—Review—

Review Series: Frontiers of Model Animals for Human Diseases

Recent Findings in Mouse Models for Human Atopic Dermatitis

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Abstract: Atopic dermatitis (AD) is a chronic inflammatory skin disease, with its major clinical feature being persistent itch sensation in the skin. There are extremely few animal models to reproduce the complicated condition of a patient with AD; therefore researchers have been confronted with some difficulties in pathologic analysis and drug development for AD. Although various models have been proposed and developed, there is no doubt that the spontaneous mouse model, NC mice, gave the greatest impact. NC mice enabled us to analyze pathogenesis of allergic skin abnormalities as well as development of new drugs for AD. However, many questions still remain in the pathogenesis of AD. In recent years, the study of the itch has attracted our attention because itch is one of the most unbearable symptoms of AD. For development of an effective treatment to overcome the itch, not only a precise animal model but also an accurate evaluation protocol are needed. This review summarizes some mouse models of AD, particularly focusing on NC mice, together with a novel evaluation system for scratching behavior of mice to help the understanding of researchers.

Key words: atopic dermatitis, immune response, itch, skin barrier

Introduction

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disorder in which most of the patients have some genetic background. Dysfunction of the skin barrier, overreaction and abnormal reaction of the immune system against external antigens and/or autoantigens, and immunoglobulin (Ig) E-mediated sensitization to food and environmental allergens have been proposed as atopic cofactors in patients with AD [2, 3]. In recent years, an opinion that AD is a complicated syndrome that is initiated by the interaction of genetic cofactors and environmental factors has been accepted [2]. Some abnormalities in genes related with the epidermal barrier have been pointed out, including the loss of function mutations in filaggrin, which is a key molecule for main-

tenance of the barrier function of the skin [17]. Deficiency in filaggrin formation and/or its processing leads to the disorganized construction of epidermal sheets, allowing unfavorable penetration of allergens and irritants into the skin. Severe exposure to allergens induces hyperplasia of the epidermis resulting in overproduction of thymic stromal lymphopoietin (TSLP) from proliferating keratinocytes. TSLP drives proallergic responses via activation of dendritic cells (DCs) in the epidermis and dermis [32]. Matrix metalloproteinases (MMP)-2 and MMP-9 produced from activated-DCs are involved in the migration of DCs [18]. Activated DCs express various costimulatory molecules and migrate to local lymph nodes where they accomplish their roles as antigen-presenting cells. OX40 ligand (OX40L) expressed on DCs engages with OX40 on activated naïve T cells.

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OX40-activated T cells induce the production of IL-4 and IL-13 and suppress IFN- γ production. In the process, development into Th2-like effectors is progressed. The majority of the AD patients manifest strong polarization to Th2-type responses, thereby leading to the hyperproduction of IgE. In the chronic phase, Th1 IFN- γ response and tissue remodeling processes are driven, and the biphasic response against allergens switches from an initial Th2 response to a Th1 response [24]. Moreover, insensitivity to bacterial exclusion may take part in colonization of *Staphylococcus aureus* (*S. aureus*) on the skin of AD subjects [15]. *S. aureus* acts as a superantigen by which skin symptoms of AD are accelerated. Increased numbers of activated circulating CD4⁺ and CD8⁺ T cells and marked infiltration of CD4⁺ T cells into the dermis are characteristic features of patients with AD. The initial phase of AD is dominated by Th2 type T cells producing IL-4, IL-5, and IL-13, and at the subsequent chronic phase, the number of Th1 cells producing IFN- γ is increased. Keratinocytes are capable of aggravating the inflammatory reaction in the skin through upregulated release of various cytokines and chemokines. Keratinocyte-mediated factors, not only TSLP but also nerve growth factor (NGF) and semaphorin 3A, contribute to the balance of sensory cognition on the skin [27]. Mast cells play crucial roles in both the early development and the chronic persistence of AD by IgE-dependent activation and release of various cytokines, proteases, and chemical mediators. Particularly, mediators produced from mast cells are widely associated with induction of itch sensation, the most unbearable clinical symptom of AD.

Various models that represent allergic dermatitis have been proposed so far. However, as mentioned, AD is not simple but is a quite complicated syndrome with a sophisticated condition originating from different types of allergic reaction. Therefore, researchers have to pay great attention to the characteristics of each model and choose the model that is suitable for their research needs. In the current review, some well-known models of human AD are discussed.

Induced Models of Allergic Dermatitis

Passive sensitization model

Passive sensitization is carried out by intravenous injection of murine monoclonal IgE antihapten antibodies before antigen challenge, by which IgE-mediated

cutaneous inflammatory reactions can be produced easily [8]. Inflammatory responses are assessed by measuring ear thickness, dye exclusion, and pathologic techniques in mice challenged with its specific antigen. Cutaneous inflammatory reactions become evident within several minutes after antigen challenge (the early phase reaction), and last for 24–48 h (the late phase reaction). The immediate reactions include the antigen-triggered release of vasoactive mediators from IgE-sensitized mast cells. On the other hand, the late phase reaction is induced by the antigen-specific triggering of sensitized T cells. The passive sensitization model simply represents the immediate hypersensitivity, and this model is quite helpful for research on mast cell-mediated reaction.

Epicutaneous sensitization model

Epicutaneous (EC) sensitization to allergens, which requires recruitment of skin DCs to draining lymph nodes, is believed to play a critical role in the pathogenesis of AD. Repeated EC sensitization to tape-stripped skin with allergen including ovalbumin (OVA), house dust mite, and haptens can induce antigen-mediated allergic dermatitis. Tape stripping mimics skin barrier destruction inflicted by scratching in patients with AD. EC-sensitized mice exhibit increased scratching behavior, and develop skin lesions characterized by epidermal hyperplasia, infiltration of CD4⁺ T cells and eosinophils, and upregulation of Th2 cytokines including IL-4, IL-5, and IL-13, with little or no change in Th1 cytokines [19]. In addition, OVA-sensitized mice have developed airway hyperresponsiveness following inhalation challenge with OVA. On the other hand, mice sensitized by the recombinant house dust mite allergen develop features of dermatitis with epidermal hyperplasia and spongiosis, infiltration of CD4⁺ and CD8⁺ cells, and a skewed Th2 response locally and systemically [6]. These features are similar to those observed with EC sensitization models with OVA. Haptens including oxazolone and trinitrochlorobenzene are used to induce allergic contact dermatitis and have been thought to evoke primarily a Th1-dominated response. However, it has been reported that multiple challenges with haptens of the skin over an extended period cause the skin inflammation to shift from Th1-dominated responses to chronic Th2-dominated inflammatory responses that are similar to those in human AD patients [10]. Furthermore, repeated challenge with oxazolone has reported to induce epidermal hyper-

plasia and suppress expression of the skin differentiation proteins including filaggrin, loricrin, and involucrin.

Mutant Models of Allergic or Inflammatory Dermatitis

Flaky tail mice

Filaggrin is important for maintenance of skin barrier function, and loss-of-function mutation of its gene is found in 15–20% of patients with AD. Filaggrin protein is localized in the granular layers of the epidermis, and profilaggrin, a 400-kDa polypeptide, is the main component of keratohyalin granules. In the differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into filaggrin molecules, which aggregate in the keratin cytoskeleton system to form a dense protein-lipid matrix.

Flaky tail (FLGft) mice, first introduced in 1958, are spontaneously mutated mice with abnormal small ears, tail constriction, and a flaky appearance of the tail skin, which is most evident between 5 and 14 days of age [13]. Mice of the FLGft genotype express an abnormal profilaggrin polypeptide that does not form normal keratohyalin F granules and is not proteolytically processed to filaggrin. Therefore, in flaky tail mice, functional filaggrin is absent from the cornified layer in the epidermis. These mice exhibit eczematous skin lesions mimicking human AD after age 28 weeks under specific pathogen-free (SPF) conditions and a progressive increase in serum IgE and IgG₁ levels. Acute AD skin lesions develop Th2-dominated inflammation characterized by infiltration of CD4⁺ T cells and increased Th2 cytokines. Subsequently, the chronic phase demonstrates a local Th1 IFN- γ response and tissue remodeling. Eight-week-old flaky tail mice demonstrate epidermal hyperplasia, and enhanced dermal infiltration of CD4⁺ cells, and expression of mRNA for IL-17, IL-6, and IL-23, but not IL-4, IL-13, or IFN- γ . Lesional skins of 32-week-old filaggrin-deficient mice exhibit more pronounced changes, and elevated IL-4 mRNA levels. These findings indicated that flaky tail mice demonstrate Th17-dominated skin inflammation and eczematous dermatitis with advancing age change [16].

NOA (Naruto Research Institute Otsuka Atrichia) mice

The phenotype of NOA mice is characterized by ulcerative skin lesions with the accumulation of mast cells, elevated serum IgE, and scratching behavior. NOA mice

are hairless, and a specialized diet exacerbates the dermatitis. A major gene responsible for the dermatitis is present on murine chromosome 14 [29].

Apolipoprotein C1 transgenic mice

Human apolipoprotein C1 transgenic (APOC1Tg) mice were developed as a model for hyperlipidemia. In addition to showing systemic increases in free fatty acids, cholesterol, and triglycerides, APOC1Tg mice are expected to be a useful model for inflammatory dermatitis. Because the composition of the stratum corneum is dependent on lipid homeostasis, APOC1Tg mice spontaneously develop moderate epidermal hyperplasia, hyperkeratosis and parakeratosis, scaling, lichenification, dermal infiltration of inflammatory cells, and pruritus [14].

Spontaneous Model for AD, NC/Tnd Mice

NC mice were established as an inbred strain from Japanese fancy mice by Kondo *et al.* [9]. Several sublines of NC mice, including NC/Nga, NC/Jic, and NC/Tnd (A registration name of NC/NgaTnd mice was changed to “NC/Tnd” from Dec. 2010), have been developed, and there are differences in each. NC/Tnd mice, which are an inbred strain originating from NC/Nga, have been maintained by inbreeding at the Tokyo University of Agriculture and Technology and express spontaneous AD most precisely. Itchy dermatitis develops in NC/Tnd mice kept under conventional conditions without air regulation from 6 to 8 weeks of age (Fig. 1). On the other hand, skin lesions are not apparent when they are raised under air-regulated SPF conditions (Fig. 1A). We have analyzed aspects manifested in NC/Tnd mice immunologically, pathologically, dermatologically, and molecular biologically and found that clinical symptoms in NC/Tnd mice are quite similar to those found in human AD [9, 11, 12]. In affected skins of NC/Tnd mice, epidermal hyperplasia, degranulation of mast cells, and recruitment of inflammatory cells are obvious (Figs. 1B and 2B). DCs are accumulated into the hyperplastic epithelia (Fig. 2B). In addition, IL-4 is produced from CD4⁺ T cells and mast cells, and Th2-specific chemokines are overproduced [28], indicating that Th2-type immune responses are upregulated as well as in human subjects with AD in the initial phase. Exacerbation of dermatitis is related to the increase in levels of total IgE and eosinophil numbers in circulation. The content of

Fig. 1. Conventional NC/Tnd mice. (A) Typical features of conventional NC/Tnd mice. Inflammatory dermatitis that is similar to AD is obvious. (B) Microscopic feature of conventional NC/Tnd mice. Hyperkeratosis, parakeratosis, acanthosis, and infiltration of inflammatory cells in the dermis are visible.

Fig. 2. Histopathologic manifestation of conventional NC/Tnd mice. (A) Toluidine blue staining for identification of mast cells. (B) Immunohistochemistry for Langerin⁺ cells that represent DCs. (C) Immunohistochemistry for CD4⁺ cells that represent effector T cells. (D) Immunohistochemistry for TSLP in hyperplastic epidermal keratinocytes.

ceramide in the skin is decreased in NC/Tnd mice before dermatitis becomes remarkable, and it became clear that trans-epidermal water loss was promoted in the affected skin as a result [1]. The itch-scratch cycle contributes to the development of AD as a cofactor. Decreased production of semaphorin 3A, which has activity that inhibits

NGF-induced sprouting of sensory neurons, has recently been reported in the skin lesions of conventional NC/Tnd mice as well as patients with AD [25, 31]. The contribution of nuclear factor- κ B to the inflammatory process has been confirmed in various aspects, and inhibition of its activity in the skin has been demonstrated to

be effective in controlling AD symptoms [23]. More recently, we have demonstrated that TSLP released from keratinocytes of the skin lesions (Fig. 2D) contributes to the early onset of AD in NC/Tnd mice through the maturation and migration of DCs in the skin and also found that the ignition of peroxisome proliferator-activated receptor gamma with a synthetic agonist reduces the onset of AD via inhibition of dendritic cell functions activated by TSLP [7].

Researchers have to be careful because there are some sublines of NC strains that rarely develop AD spontaneously. When experiments under conventional circumstances are not applicable, allergic dermatitis can be induced by using repeated application protocols with haptens, including FITC or picryl chloride. However, the hapten-induced dermatitis is initiated by a Th1-mediated immune response. Recently, AD-like skin lesions have been reproduced by applying an extract of house dust mites to SFP NC/Nga or NC/Tnd mice as an ointment. Furthermore, hairless NC/Nga mice are available by using the Toxin Receptor Mediated Cell Knock-out (TRECK) method [21]. Hairless NC/Nga mice allow us to apply therapeutic reagents onto the skin without shaving and to observe clinical symptoms quite easily.

Attempts to Develop New Approaches to AD Using NC Mice

Using NC mice, two therapeutic approaches have been undertaken; immunomodulation therapy based on the Th1/Th2 concept and anti-itch therapy.

Approaches based on a Th1/Th2 balance paradigm

Attempts to manipulate the Th1/Th2 balance have been made towards allergic diseases including AD. Neutralization of IL-31, which is a cytokine produced predominantly by Th2 subsets in the acute phase of AD, led to the reduction of scratching behavior in NC/Nga mice [4]. Interestingly, although the scratching behavior reduced during the treatment period, clinical skin scores were not significantly improved. In contrast, Hattori *et al.* [5] demonstrated that AD-like symptoms were ameliorated by sustained IFN- γ expression. They injected the IFN- γ -expressing plasmid vectors intravenously to maintain high IFN- γ concentrations in blood for weeks, bringing Th1-prone immune responses. In their study, the scratching frequency, clinical skin scores, and histological features, were significantly improved over 10

weeks. From these results, the Th2-dominant responses at the onset seem to be the key factor for development of AD in NC/Nga mice. However, stat6-deficient NC/Nga mice also develop AD-like histological features though the splenocytes in stat6-deficient NC/Nga mice highly express IFN- γ and less Th2 cytokines [30]. Moreover, exogenous injection of IFN- γ into 4-week-old NC/Nga mice induces more severe dermatitis as compared to controls [12]. These findings demonstrated that the blocking of Th2-mediated immune response did not ameliorate AD, suggesting the limitation of the Th1/Th2 balance theory. Since Th17 and regulatory T cell subsets play a crucial role in regulation of allergic inflammation, these cells may contribute to the pathogenesis of AD. Therefore, total reevaluation of immune responses in NC mice will provide a better understanding of AD.

Approaches targeting itch sensation

Recently, much attention has focused on the correlation between itch and sensory neurons. In the peripheral and central system, unmyelinated nerve fibers (C-fibers) and a gastrin-releasing peptide receptor-expressing spinal cord play an important role in itch sensation [20, 26]. NGF produced from keratinocytes and fibroblasts in the skin contributes to the massive development of C-fibers in AD patients and NC/Nga mice. Takano *et al.* [22] demonstrated that blocking of high affinity NGF receptors (TrkA) improved the dermatitis and scratching behavior in NC/Nga mice, indicating the therapeutic effect of anti-NGF treatment on severe dermatitis. Repeated topical injection with recombinant semaphorin 3A improved AD in NC/Nga mice [23]. Therefore, semaphorin 3A will be one of therapeutic factors for AD. Although both anti-TrkA and semaphorin3A had a direct effect on nerve sprouts, they reduced the number of immune cells in the regional skin. This is probably due to the reduction of mechanical stimulation that was brought about by less itch sensation, indicating that the itch-scratch cycle is closely related to the severity of dermatitis in NC mice. Thus, targeting itch-specific/dominant neurons may be a promising strategy for AD therapy.

Attempt to Evaluate Scratching Behavior in Mice

Intradermal injection of pruritogenic reagents into mice induces scratching behavior that can be used to evaluate the efficacy of new drug for AD. By using NC

Fig. 3. Outline of the SCLABA-Real[®] system. (A) A high-speed camera (250 frames/s) with computer, an infrared light box, and acryl boxes for mice. Black and white pictures are collected, and frame-to-frame differences are extracted by the system. (B) Acryl boxes with mice. After placing mice in each compartment without any labeling or operation, measurement of the scratching behavior can be immediately started. (C) Analysis and evaluation windows in the exclusive software. The flame-to-flame difference extracts quick movements of scratching behavior with abnormal thresholds.

mice, spontaneous development of AD with itching behavior can be applied for evaluation of new drugs. However, precise measurement and quantification of high-speed scratching behavior in mice are still complicated. SCLABA-Real[®] enabled us to analyze scratching behavior in real-time without using any kinds of stressful operations on a mouse (Fig. 3) [7]. The behavior of 4 mice is simultaneously recorded with a high-speed digital camera from the top of individual cages placed on invisible near-infrared light. The SCLABA-Real[®] software accurately detects the algorithm that is specific for scratching behavior in mice and records data in real time. Effective antipruritic reagents can be selected by accurate evaluation of scratching behavior in laboratory mice using the SCLABA-Real[®] system.

Although itch is the critical target for drug development, quantification of scratching behavior by visual examination is quite subjective. This innovative product, the SCLABA-Real[®] system, provides automatic analysis of scratching behavior in an objective manner. Accurate analysis of the itch mechanism is realized by accurate quantification protocols.

Final Remarks

AD is an extremely complicated condition of patients as described. It is necessary to investigate the mecha-

nisms of skin barrier dysfunction, abnormal immune responses, itch sensation, and continuous infection of *S. aureus* to develop an effective treatment; however, the mechanisms have not yet been fully elucidated. There are limitations on studies using human subjects, and application of the experimental approach to patients is quite difficult. Therefore, analysis using laboratory animals is essential.

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