to HLA-DQ8. Blister formation, neutrophil infiltration in the dermis, and deposition of IgA antibodies at the dermal-epidermal junction were observed in 16% of HLA-DQ8–transgenic nonobese diabetic mice that were sensitized to gluten, whereas no blistering or IgA deposition was observed in non–HLA-DQ8–transgenic mice, indicating that HLA-DQ8 is required for blister formation (Marietta et al., 2004).

CONCLUSION

The current understanding of the pathophysiology of AIBDs has been greatly increased by studies that have been performed in mouse models for these disorders. The current models summarized in this article are based on the active immunization with recombinant autoantigens. The mouse models focus on various aspects of the autoimmune cascade finally resulting in the production of antibodies directed against the antigen of interest. In most cases, the mice develop a clinical phenotype resembling certain aspects of the human disease. However, so far there is no spontaneous mouse model for AIBD, which limits the significance of the established in vivo systems with regard to modeling the human situation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to this paper. Teaching slides are available as supplementary material.

REFERENCES