Research Techniques Made Simple: Mouse Models of Atopic Dermatitis

Doyoung Kim, Tetsuro Kobayashi and Keisuke Nagao

Atopic dermatitis (AD) is a common, chronic inflammatory skin disease characterized by impaired barrier function, eczematous dermatitis, and chronic pruritus. Mouse models have been heavily used to deepen our understanding of complicated disease mechanisms in AD and to provide a preclinical platform before performing clinical interventional research on novel therapeutic agents in humans. However, what aspects of human AD these mouse AD models faithfully recapitulate is insufficiently understood. We categorized mouse AD models into three groups: (i) inbred models, (ii) genetically engineered mice in which genes of interest are overexpressed or deleted in a specific cell type, and (iii) models induced by topical application of exogenous agents. To maximize benefits from current murine AD models, understanding the strengths and limitations of each model is essential when selecting a system suitable for a specific research question. We describe known and emerging AD mouse models and discuss the usefulness and pitfalls of each system.

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic inflammatory skin disease with underlying barrier impairment and is accompanied by severe pruritus and associated with type 2/22-mediated inflammation. Recent studies have begun to unveil and dissect the complex pathophysiology in AD, including the genetic basis for barrier impairment, diverse aspects of the dysregulated immune system, and the involvement of commensal microbiota, particularly Staphylococcus aureus. Numerous AD mouse models have been generated over the years, each recapitulating one or more aspects of human AD (Figure 1a). However, a considerable gap remains between what has been learned in mouse models and what information can be translated into humans. Better understanding of each AD mouse model may enable researchers to perform studies directly relevant to human AD pathogenesis and to identify or validate novel therapeutic targets. To reflect the spectrum of inflammation involved in...
classic and monogenic AD, as well as in AD mouse models, skin inflammation discussed herein is referred to as eczema-tous dermatitis.

**MOUSE MODELS OF AD**

Mouse AD models can be categorized into three groups: (i) inbred strains of mice that develop AD-like phenotypes; (ii) genetically engineered models with either ablation or over-expression of a single gene, either ubiquitously or in a certain cell lineage; and (iii) AD-like phenotypes induced by exogenous agents. Understanding the strengths and limitations of each model would allow researchers to select a system that is suitable for a particular research question and to be aware of the caveats that need be considered.

**Inbred models**

Impaired skin barrier is a fundamental component of AD pathogenesis. Genetic studies have linked several chromosomal loci or genes involved in epidermal differentiation to risk of AD. FLG mutations (a genetic cause for ichthyosis vulgaris) contribute to barrier defect and represent a major predisposing factor for AD development in humans (Brown et al., 2012; Kezic et al., 2011). The flaky tail mice (ma/ma, Flg<sup>ft/ft</sup>) harbor mutations in genes involved solely in keratinocyte homeostasis. These mice develop spontaneous eczematous dermatitis under specific pathogen-free conditions, with enhanced immune responses against percutaneous antigens (Fallon et al., 2009). Mutations in Flg and Tmem79 have been identified, the latter causing a defect in a component of lamellar granule assembly machineries, conferring both matted hair and spontaneous AD-like phenotypes (Sasaki et al., 2013; Saunders et al., 2013). Segregation of the two mutated genes determined Tmem79, but not Flg, as the causative gene mutation that drove eczematous dermatitis. Consistently, genomic ablation of Flg is not sufficient for spontaneous onset of the AD-like phenotype, either under specific pathogen-free conditions or upon S. aureus inoculation (Kobayashi et al., 2015), further indicating that at least one additional defect is required for the development of eczematous dermatitis (Kawasaki et al., 2012; Sasaki et al., 2013).

Another inbred strain is the NC/Nga mouse, in which pruritic skin lesions develop when mice are maintained under conventional housing conditions (Matsuda et al., 1997). NC/Nga mice, like the flaky tail mice, exhibit pronounced type 2 immune responses. The genetic determinant in these mice appears to be localized in chromosome 9, which includes genes involved in immunity, such as Thy1, Cd3d, Cd3e,

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**Figure 1. Examples of AD mouse models.** (a) Features of AD mouse models that may be considered for phenotype analyses. (b) Gross phenotype and histology of an 8-week-old *Adam17<sup>+/+</sup>* Sox9-Cre mouse and a littermate control. (c) Phenotype and histology of MC903-induced AD-like inflammation; 45 μmol/L of MC903 in ethanol was applied onto back skin of a C57BL/6 mouse every other day for 14 days. Scale bars = 50 μm. AD, atopic dermatitis.
Inbred models

Pros: resembles natural course of human AD; enhanced percutaneous sensitization to hapten and allergens
Cons: lack of genetic information in some strains; variable induction protocols when combined with hapten or allergen challenges; some models do not spontaneously develop dermatitis under specific pathogen-free conditions

- Flaky tail (ma/ma, Flg<sup>–/–</sup>)
  - Recapitulates barrier defect in a subset of human AD
  - Combined genetic alteration of Flg and Tmem79

- NC/Nga
  - Spontaneous onset in conventional housing condition
  - Genetic determinant linked to immune-related genes

Genetically engineered models

Pros: useful in elucidating gene-specific functions in vivo; powerful when crossed with other strains
Cons: time consuming and expensive to generate; undesirable effects from unexpected gene expression or alteration (i.e., variable penetrance, inefficiency of Cre)

Overexpression

- IL-4 (K14-IL4 Tg) / IL-13 (K5-tTA-IL13 Tg)
  - Keratinocyte-specific overexpression of type 2 cytokines, recapitulating human AD, including chronic parenchymal changes
  - Chan et al., 2006
  - Zheng et al., 2009

- IL-31 (EF1α-IL31 or Epi-Lck-IL31 Tg)
  - Pruritus and disruption of the skin barrier
  - Dillon et al., 2004

- TSLP (K5-rTa-TSLP Tg) / IL-18 (K14-IL18 Tg) / IL-33 (K14-IL33 Tg)
  - Keratinocyte expression of type 2 cytokines that activate innate lymphoid cells
  - Imai et al., 2013
  - Konishi et al., 2002
  - Yoo et al., 2005

- JAK1 (Jak1<sup>madh/spade</sup>)
  - JAK1 hyperactivation leading to barrier dysfunction
  - Yasuda et al., 2016

Ablation

- ADAM17 (Adam17<sup>fl</sup>/spade)
  - Spontaneous dysbiosis and eczematous skin inflammation
  - Kobayashi et al., 2015

- CARMA-1 (unmodulated)
  - N-ethyl-N-nitrosourea—induced, genome-wide mutagenesis model
  - Mutation in mouse ortholog of CARMA-1/CARD11
  - Jun et al., 2003

Models induced by exogenous agents

Pros: time-controlled induction; applicable to various mouse strains
Cons: nonstandardized products for some allergens; variable protocols (doses and durations); labor-intensive (daily applications)

- Hapten-induced (e.g., oxazolone, trinitrochlorobenzene (TNCB)) / Allergen-induced (e.g., ovalbumin, house dust mite)
  - AD-like inflammation induced by repeated challenge
  - Ambiguous distinction between AD and allergic contact dermatitis
  - Kitagaki et al., 1995, 1997
  - Matsuoka et al., 2003
  - Spergel et al., 1998
  - Wang et al., 2007

- MC903 (calcipotriol)-induced
  - High reproducibility of AD-like responses in various strains
  - Kim et al., 2013
  - Li et al., 2006
  - Myles et al., 2016
  - Naidoo et al., 2018
  - Oetjen et al., 2017

**Cd3g, Il10ra, Il18, and Csk (Kohara et al., 2001).** Thus, these mice, in contrast to the flaky tail mice, might reflect the altered immune component of AD. Although the spontaneous nature of inbred mice may reflect the natural course in human AD, it is not trivial to pinpoint the underlying genetic defect. It should also be noted that genetic background-unique modifiers may either attenuate or aggravate phenotypes in any mouse model. Therefore, it is important that researchers be cognoscenti regarding the genetic backgrounds of the mice and choose appropriate controls.

**Genetically engineered models**

The overall complexity of AD pathogenesis and the vast numbers of secondary gene changes downstream of chronic inflammation hampers the narrowing down of genes that play central roles in AD pathogenesis. In this regard, transgenic and knockout or conditional knockout mice are valuable in elucidating the biological significance of the targeted molecules. Genetically engineered mice with altered expression of AD-related genes would provide an approach to investigating the biological function of each molecule. The generation of genetically engineered mice is time consuming and costly, requiring strategic planning. A list of selected mouse strains with genetic modification is shown in Table 1.

Transgenic mice overexpressing type 2 cytokines, IL-4 or IL-13 in epidermis, develop spontaneous pruritus and chronic dermatitis. In both strains, skin lesions are characterized by prominent infiltration of T cells, mast cells, eosinophils, and macrophages, and total IgE and IgG1 are elevated in serum (Chan et al., 2001; Zheng et al., 2009). These models also
recapitulate chronic epithelial and stromal changes observed in human AD, such as acanthosis or dermal remodeling with fibrosis and increased vasculature. The efficacy of dupilumab, a monoclonal antibody that blocks the binding of these cytokines to their cognate receptor, emphasizes that these transgenic mice are effective AD models. However, lymphoid cells, rather than keratinocytes, produce IL-4 and IL-13 in both mice and humans under physiological conditions.

IL-31, the predominant source of which are T helper type 2 cells, is associated with pruritus and disruption of the physical skin barrier, and it has recently gained attention as a novel therapeutic target in AD (Dillon et al., 2004; Feld et al., 2016; Ruzicka et al., 2017). Transgenic mice overexpressing IL-31, driven by the ubiquitous promoter for EF1, develop hair loss by 2 months of age and display dermatitis with prominent scratch behavior (Dillon et al., 2004).

Keratinocyte-derived cytokines may also play crucial roles during atopic inflammation. TSLP is a keratinocyte-derived type 2 cytokine. A doxycycline-inducible, keratinocyte-specific transgenic expression of TSLP (K5-TSLP) in mice leads to the onset of AD-like skin lesions after 2–3 weeks of doxycycline treatment, with concomitant increase in serum total IgE and the type 2 immunity-associated chemokine CCL17 (Yoo et al., 2005). Keratinocyte-specific expression of the IL-1 family of cytokines, IL-18 and IL-33, each also exhibit AD-like phenotypes (Imai et al., 2013; Konishi et al., 2002). Given the fact that TSLP transgenic mice lacking conventional T cells (K5-TSLP, TCRB+/-) still develop skin inflammation and that the three keratinocyte-derived cytokines, TSLP, IL-18, and IL33, are important tissue-derived cytokines that activate group 2 innate lymphoid cells (i.e., ILC2), these models might be useful in studying the crosstalk between keratinocytes and innate immunity. An anti-IL-33 antibody is currently under clinical trial (NCT03738423, NCT03736967). Although IL18 has not been associated with human AD in genome-wide association studies, loci including IL18R1 and IL18RAP have been reported, implicating the involvement of this cytokine (Tamari and Hirota, 2014).

The imbalance of skin commensal microbiota, termed dysbiosis, is now a recognized feature of human AD. Although S. aureus colonization in AD skin has been known for more than half a century, whether it contributed to pathogenesis, or was merely a result of chronic inflammation, had been debated. A mouse model that recapitulated this condition had been lacking. We recently reported that Adam17fl/fl Sox9-Cre mice, which lack ADAM17 in keratinocytes, spontaneously developed dysbiosis that was dominated by Corynebacterium species and S. aureus (Kobayashi et al., 2015). These mice display dry skin at approximately 3–4 weeks after birth, then develop overt eczematous dermatitis at approximately 6 weeks (Figure 1b). Eczematous dermatitis is preceded by the emergence of S. aureus, and targeting of the dysbiotic organisms with antibiotics extinguishes skin inflammation (Kobayashi et al., 2015). Although eczematous dermatitis is less prominent in the absence of S. aureus in mice housed in facilities with stringent health status (unpublished observation), this can be taken advantage of by inoculating S. aureus to induce eczematous dermatitis in a time-controlled manner.

AD mouse models have also been established through screening libraries following chemically induced, genome-wide mutagenesis. Heterozygous mutations in CARD11, encoding a scaffolding protein involved in lymphocyte receptor signaling, are linked with monogenic AD in humans (Ma et al., 2017). Growing evidence suggests a benefit of targeting Janus kinase in AD (Guttman-Yassky et al., 2019). In these contexts, two N-ethyl-N-nitrosourea–derived models, CARMA-1/CARD11-mutant mice (Jun et al., 2003) and JAK3spade/Spade mice (Yasuda et al., 2016) might be interesting models for understanding atopic inflammation from the immune signaling perspective.

Models induced by epicutaneous application of exogenous agents

Induced mouse models are perhaps the most frequently used systems in the fields of dermatologic research such as immunology and carcinogenesis. Although topical application can be labor intensive, it enables time- and dose-controlled induction of a phenotype and can be used in a variety of mouse models, including genetically modified mice.

Haptens are small molecules that penetrate intact mouse epidermis and provoke adaptive immune responses upon subsequent exposures, resulting in contact hypersensitivity responses that model allergic contact dermatitis in humans. Repeated hapten challenge is reported to induce AD-like dermatitis by shifting type 1 to type 2 responses (Kaplan et al., 2012; Kitagaki et al., 1995, 1997). Allergic contact dermatitis and AD are distinct entities, and whether dermatitis induced by chronic hapten application recapitulates eczematous dermatitis remains to be determined.

Sensitization to protein antigens is thought to occur in patients with AD and may contribute to the onset of food allergy and asthma, known as the atopic march. Multiple epicutaneous exposure to ovalbumin can induce AD-like symptoms (Spergel et al., 1998) with ovalbumin-specific IgG1, IgG2a, and IgE humoral responses (Wang et al., 2007). Human AD-like symptoms can also be induced by applications of house dust mite extract onto mouse skin (Matsuoka et al., 2003). Skin changes in both models are enhanced when the barrier disruption is induced mechanically or by using mice that exhibit spontaneous skin barrier perturbation, such as NC/Nga or flaky tail mice. The relevance of skin inflammation induced in the house dust mite model has yet to be determined, because humans presumably are not exposed to high doses of house dust mite antigens percutaneously. Commercially available house dust mite and ovalbumin allergen products can vary in their allergen composition and concentration, depending on how they are prepared (Casset et al., 2012).

Although rash observed during topical application of calcemic vitamin D3 analogs in psoriasis patients is clinically distinct from AD, topical application of MC903 (calcipotriol) to mouse skin recapitulates features of AD (Figure 1c), such as inflammation, itch, and barrier dysfunction (Li et al., 2006; Naidoo et al., 2018). Mice treated with MC903 also have increased serum IgE. Conveniently, these AD-like responses can be induced regardless of genetic backgrounds, enabling the use of this model in mice that carry multiple transgenes without the necessity for backcrossing, which may facilitate their use in preclinical studies. Emerging concepts of AD pathogenesis such as innate lymphoid cells, sensory neuron,
Comparison of murine AD models to human AD

To date, the gross phenotypes of mouse models have been correlated with human AD by comparing clinical manifestations, histology, and expression of a limited number of markers. However, emerging cutting-edge technologies with transcriptomic analysis now deepen our understanding of each model and should allow us to compare complex molecular networks between species. Comparison of gene expression data from mouse models to a differentially expressed list (meta-analysis derived AD: “MADAD”) of 595 genes from human AD skin defined by meta-analysis showed that the IL-23— injection model, a cytokine that is usually associated with psoriasis, exhibited the highest degree of overlap (Chan et al., 2006; Ewald et al., 2017). Our analysis of Adam17fl/fl Sox9-Cre mice showed overlap with the human AD transcriptome to a degree that was comparable to the IL-23— injected model (Woodring et al., 2018). However, the maximum overlap of genes remains under 40% in any model, suggesting that animal models each reflect limited aspects of human AD. These observations warrant further evaluations of the predictive power of each as preclinical models. One approach to identifying a model that reflects human AD might be to test whether therapeutics with known clinical efficacy in humans are also effective in AD mouse models. It is possible that mouse models reflect certain subsets of classic human AD and that further clinical subcategorization of AD is needed. More than 20% of protein coding genes are not shared between mice and humans, suggesting that the two species may have developed unique immune systems after divergence from common ancestors. Future analysis may require the establishment of bioinformatics analytical frameworks with an evolutionary systems biology approach.

CONCLUSIONS AND FUTURE PERSPECTIVES

We have highlighted the diversity of current murine AD models and their advantages and limitations that should be considered when selecting a model that is appropriate for each research question or interpreting published studies. To increase the translatability of AD mouse models, it may be beneficial to establish phenotype criteria and accumulate transcriptome data, which should facilitate distinction of eczematous dermatitis from other forms of skin inflammation (Figure 1a), such as psoriasis and contact hypersensitivity. Practical and reproducible approaches for evaluating the degree of inflammation are also essential, because ear thickness, transepidermal water loss, and other laboratory assays are variably used. A standardized clinical scoring system should be useful in reducing variability between studies (Kobayashi et al., 2015; Plant et al., 2012). Finally, beyond mouse models, nonmurine animal models for AD such as canine AD may better recapitulate human AD and thus be powerful models for preclinical studies (Cosgrove et al., 2013; Michels et al., 2016).

ORCIDs
Doyoung Kim: http://orcid.org/0000-0002-0194-9854
Tetsuro Kobayashi: https://orcid.org/0000-0003-2316-4748
Keisuke Nagan: http://orcid.org/0000-0002-7005-3138

CONFLICT OF INTEREST
The authors state no conflict of interest.

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

REFERENCES
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DETAILED ANSWERS

1. The MC903 model used to study human atopic dermatitis represents which category of mouse model?

   **Answer:** D. Model induced by an exogenous agent
   
   Repetitive application of MC903 (calcipotriol), a topical antipsoriatic agent, onto mouse skin can induce AD-like inflammation.

2. Which of the following mutations is the most responsible for atopic dermatitis-like inflammation in flaky tail mice?

   **Answer:** B. *Tmem79*
   
   A recent report with segregation of the two mutated genes identified *Tmem79*, but not *Flg*, as the causative gene mutation of skin inflammation in flaky tail mice.

3. Which of the following cytokines from keratinocytes is responsible for the activation of group 2 innate lymphoid cells 2 (ILC2)?

   **Answer:** D. All of the above
   
   Group 2 innate lymphoid cells (ILC2s) are activated in response to a variety of stimuli, including epithelial cytokines IL-18, IL-25, IL-33, and TSLP.

4. Which of the following microbes is responsible for the development of skin inflammation in *Adam17fl/fl Sox9Cre* mice?

   **Answer:** D. *Staphylococcus aureus*
   
   *S. aureus* is primarily responsible for driving skin inflammation in *Adam17fl/fl Sox9Cre* mice.

5. Which of the following sentences highlights a lesson from a recent study that compared transcriptomic profiles between human atopic dermatitis (AD) and mouse models?

   **Answer:** D. Each animal model reflects limited aspects of human AD.
   
   The IL-23—injection model has been reported to show the highest degree of overlap with human AD among the tested models. However, the maximum overlap of genes between human AD and mouse models remains under 40%. In general, more than 20% of protein coding genes are not shared between mice and humans.