

Lessons from Murine Models of Atopic Dermatitis

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Atopic dermatitis (AD) is a complex disease that manifests immunologic abnormalities in the skin. The immunologic changes of AD are characterized by a T helper cell type 2 (Th2)–dominated immune response in its acute phase and a Th1/Th2 mixed immune response in its chronic phase. Although the clinical presentation and pathologic changes are initially in the skin, AD may be, in part, a systemic disorder. In fact, AD is often considered to be the initial manifestation of the “atopic march” that progresses from AD to allergic rhinitis and asthma. In the past several years, a number of murine models of AD have been developed or discovered, and studies on these models have contributed greatly to our understanding of the immunopathogenesis of this disease. This review is focused on these recent, illuminating advances in animal models of AD.

Introduction

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease that affects 10% to 20% of children and 1% to 3% of adults [1••]. During the past decade, its increasing prevalence and its burden to health care costs have attracted the attention of many researchers and health workers. Atopic dermatitis is a complex disease that manifests immunologic abnormalities in the skin. The immunologic changes of AD are characterized by a T-helper cell type 2 (Th2)–dominated immune response in its acute phase and a Th1/Th2 mixed immune response in its chronic phase. Although the clinical presentation and pathologic changes are initially in the skin, AD may be partly a systemic disorder. In fact, AD is often considered the initial manifestation of the “atopic march” that progresses from AD to allergic rhinitis and asthma. Both genetic susceptibility and environmental influence are believed to contribute to the pathogenesis of AD.

Immunologic Responses in Atopic Dermatitis

Systemic immune responses

Two types of AD have been proposed: allergic or extrinsic AD and nonallergic or intrinsic AD. Of all the AD patients, between 70% and 80% of adult patients have allergic AD, which is characterized by elevated serum levels of total and specific immunoglobulin E (IgE) and skin sensitization to environmental allergens. The intrinsic type of AD affects 20% to 30% of patients and is not associated with any increase in the levels of total IgE or specific IgE to environmental allergens [2,3]. Immunologic differences between the allergic type of AD and the nonallergic type can be found by phenotyping characteristics of epidermal dendritic cells. It has been shown that there is a higher expression of high affinity IgE receptor I (FcεRI) on epidermal dendritic cells in allergic (extrinsic) AD than that in nonallergic (intrinsic) AD patients [4] and a ratio of FcεRI/FcεRII expression in epidermal dendritic cells above 1.5 is seen in extrinsic AD, whereas a ratio of only 0.5 was found in intrinsic AD [2,5]. Both types of AD are associated with peripheral eosinophilia. It has been proposed that atopic dermatitis may be an initial step of the systemic disorder “atopic march” that predates other atopic entities, such as allergic rhinitis, asthma, and food allergy [6,7,8••]. This concept of “atopic march” has been supported by the findings in a murine model of AD demonstrating that epicutaneous sensitization with allergen predisposes mice to airway hyperresponsiveness [9••].

Immune responses in the skin

The immunologic features of AD may vary depending on the duration of the lesion. Studies of biopsy specimens of acute lesions from AD patients showed infiltration of activated CD4⁺ memory T cells in the dermis compared with normal or nonlesional skin of AD patients. There was an increased number of Th2 cytokine-producing cells (interleukin [IL]-4, -5, and -13) [10]. However, in chronic skin lesions of AD, the number of cells expressing IL-5, granulocyte macrophage colony-stimulating factor (GM-CSF), IL-12, and interferon (IFN)-γ messenger RNA (mRNA) is significantly higher, and the number of cells expressing IL-4 and IL-13 mRNA is lower than that seen in the acute skin lesions. There is infiltration by inflammatory dendritic epidermal cells (IDECs), macrophages, and eosinophils in chronic skin lesions. These cells are important sources of the Th1 cytokine (such as IL-12) production that can lead

to the switch to a Th1-type cytokine milieu with enhanced IFN- γ expression [1••]. Increased numbers of IgE-bearing Langerhans cells (LCs) and IDECs in the lesional skin of AD play a central role in antigen presenting to Th1 and Th2 cells and in amplifying inflammatory responses. Fc ϵ RI⁺ LCs are important in the initiation of an eczematous skin lesion in the acute phase, whereas Fc ϵ RI⁺ IDECs play a central role in switching Th2 immune responses to Th1 immune responses seen in the chronic phase by priming naïve T cells into IFN- γ -producing T cells and the release of IL-12 [1••,11].

Murine Models of Atopic Dermatitis-like Skin Disease

Spontaneous development of atopic dermatitis-like skin lesions

There are several murine strains that spontaneously develop AD-like skin disease. One strain that has received much attention recently is NC/Nga mice because the clinical and immunopathologic features of the skin lesions in these mice resemble those seen in human AD. These mice originally came from inbred strains of Japanese fancy mice. In nonspecific, pathogen-free (non-SPF) environments, these mice have been shown to develop spontaneous chronic AD-like skin lesions [12•]. In addition, NC/Nga mice showed high susceptibility to x-ray irradiation as well as high susceptibility to anaphylactic shock when sensitized and challenged by allergen ovalbumin (OVA). Starting at 8 weeks of age, the NC/Nga mice develop skin lesions with increasing severity that peaks at 17 weeks of age. The occurrence of the skin lesions is associated with markedly elevated plasma levels of total IgE [12•,13]. Immunopathologic findings in these mice are characterized by massive infiltration of IL-4- and IL-5-producing CD4⁺ T cells, eosinophils, and degranulation of mast cells in the lesional skin [12•]. Highly expressed Th2 chemokines, thymus- and activation-regulated chemokine (TARC), macrophage-derived chemokine (MDC) in keratinocytes, and dermal dendritic cells have been noted in the lesional skin of NC/Nga mice. Both TARC and MDC have been known to be responsible for recruiting CCR4-bearing Th2 cells to the lesional skin [14,15]. The complexity of the pathogenesis of the AD-like lesions in these mice has been illustrated when both genetic and environmental factors were examined. As a control, BALB/c mice were housed with NC/Nga mice in the same non-SPF conditions. Interestingly, BALB/c mice did not develop AD-like dermatitis, suggesting a possible genetic predisposition in the development of AD in NC/Nga mice [12•]. However, when NC/Nga mice were kept in SPF conditions, the mice failed to develop the skin lesions, suggesting that microbial flora on the skin or related factors in the environment may play a role in inducing AD in these mice.

The molecular mechanisms underlying AD-like dermatitis in NC/Nga mice are not well understood. It has been

suggested that B cells from NC/Nga mice have higher sensitivity to CD40L and IL-4, resulting in increased IgE production. Furthermore, constitutively augmented phosphorylation of Janus-activated kinase 3 (JAK3) in B cells was noted, which may be an underlying molecular mechanism for IgE hyperproduction in NC/Nga mice [16]. However, an impaired Th1 immune response may contribute to a Th2-dominant immune response seen in NC/Nga mice. It was shown that IL-12 was unable to downregulate IgE synthesis in splenocytes of NC/Nga mice, possibly due to decreased phosphorylation of signal transducers and transactivators of transcription (STAT)-4. Furthermore, there was a defective production of IFN- γ by T cells of NC/Nga mice when the cells were stimulated with recombinant IL-12 [17]. In another study, it has been noted that a defective IFN- γ response to bacterial stimulation can lead to a Th2-dominant state, overproduction of IgE, and increased susceptibility to dermatitis in NC/Nga mice [18].

Interesting observations were made from a recent study on NC/Nga mice that are also STAT-6 deficient (in non-SPF conditions). Despite a lack of production of IgE and Th2 cytokines, these mice nevertheless developed the AD-like skin disease as did their STAT-6-intact NC/Nga littermates [19], suggesting that the development of AD-like skin lesions in NC/Nga mice may occur via an IgE- and STAT-6-independent pathway and that Th2 immune responses may not be necessary in the initiation of the skin disease in these mice [19]. It is possible that an alternative Th2 development pathway, independent of IL-4 receptor alpha, mediated the signaling. It has been shown that GATA-3 exhibits STAT-6-independent autoactivation, creating a feedback pathway stabilizing Th2 commitment in antigenic stimulation systems [20] and that an unimpaired Th2 polarization in response to *Leishmania major* led to infection in interleukin-4 receptor alpha-deficient BALB/c mice [21]. More recently, it has been noted that the AD-like lesional skin of NC/Nga mice displays oligoclonal T-cell accumulation, suggesting antigen-specific immune responses [22]. It would be interesting to know which antigens stimulate T cells to expand in the AD-like lesions of NC/Nga mice.

Most recently, interesting data derived from NC/Nga mice [23] showed that sensitization of the skin under SPF environments followed by repeated challenges with hapten, such as dinitrofluorobenzene (DNFB), results in chronic dermatitis associated with high levels of serum IgE. These mice demonstrated a significantly increased production of Th1 cytokines IL-12, IL-18, and IFN- γ by CD4⁺ T cells. High levels of IgE in the absence of IL-4 production in these mice challenged with DNFB suggest that hypersecretion of IgE is attributed to constitutive hyperphosphorylation of JAK3, a nonreceptor protein tyrosine kinase involved in IL-4 signaling [16]. Another Th2 cytokine, IL-13, is known to induce IL-4-independent IgE production [24]. However, the role of IL-13 was not evaluated in this study. Because IL-4 can function as a

suppressor for Th1 development, insufficiency of IL-4 expression may have contributed to the chronic skin lesions induced by Th1 cytokines.

The data generated from the NC/Nga mouse model demonstrate that the main features of AD in these mice are similar to those observed in human AD, and these studies provide very useful insights into mechanisms by which Th2 immunity plays a central role in the pathogenesis of spontaneous AD-like skin disease developed in non-SPF environments. The Th2 immune responses may occur via a STAT-6-independent pathway. The relevance and importance of environmental factors, such as microbes and related factors, as well as genetic factors in the generation of AD-like skin lesions, are demonstrated in this murine model.

Atopic dermatitis-like lesions in genetically modified mice

Transgenic mice and mice with targeted gene disruption (knockout mice) are important and valuable research tools commonly used in many fields of biology and medicine. Studies using transgenic and knockout mice have greatly contributed to our understanding of the roles of a variety of individual cytokines, enzymes, and other molecules in the pathogenesis of AD. In general, transgenic or knockout mice can be generated with target genes believed or suspected to be important in the pathogenesis of AD. But a more commonly used approach is to examine the role of individual cytokines/factors in knockout mice in combination with other AD models, such as allergen sensitization and challenge models.

Recently, transgenic mice have been generated that express IL-4 under the control of keratinocyte-specific promoter (K14), which selectively targets the expression of IL-4 in the skin. These mice exhibited spontaneous chronic AD-like lesions when housed in either SPF or non-SPF conditions [25•]. Overexpression of IL-4 in mice elicits all of the main features characteristic of human atopic dermatitis, namely, xerosis, inflammatory skin lesions, staphylococcus colonization and infiltration of T cells, mast cells, eosinophils, and macrophages in the skin lesions. The immune response seen in IL-4 transgenic mice is characterized by an upregulation of Th2 cytokines in the early phase and enhanced expression of Th1 cytokines in the late phase of the skin lesion [26], suggesting that at different stages, both Th2 and Th1 inflammation are present for the initiation and maintenance of chronic skin inflammatory responses in this model. Further studies of these transgenic mice by the same group have shown that chronic inflammation in the skin lesion is driven by a continuous and incremental migration of activated inflammatory cells from the secondary lymphoid organs into the skin [27].

Transgenic overexpression of caspase-1, also known as IL-1 β -converting enzyme (ICE), specifically in keratinocytes (KCASP-1), causes spontaneous release of active IL-18, chronic dermatitis, and production of total IgE

under SPF conditions [28,29]. The role of STAT-6 in these mice was evaluated in STAT-6-deficient KCASP-1 mice. In the absence of STAT-6, KCASP-1 transgenic mice still displayed chronic inflammatory dermatitis but without detectable IgE. However, depletion of IL-18 in KCASP-1 transgenic mice resulted in diminished pruritic dermatitis by abolishing the infiltration of mast cells to the skin and the levels of plasma histamine, although significant serum levels of total IgE were present [30]. It is known that the main function of caspase-1 is to convert pro-IL-18 to active IL-18. Therefore, the pathologic changes of the skin seen in KCASP-1 mice may be a result of increased production of active IL-18. IL-18 initially was thought of as a Th1 cytokine. However, later studies demonstrated that IL-18 is a pleiotropic cytokine that plays an important role in both Th1- and Th2-mediated immunity [31,32] and has been shown to be important in the pathogenesis of asthma [33]. The possible involvement of IL-18 in human AD has been suggested in a study that showed that the levels of serum IL-18 correlates with disease severity in young children with atopic dermatitis [34]. Evidence for a possible role of IL-18 in chronic inflammatory skin disease came from studies of an IL-18 transgenic model (KIL-18 mouse) in which IL-18 is selectively expressed in the skin under the control of keratinocyte promoter (K5) [30]. Overexpression of IL-18 in the skin caused AD-like skin lesions associated with high levels of plasma histamine, intense pruritis, and elevated total IgE. These findings indicated that IL-18 induces a hallmark of chronic AD-like inflammatory skin disease independent of STAT-6/IgE in SPF conditions.

The role of Th2 immunity in generating chronic dermatitis is also supported by a recent study of IL-31 [35]. IL-31 was recently identified as a Th2 cytokine, primarily produced by Th2 cells. IL-31 signals through its receptor consisting of IL-31 receptor A (IL-31RA) and through the oncostatin M (OSM) receptor, both expressed constitutively in epithelial cells and keratinocytes. When IL-31 was overexpressed under the control of the lymphocyte-specific promoter-enhancer (Eu-Lck) or the ubiquitous promoter from elongation factor-1 α (EF1 α), both types of transgenic mice displayed chronic pruritic dermatitis followed by alopecia and dermal infiltration of mast cells and other inflammatory cells. However, there was a lack of increased serum IgE [35]. This low-serum IgE level in IL-31 transgenic mice suggested that IL-31 induces pruritic dermatitis in an IgE-independent pathway as seen in patients with intrinsic atopic dermatitis. It has been reported that IL-31 stimulates cultured keratinocytes to produce Th2 chemokines TARC and MDC, known to be important in human atopic dermatitis [14]. IL-31 receptor A is also noted to be upregulated in lung epithelial cells of OVA-challenged, wild-type mice [35].

One mouse model with AD-like skin lesions resulted from gene-targeted disruption of transcription factor RelB (RelB-deficient mice) [36]. Besides impaired hematopoiesis, these RelB-deficient mice developed itchy dermati-

tis with many features similar to extrinsic atopic dermatitis in humans, including infiltration of CD4⁺ T cells, eosinophils in the skin, high expression of Th2 cytokines (IL-4 and IL-5) and Th1 cytokine IFN- γ in skin lesions and elevated serum IgE levels and eosinophilia. However, no dermatitis can be found in RelB-deficient mice that were also deficient in T cells, indicating that the dermatitis in these RelB-deficient mice is T-cell dependent [36].

Atopic dermatitis-like lesions induced by topical allergen sensitization

It has been shown that contact with specific aeroallergens can cause flares of AD and that topical application of aeroallergens can elicit an IgE response, resulting in an eczematous reaction in AD [37], and that avoidance of the same aeroallergens resulted in an improvement of dermatitis [38,39]. A role for cutaneous exposure to aeroallergens in the pathogenesis of AD is suggested by the studies showing epicutaneous application of allergens to wild-type or genetically altered mice induces atopic dermatitis-like lesions [9••,40]. Spengel *et al.* [9••] showed that wild-type BALB/c and C57BL/6 mice developed local inflammatory dermatitis after repeated epicutaneous sensitization with OVA. The dermatitis was associated with marked skin thickening, a dermal cellular infiltrate of T cells and eosinophils, increased expression of IL-4 and IL-5, and a significant elevation of total serum IgE levels. Furthermore, this epicutaneous sensitization with protein antigen OVA led to antigen-specific airway hyperresponsiveness. Thus, this mouse model provided evidence that suggests a connection between epicutaneous sensitization with allergen and the development of airway hyperresponsiveness.

The availability of numerous targeted gene deletion mice provided abundant opportunity for dissecting specific pathways and molecular mechanisms that underlie AD. The roles of individual cytokines, chemokine receptor CCR3, anaphylatoxin C3a, T-cell subpopulation, and mast cells in the pathogenesis of AD [40–44] are demonstrated in targeted-gene deletion murine models of AD elicited by repeated epicutaneous sensitization with OVA or superantigen (staphylococcal enterotoxins B [SEB]) [45]. Spengel *et al.* [40] showed that OVA-sensitized skin from IL-5-deficient (IL-5(-/-)) mice had undetectable eosinophils and exhibited decreased skin thickening. OVA-sensitized skin of IL-4(-/-) mice had normal thickening of the skin associated with significantly reduced eosinophils and a significant increase in infiltrating T cells and increased expression of macrophage-infiltrating protein (MIP)-2, MIP-1a, and RANTES (released on activation, normal T-cell expressed and secreted). In contrast, sensitized skin from IFN- γ (-/-) mice displayed a reduced dermal thickening. Interestingly, IgE-deficient mice (IgE(-/-)) developed the same degree of allergic skin inflammation as wild-type mice [40], indicating that IgE may not be as essential in the development of inflammatory skin disease in this model as in human intrinsic atopic dermatitis. A recent study [44] evaluated the

role of IL-10 in a mouse model of allergic dermatitis induced by epicutaneous sensitization with OVA on tape-stripped skin in IL-10 deficient mice. Infiltration of eosinophils, expression of eotaxin, IL-4, and IL-5 mRNA in OVA-sensitized skin were diminished in IL-10(-/-) mice, but higher IFN- γ was found in the splenocytes of these mice when compared with that of wild-type mice, suggesting that IL-10 is important for Th2 immunity in allergic dermatitis.

The role of α/β T cells, B cells, or CD40-CD40L interactions in skin inflammation and Th2 response in AD has been demonstrated in mice with targeted gene deletions [46]. When these mice were epicutaneously sensitized with OVA, B cell-deficient IgH(-/-) mice developed remarkable dermal cellular infiltration, induction of IL-4, and elevated levels of serum total IgE. However, all of these findings were absent in RAG-2(-/-) mice, which are deficient in both T cells and B cells. These responses were also elicited in T-cell receptor (TCR) δ -deficient mice after sensitization, but not in OVA-sensitized skin sites from TCR α -deficient mice. OVA-sensitized skin from CD40-deficient (CD40(-/-)) mice exhibited significant infiltration with eosinophils and an increased expression of IL-4 mRNA [46]. These results suggest that CD40L-CD40 interaction is not critical for the development of skin inflammation and for enhanced expression of IL-4 after epicutaneous OVA sensitization. Taken together, these findings indicate that α/β T cells, B cells, and CD40-CD40L play critical roles in Th2 immune responses in the development of AD in these particular animal models.

The CC chemokine receptor 3 (CCR3) mainly binds eotaxins and is expressed on Th2 cells, eosinophils, and mast cells. CCR3 plays an important role in allergic inflammatory responses. After repeated epicutaneous sensitization to OVA, CCR3-deficient (CCR3(-/-)) mice showed diminished recruitment of eosinophils to the skin but the same recruitment of mast cells and T cells as seen in sensitized wild-type mice. Furthermore, CCR3(-/-) mice failed to recruit eosinophils to the lung and to induce airway hyperresponsiveness, suggesting that CCR3 and its ligands are essential in recruiting eosinophils to the skin and lung and in inducing airway hyperresponsiveness [41].

Mast cells are important effector cells in IgE-mediated allergic diseases and have been shown to appear in the skin lesions of AD patients and in many of the AD-like lesions in mouse models. The role of mast cells in allergic skin inflammatory disease was evaluated using mice deficient in mast cells (W/W^v) [42]. OVA-sensitized skin sites from W/W^v mice exhibited infiltration of eosinophils, T cells, and mononuclear cells. Expression of IL-4 mRNA in sensitized skin sites was similarly increased in wild-type and W/W^v mice. However, the serum total IgE and expression of IFN- γ mRNA was significantly enhanced in the sensitized skin of W/W^v mice, but not in that of wild-type controls [42]. These results suggest that mast cells may have a regulatory role in the expression of IFN- γ and total IgE levels in this AD model.

The skin of patients with AD is frequently colonized with enterotoxin-secreting *Staphylococcus aureus* [47], which exacerbates skin lesions in patients with AD [48]. Epicutaneous sensitization of wild-type mice with superantigen (SEB) caused allergic skin inflammation, Th2-dominant immune responses, and increased IgE synthesis [45]. These findings are characteristic of patients with AD.

Mechanical injury induces the accumulation of C3a in skin blisters [35], and itchy skin is a main feature of AD. A similar animal model of epicutaneous sensitization of OVA in mice deficient in anaphylatoxin C3a receptor (C3aR (-/-)) was used to assess the role of C3a in allergic inflammatory skin disease [43]. C3aR(-/-) mice developed an exaggerated Th2 response to epicutaneous sensitization to OVA and an inability of splenocytes from C3aR(-/-) mice to secrete Th2 cytokine in response to TCR ligation, suggesting that C3a-C3aR interaction inhibits the ability of antigen-presenting cells to drive Th2-cell differentiation in response to epicutaneously introduced antigen.

Atopic dermatitis-like diseases can also be induced by epicutaneous sensitization with house dust mite allergens such as HDM (*Dermatophagoides farinae* [DF]). Epicutaneous sensitization with house dust mite allergens in wild-type BALB/c mice induced allergic dermatitis and neurogenic inflammation in the sensitized skin and high levels of total and specific IgE [49]. Epicutaneous sensitization by a crude extract of DF in NC/Nga mice housed in non-SPF conditions elicits itchy dermatitis and increased total and specific IgE [50].

Murine models of atopic dermatitis after oral sensitization of food allergens

The cause of AD in human subjects remains enigmatic. Recent studies showed a strong association between IgE-mediated food allergy and atopic dermatitis in children [51]. Food hypersensitivity exacerbates the skin lesions in more than 50% of all children with atopic dermatitis [52]. Elimination of specific foods from the diet of patients with food allergy and AD resulted in a significant improvement in their eczematous skin diseases [53]. The role of food hypersensitivity in the pathogenesis of AD has been demonstrated in a mouse model [54••]. In this study, C3H/HeJ mice were sensitized orally to cow's milk and peanut with a cholera toxin adjuvant and then subjected to repeated injection of low-grade food allergens. One third of these mice had elevated levels of food-specific IgE antibodies; peripheral blood eosinophilia; an inflammatory skin eruption characterized by an infiltration of CD4⁺ Th2 lymphocytes and increased numbers of eosinophils and mast cells; and enhanced expression of Th2 cytokines (IL-5 and IL-13) [54••]. This study demonstrates that oral sensitization to food allergens elicits systemic immune responses and inflammatory skin lesions that resemble human AD.

Conclusions

In the past decade, a number of mouse models of AD have been discovered or developed. Inbred strains, genetically modified mice, and allergen sensitization models are all valuable tools for studying AD. The combined approach of using genetically modified mice with allergen sensitization is particularly powerful. Together, these murine models have provided us with valuable insights into the pathogenic mechanisms responsible for atopic dermatitis and have contributed greatly to our understanding of the immunopathogenesis of atopic dermatitis. These studies demonstrated the importance of genetic and environmental factors in generating AD. Studies from some of the mouse models provided evidence that the development of skin lesions may precede or lead to the development of systemic allergic responses, such as asthma, and that exposure to food allergens and other allergens (such as OVA and HDM) can induce allergic inflammatory skin diseases. Another important role these animal models play in the study of AD or in other diseases in general is that they provide excellent tools for testing potential therapeutic targets that may be further developed for the treatment of AD and other allergic diseases. Although mouse models of AD resemble human AD in many aspects, these models, like many other animal models, are not perfect. New and better animal models of AD still need to be generated or discovered.

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This study demonstrated in a murine model that AD could be induced by food hypersensitivity.

