

Animal Models of Atopic Dermatitis

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Abstract: Atopic dermatitis (AD) is a chronic, recurrent eczematous skin disease with itching. Many AD patients have a family history of asthma, allergic rhinitis, and a tendency to IgE overproduction. AD is recognized as a form of persistent antigen-specific dermatitis with a Th2 cytokine profile. Various antigens induce AD, but uniformity of their clinical manifestations suggest the involvement of a common non-antigen-specific pathway. We have generated a keratinocyte-specific caspase-1 transgenic mouse (KCASP1Tg) using the keratin 14 promoter. KCASP1Tg over-secreted IL-1alpha/beta and IL-18 from the skin and developed persistent itching dermatitis with marked serum IgE and histamine elevation at age 8 weeks in specific pathogen-free conditions. We also produced a keratin 14-driven mature IL-18 transgenic mouse (KIL-18Tg). KIL-18Tg developed similar AD-like skin changes with IgE elevation at 24 weeks. KCASP1Tg crossed with stat6^{-/-} developed skin manifestations at age 8 weeks without IgE elevation; however, KCASP1Tg crossed with IL-18^{-/-} did not develop dermatitis. KCASP1Tg crossed with IL-1alpha/beta^{-/-} developed dermatitis at age 24 weeks. Thus, epidermal IL-18 secretion induces AD-like dermatitis, and epidermal IL-1s enhances AD-like changes. Both KCASP1Tg and KIL-18Tg showed Th2-type cytokine profiles and satisfied the human AD criteria. These mice models indicated the involvement of an antigen-independent innate-type pathway in addition to the antigen-specific acquired-type pathway in AD. These models responded to various therapeutic agents for AD, and are ideal, potent tools for the development of gene therapy and other new therapies for AD.

Key words: Atopic dermatitis; Interleukin 18 (IL-18); Mast cell; Mouse; Caspase

Introduction

According to the definition by the Japanese

Dermatological Association (JDA), atopic dermatitis (AD) is “a chronic and recurrent eczematous skin disease with itching; many AD

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patients have a family history of atopic predisposition (bronchial asthma, AD or allergic rhinitis) and a tendency to IgE overproduction.”¹⁾ Understanding AD pathology is very difficult because it involves immediate type allergic reactions despite its basically eczematous reaction.

The clinical efficacy of topical steroids has been remarkable, but the uncritical use of corticosteroids has resulted in frequent adverse effects. A flood of incorrect information that ensued created mistrust of corticosteroids therapy, which continues to hinder the proper treatment of AD. In this situation, animal models of AD are essential for answering questions about AD and developing new therapies. The mouse is the most suitable species for this purpose since it is a mammal, the reproduction cycle is short, the immunological background of this species has been established, and diagnostic reagents are available.

Several AD mouse models have been used for years. Mutant mouse models include the Nc/Nga mouse,²⁾ which is constantly infested by mites on the skin, and the NOA (Naruto Research Institute Otsuka Atrichia) mouse.³⁾ Other models have been produced by repeated sensitization with antigens.⁴⁾ The production of a sensitization model takes a long time, and can be achieved only in a few strains of mice such as BALB/c. These mice have a different genetic background from commonly used genetically manipulated mice based on C57BL/6 mice, and this causes difficulty in cross breeding analysis. The genetic background of Nc/Nga mice has not been well characterized, and these mice do not develop dermatitis under specific pathogen-free (SPF) conditions because dermatitis in this model depends on the presence of mite infestation.²⁾ Thus, it is difficult to use these mice to develop therapeutic drugs.

The NOA mouse, a hairless strain created by mutation, is considered an AD model because the mouse develops ulcerating skin lesions mainly on the trunk, shows scratching behavior, and presents elevated serum IgE levels. This

mouse has been found to show an elevated platelet factor 4 and eotaxin, an eosinophil chemotactic factor, in the skin.³⁾ However, the genetic background of this mouse has not been established. While backcrossing with Th2-dominant BALB/c mice results in the development of symptoms, backcrossing with Th1-dominant mice such as C57BL/6 results in low occurrence of dermatitis. Because of these problems, it has been necessary to develop new genetically modified AD mouse models.

Clinically, AD has been regarded as a persistent antigen-specific eczematous reaction to environmental antigens such as food antigens and mites. The pathogenesis of AD is believed to involve Langerhans cells and activated T cells. The overproduction of IgE antibodies in AD has led researchers to pay attention to the roles of B cells, mast cells, and basophils, and AD has been considered a Th2-type disease involving cytokines such as IL-4, IL-10, IL-13, and IL-5. Although various antigens induce AD, the uniformity of their clinical manifestations suggests the presence of a non-antigen specific pathway in the pathogenesis of AD.

The authors produced a mouse model that showed continuous secretion of proinflammatory cytokines in the skin,⁵⁾ which developed AD-like dermatitis under SPF conditions.⁶⁾ We identified IL-18 as a cytokine involved in AD pathogenesis,⁷⁾ and proposed a new concept of AD.⁸⁾

Skin-Specific Caspase 1 Transgenic Mouse (KCASP1Tg)

Inflammation is believed to involve proinflammatory cytokines including IL-1 α , IL-1 β , tumor necrosis factor (TNF) α , and IL-18. These substances are produced in the cell as inactive precursors and released from the cell after enzymatic activation. The IL-1 β -converting enzyme that activates IL-1 β was later found to be coded by a member of a gene family related to apoptosis. This enzyme, named caspase, is a cysteine protease that mediates proteolysis at

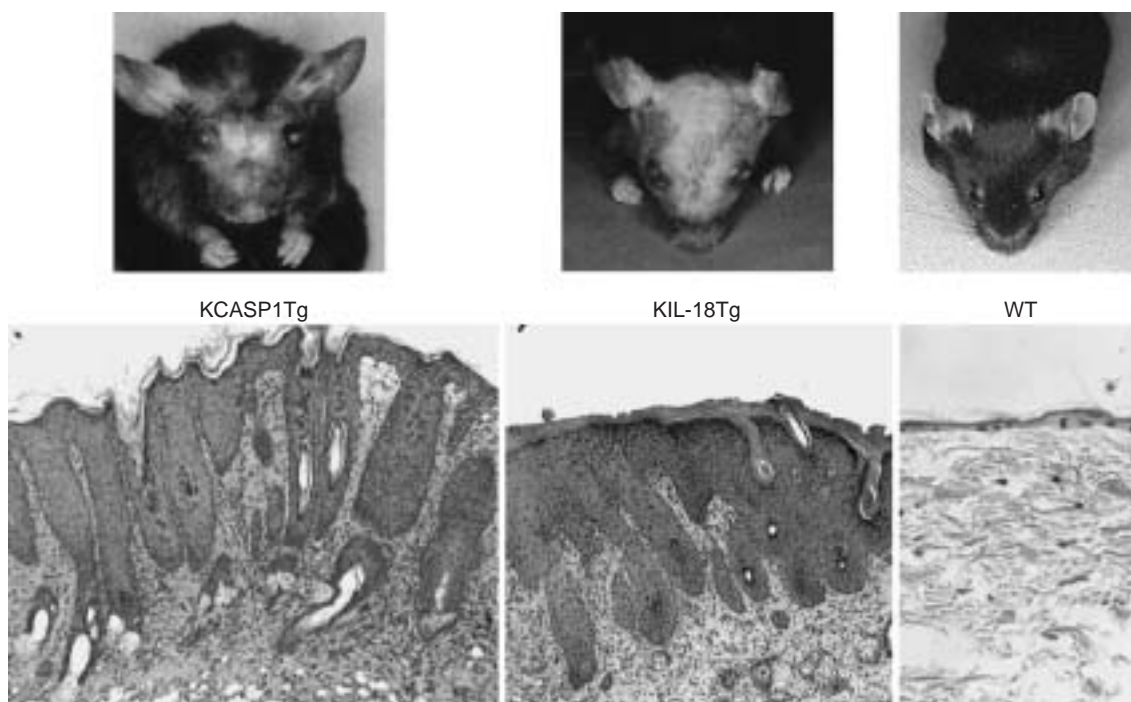


Fig. 1 Clinical and histopathological findings in KCASP1Tg and KIL-18Tg

an aspartate site.

Monocytes produce caspase 1, which promotes the activation and secretion of IL-1 β and IL-18. While epidermal keratinocytes produce pro-IL-1 β and IL-18, they do not secrete these cytokines because they normally lack caspase 1 activity.⁹⁾ We attached a keratin14 promoter to human caspase 1 DNA segments and introduced the DNA into fertilized eggs so that caspase 1 would be expressed only in the epidermal basal cells.⁵⁾

Clinical Symptoms in Model Mice

About 8 weeks after birth, the mice started to show dermatitis around the eyes and neck. The lesions extended to the ears and legs and finally became generalized. The dermatitis started as erosive erythematous patches and moist crusted areas. After repeated re-epithelization and inflammation, the lesions progressed to chronic lichenified plaques (Fig. 1). Almost all the mice developed cataract, as is the case with AD.

The frequency of scratching behavior increased remarkably with the onset of the skin lesions. In week 10 and after, the frequency of scratching per 10 minutes was 10 times as high as that in normal mice (Fig. 2b).

Histopathological Changes in Model Mice

The skin before the onset of skin symptoms showed no histopathological changes. After onset, it presented remarkable epidermotropic cell infiltration, papillomatous proliferation and thickening of the epidermis, partial epidermal defects, crusts, and parakeratosis. These changes resembled those observed in the acute stage of human atopic dermatitis (Fig. 1). The dermis showed an increase in the number of infiltrating CD4+ T cells and a remarkable increase in toluidine blue-positive mast cells, similar to the findings in AD⁶⁾ (Fig. 2c).

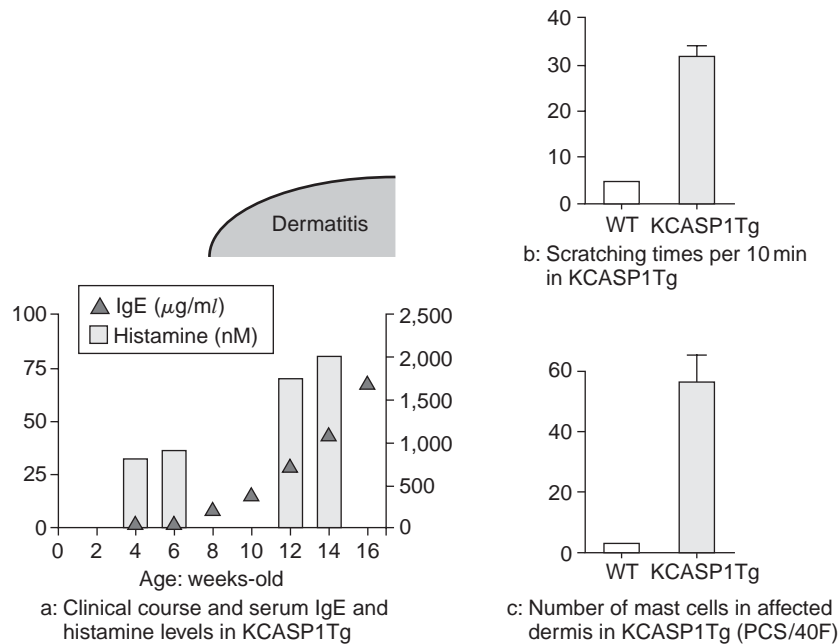


Fig. 2 Immunological profiles of KCASP1Tg

Serological Changes in Model Mice

Serum histamine levels correlate with the severity of AD in humans. The serum histamine levels in the model mice started to increase before the onset of symptoms, and it increased according to the extension of the skin lesions (Fig. 2a). Serum histamine levels corresponded with an increase in mast cells in the skin. Serum IgE levels were as low as that of wild-type mice. It increased by the time of symptom onset, and showed remarkable elevation to $50\mu\text{g/ml}$ or more, correlating with the severity of the skin rash (Fig. 2a). IgG1 increased remarkably, however, increases in IgG2 and IgM were limited, suggesting the allergic nature of the observed changes.

Cytokine Production and Immunological Profile of Model Mice

The sera of the model mice were found to contain high levels of active IL- 1β and IL-18. The expression of cytokine mRNAs in the affected skin showed an elevation of IL-4 and

IL-5, which are not detected in normal mice, and IFN- γ was not detected. The increase in IL-4 production was confirmed with an enzyme-linked immunosorbent assay (ELISA) for the culture condition medium of the lesions.

In addition, we measured the cytokine production in splenic cells after stimulation with anti-CD3 antibodies. We detected elevation of IL-3, IL-4, and IL-5, as well as the suppression of IFN- γ , indicating a systemic Th2 shift of cytokines. These mice showed an elevation of CD40L expression, implying the promotion of IgE production from B cells.

Appropriateness as a Model of Atopic Dermatitis

Because the definition of atopic dermatitis is based on clinical findings in humans, it is somewhat controversial whether the same criteria can be applied to mice. The definition by the Japanese Dermatological Association, i.e., recurrence, itching eczema, family history, and elevated serum IgE levels, applies to our mouse model.¹⁾

Our model satisfies all the essential elements of the internationally accepted diagnostic criteria by Hanifin-Rajka,¹⁰⁾ i.e., (1) itching, (2) typical distribution of skin rash, (3) chronic recurrent dermatitis, and (4) family history. At least three of these major criteria are required. Our model satisfies 11 of 22 minor elements, including xeroderma, elevated serum IgE, early onset, dermatitis of the hands and feet, cheilitis, conjunctivitis, cataract, facial erythema, lesions around the eyes, and aggravation due to environmental changes. We could not detect environmental antigen specific IgE in their sera.

As discussed above, these mice are qualified as an animal model of AD.

Onset Mechanism of AD Studied in Model Mice

There has been a long debate as to whether the onset of AD involves type I or type IV allergy. However, it has been accepted that the distinction between type I and type IV does not have much significance in this context. The role of IgE and the mechanism for IgE elevation are nevertheless important issues.

KCASP1Tg mice produce IgE without antigen-specific reactions, and the skin of these mice secretes IL-1 and IL-18. IL-18 was originally discovered as a factor inducing IFN- γ in hepatitis, and thus had been considered a Th1 cytokine. This assumption contradicts the symptoms seen in these mice.⁵⁾ To answer this question, we injected recombinant IL-18 into wild-type mice, and observed the elevation of IgE. The results indicated that IL-18 is a Th2 cytokine and suggest the possibility that Th2 induction in these mice may be mediated by IL-18.⁷⁾

On the other hand, the class switching and production of IgE essentially requires signal transduction via the stat6 system locating the downstream of IL-4. We therefore crossed KCASP1Tg with stat6 knockout mice. Because this resulted in the complete suppression of IgE production, we concluded that the IgE

induction by IL-18 is mediated by stat6. On the other hand, we crossed our mice with IL-18 knockout mice to see whether IgE production in KCASP1Tg depends on IL-18. This experiment showed that the IgE level in KCASP1Tg IL-18^{-/-} mice is as low as one-tenth of the reference IgE level. These mice possessed IL-4, suggesting that there are minor IgE induction pathways other than those involving IL-18.

While the stat6-dependence of IgE production was confirmed, this study produced the surprising results that the stat6^{-/-}-KCASP1Tg mice lacking IgE developed similar dermatitis with the same timing as the KCASP1Tg mice. Furthermore, the IL-18^{-/-}-KCASP1Tg mice did not develop dermatitis.

The above findings indicate that IgE is not essential to the development of dermatitis, and IL-18 plays a key role.

IL-18 Transgenic Mice

Next, it was necessary to examine the *in vivo* function of epidermal IL-18. We produced epidermis-specific mature IL-18Tg (KIL-18Tg) for this purpose. While KCASP1Tg developed symptoms within 8 weeks, KIL-18Tg took 6 months before the onset of symptoms.⁸⁾ These mice developed lichenification of the skin from the early stage of the skin rash (Fig. 1).

Because KCASP1Tg mice also secrete IL-1 β , we crossed the model mice with IL-1 α/β knockout mice to examine the effect of IL-1 β . While the IL-1s^{-/-}-KCASP1Tg mice presented a similar phenotype to the KCASP1Tg mice, they resembled KIL-18Tg mice in that 6 months was required before the onset of symptoms. These observations suggest that IL-18 is essential for the onset of skin rash and that IL-1s acts as an onset booster.

Suggestions from AD Model Studies and Future Prospects

The model mice described above introduced a new concept of AD. High IgE levels and

RAST (radioallergosorbent test) scores have been emphasized in the clinical management of AD. These indices sometimes cause excessive dietary restriction and limitation on bedding and clothing, and these may be exploited as an opportunity for home reform businesses. In addition to the conventional antigen-specific mechanism, it is possible that epidermal damage due to scratching promotes the release of epidermal proinflammatory cytokines (in particular, IL-18), that activates mast cells, and results in aggravation of AD.

We proposed a new concept that the part of AD caused by environmental antigen-specific eczema reaction as “acquired-type AD” and the part consisting of non-antigen specific dermatitis seen in these mice as “innate-type AD.”⁸⁾ In Europe, the term “extrinsic AD” was proposed to describe AD showing antigen-specific IgE antibodies and the term “intrinsic AD” for AD lacking these antibodies, and the latter has been reported to be increasing recently.¹¹⁾ In our daily practice, about 10 to 20% of AD cases do not have high serum IgE levels. It is likely that these cases are affected by epidermal injury due to scratching caused by psychological factors or ichthyosis.

Therapeutically, KCASP1Tg mice responded well to topical steroids and steroid injections. These mice showed disappearance of erosions and remarkable improvement in dermatitis. The effectiveness of the topical use of anti-allergic drugs and immunosuppressive agents was also confirmed. Because KCASP1Tg mice are tested in SPF conditions, they are expected to be ideal tools for the development of new drugs and the evaluation of existing drugs.

The past pococurante use of corticosteroids and the flood of incorrect information about adverse effects once caused much confusion about the treatment of AD. Owing to the efforts of the JDA to promote correct information, we have overcome most of the misconceptions. The development of gene therapy and other new future therapies will require animal models. These two types of model

mouse discussed here are expected to open new possibilities in the development of new AD therapies.

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