Animal Models of Atopic Dermatitis

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Atopic dermatitis (AD) is characterized by allergic skin inflammation. A hallmark of AD is dry itchy skin due, at least in part, to defects in skin genes that are important for maintaining barrier function. The pathogenesis of AD remains incompletely understood. Since the description of the Nc/Nga mouse as a spontaneously occurring model of AD, a number of other mouse models of AD have been developed. They can be categorized into three groups: (1) models induced by epicutaneous application of sensitizers; (2) transgenic mice that either overexpress or lack selective molecules; (3) mice that spontaneously develop AD-like skin lesions. These models have resulted in a better understanding of the pathogenesis of AD. This review discusses these models and emphasizes the role of mechanical skin injury and skin barrier dysfunction in eliciting allergic skin inflammation.

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Introduction

Atopic dermatitis (AD) is an increasingly common pruritic inflammatory skin disorder that affects at least 15% of children and is characterized by cutaneous hyperreactivity to environmental triggers (Geha, 2003; Leung and Bieber, 2003; Novak *et al*., 2003) The diagnosis of AD is based on clinical presentation of skin erythematous plaques, eruption, and/or lichenification typically in flexural areas accompanied by intense pruritus and cutaneous hypersensitivity. The skin lesions are associated with one or more typical atopic signs such as palmar hyperlinearity and infraorbital fold. Pathological examination reveals spongiosis, hyperkeratosis, and parakeratosis in acute lesions and marked epidermal hyperplasia, acanthosis, and perivascular accumulation of lymphocytes and mast cells in chronic lesions. Most AD patients have a personal or family history of allergies or asthma. Infants with AD have an increased tendency to develop asthma and allergic rhinitis later in life, a phenomenon known as the atopic march (Spergel and Paller, 2003).

AD has a complex etiology that involves abnormal immunological and inflammatory pathways that include defective skin barrier, exposure to environmental agents, and neuropsychological factors (Fartasch, 1997; Pastore *et al*., 1997; Trautmann *et al*., 2000; Geha, 2003; Leung and Bieber, 2003; Novak *et al*., 2003; Howell *et al*., 2004). Approximately 70–80% of AD patients present with the ''extrinsic'' form of AD. They have elevated serum IgE levels with IgE antibodies to environmental and/or food allergens. The remaining 20–30% present with the ''intrinsic'' form of AD and have low serum IgE levels with no evidence of IgE antibodies (Leung *et al*., 2004). However, a number of these individuals develop evidence of allergic IgE-mediated sensitization later in life.

A hallmark of AD is dry itchy skin. It is believed that this is due to defects in skin genes that are important for maintaining skin barrier function and turgidity. In addition, genes that promote pruritus, for example, IL-31 or a Th2 response to allergens, are likely to also contribute to the pathogenesis of AD. Recently, up to 15% of patients with AD were found to have mutations in the epidermally expressed filaggrin gene, which is important for skin barrier function and turgidity (Palmer *et al*., 2006; Morar *et al*., 2007; Nomura *et al*., 2007). Intense pruritus and the resulting scratching cause mechanical skin injury that leads to cytokine and chemokine release in the skin, which in return causes a further increase in skin permeability that further promotes entry of allergens in the skin (Homey *et al*., 2006). Epicutaneous (EC) sensitization to allergens, which requires mobilization of antigen-laden skin dendritic cells (DCs) to draining lymph nodes (DLN), is believed to play an important role in the pathogenesis of the disease. This is supported by the observation that application of allergen to the abraded uninvolved skin of patients with AD provokes an eczematous rash with eosinophilic infiltration (Mitchell *et al*., 1982).

Acute AD skin lesions exhibit Th2-dominant inflammation characterized

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Abbreviations: AD, atopic dermatitis; APC, antigen-presenting cell; CASP1, caspase-1; CCR3, CC chemokine receptor 3; DC, dendritic cell; DLN, draining lymph nodes; EC, epicutaneous; OVA, ovalbumin; Ox, oxazolone; SC, stratum corneum; TSLP, thymic stromal lymphopoietin; WT, wild type

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by dermal infiltration of CD4$^+$ T cells and eosinophils with the deposition of eosinophil products and increased skin expression of Th2 cytokines. Subsequently, the chronic phase demonstrates a local Th1 IFN-γ response and tissue remodeling with increased deposition of collagen and dermal thickening. There are also increased numbers of mast cells, but virtually no accumulation of neutrophils. IgE receptor-bearing DCs, both myeloid and plasmacytoid, also accumulate in the lesion and may be instrumental in the perpetuation of AD (Novak *et al.*, 2004; Novak and Bieber, 2005).

DCs are essential for the generation of an immune response and hence play a critical role in the pathophysiology of AD. In the skin, there are two main types of immature DCs, namely Langerhans cells in the epidermis and interstitial (dermal) DCs in the dermis. Langerhans cells form a DC network in the epidermis where they sample antigens that get through the skin barrier of stratum corneum (SC). Antigens that reach the interstitial spaces are taken up by interstitial (dermal) DCs. Following antigen uptake and in the presence of a danger signal generated by microbial antigens that include normal flora and/or mechanical injury with its release of mediators such as IL-1 and tumor necrosis factor-α, immature DCs acquire the phenotype of functional antigen-presenting cells (APC). They upregulate the expression of major histocompatibility complex class II molecules and of key co-stimulatory molecules such as CD80 and CD86, reduce their capacity to capture antigen, and express chemokine receptors, particularly CCR7, the receptor for the chemokines CCL19 and CCL21 expressed in DLNs. The expression of CCR7 allows skin DCs to migrate toward DLN where they present antigenic peptides to naive T cells that continuously circulate through the DLN. Interaction between antigen-laden DCs and antigen-specific T cells leads to T-cell proliferation and differentiation. Differentiation of CD4$^+$ T cells leads to the generation of Th1 cells that secrete IFN-γ, Th2 cells that secrete IL-4, IL-5, and IL-13, or Th17 cells that secrete IL-17 and IL-22. DCs play a critical role in the polarization of T cells into Th1, Th2, or Th17 cells depending on their specific expression of cytokines and co-stimulatory molecules and the influence of the individual tissue milieu from which they originate. After EC sensitization, loss of protection by the skin barrier increases the exposure of Langerhans cells and dermal DCs to environmental antigens. Cytokines released by resident skin cells are likely to play an important role in influencing the ability of DCs to polarize T cells. Examples of these cytokines are thymic stromal lymphopoietin (TSLP) and IL-6. TSLP is an IL-7-like cytokine produced by keratinocytes, and is highly expressed in AD skin lesions. Evidence suggests that TSLP polarizes DCs to promote an inflammatory Th2 response characterized by high-production tumor necrosis factor-α with little production of IL-10 (Liu, 2007). Transforming growth factor-β and IL-6 expression is upregulated in skin after mechanical injury, which may be important in polarizing DCs to promote a Th17 response to EC sensitization injury (He *et al.*, 2007; our unpublished observations).

Our understanding of human diseases has been enormously expanded by the use of animal models, because they allow in-depth investigation of pathogenesis and provide invaluable tools for diagnostic and pharmaceutical purposes. Because AD is a common disease for which there is no satisfactory therapy, understanding AD through the study of animal models is a pressing need. Although species other than mouse, for example, dogs and guinea pigs, can develop AD-like lesions, mouse models are primarily used because of the ease of manipulation, low cost, and most importantly the availability of genetically manipulated strains. Since the description of the Nc/Nga mouse as the first spontaneously occurring model of AD in 1997 (Matsuda *et al.*, 1997), a number of mouse models have been developed. These models can be categorized into three groups: (1) models induced by EC application of sensitizers; (2) transgenic mice that either overexpress or lack selective molecules; (3) mice that spontaneously develop AD-like skin lesions. These models display many features of human AD, and their study has resulted in a better understanding of the pathogenesis of this disease.

AD models induced by EC sensitization

An animal model of AD induced by skin injury and EC sensitization with allergen. Our laboratory has developed a mouse model of AD induced by repeated EC sensitization of tape-stripped skin with ovalbumin (OVA) (Spergel *et al.*, 1998). This model operates in all five strains of mice tested to date including BALB/c and C57BL/6 mouse strains (Spergel *et al.*, 1999). The back skin of mice is shaved and tape stripped six times with 3M tape, mimicking skin injury inflicted by scratching in patients with AD. A 100 μg portion of OVA in 100 μl of normal saline or 100 μl of normal saline is placed on a 1 × 1 cm patch of sterile gauze, which is secured to the skin with a transparent biocclusive dressing. This ensures that the antigen is not accessible to licking. Each mouse has a total of three 1 week exposures to the patch at the same site; exposures are separated by 2 week intervals (Figure 1).

EC-sensitized mice develop increased scratching behavior, and their skin develops lesions characterized by epidermal and dermal thickening, infiltration of CD4$^+$ T cell and eosinophils (Figure 2a), and upregulated expression of the Th2 cytokines IL-4, IL-5, and IL-13 (Figure 2b), with little or no change in the expression IFN-γ. There is enhanced expression of eotaxin and thymus and activation-regulation chemokine, the chemokines that attract CC chemokine receptor 3 (CCR3)$^+$ eosinophils and skin-homing CCR4$^+$ CD4$^+$ T cells, respectively. There is also increased deposition of collagen. Systemically, serum OVA-specific IgG1, IgE, and IgG2a are elevated, and splenocytes from OVA-sensitized mice produce increased level of IL-4, IL-5, IL-13, and IFN-γ in response to OVA re-stimulation (Spergel *et al.*, 1998). The fact that antigen-specific IFN-γ-producing cells are present in the spleen, with no detectable upregulation of IFN-γ expression in sensitized skin sites, suggests that local factors at the site of sensitization

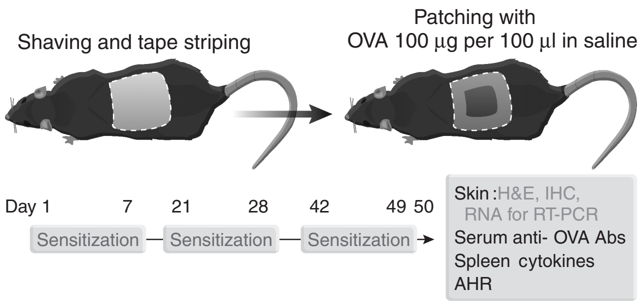

Figure 1. EC sensitization protocol. Mice were sensitized with ovalbumin (OVA) (100 μg) or saline applied in 100 μl to a sterile patch. The patch was placed for a 1-week period and then removed. Two weeks later, an identical patch was reapplied to the same skin site. Each mouse had a total of three 1 week exposures to patch separated from each other by 2 week intervals. All experiments were performed at the end of the third sensitization.

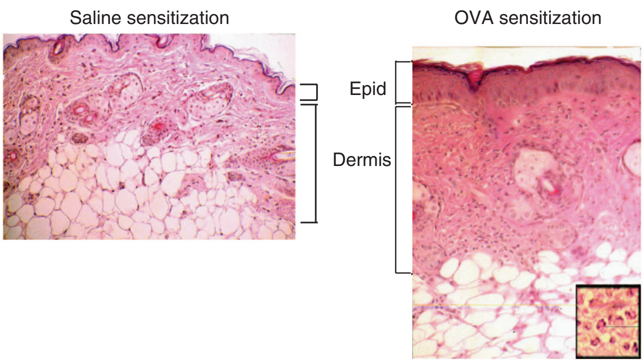

Figure 2. Histological features of ovalbumin (OVA) and saline-sensitized skin sites in BALB/c mice. Skin sections were stained with hematoxylin and eosin (H&E) and examined at original magnifications $\times$200 and $\times$400. There is marked hyperplasia of the epidermis, a dermal infiltrate, and mild spongiosis. The cellular infiltrate consists of neutrophils, eosinophils, and lymphocytes. Further magnification in the inset (bold-bordered box) shows the presence of the multiple eosinophils.

promote selectively the activation of Th2 cells. In this respect, TSLP promotes the secretion of Th2 cytokines with no detectable effect on the secretion of Th1 cytokines by TCR-OVA transgenic T cells stimulated *in vitro* with OVA peptide (R He, unpublished observations). In addition, OVA-sensitized mice develop increased airway hyperresponsiveness following inhalation challenge with OVA, a feature that parallels the occurrence of asthma in a majority of patients with AD. (Spergel *et al.*, 1998). Decreasing the cycles of sensitization from three to two or compressing the duration of the sensitization protocol by decreasing the interval between the cycles of sensitization leads to suboptimal development of allergic skin inflammation. The requirement for a 7-week protocol of EC sensitization, although cumbersome, appears to mimic the exacerbation of AD over time. Withdrawal of antigen sensitization at the end of the 7-week protocol results in decreased skin inflammation with IL-4 mRNA levels returning to baseline within 7–10 days, whereas IL-13 mRNA levels decrease over a longer period of time.

We have used this mouse model to determine the critical cellular players involved in allergic skin inflammation. Using RAG2$^{-/-}$ mice, which lack both T and B cells, B-cell-deficient IgH$^{-/-}$ mice, TCRβ$^{-/-}$ mice, and CD40-deficient mice, we demonstrated that TCRαβ^{+} T cells, but not γδ^{+} T cells, B cells, or CD40L–CD40 interactions are critical for skin inflammation and the Th2 response in AD (Woodward *et al.*, 2001). The study of mast cell-deficient mice indicated that mast cells are not important for the development of Th2-mediated skin inflammation; however, they regulate IFN expression in the skin (Alenius *et al.*, 2002) This is important given the role of IFN-γ in upregulating Fas expression on keratinocytes, thus targeting them for killing by activated FasL^{+} T cells (Trautmann *et al.*, 2000) and given the role of IgE-mediated reactions in exacerbating AD (Milgrom, 2002). A recent study showed that iNKT cells are not required for allergic skin inflammation in this model. Skin infiltration by eosinophils and CD4^{+} cells and expression of mRNA encoding IL-4 and IL-13 in OVA-sensitized skin were similar in wild-type (WT) and CD1d$^{-/-}$ mice. No significant increase in iNKT cells was detectable in epicutaneously sensitized skin. In contrast, iNKT cells were found in the bronchoalveolar lavage fluid from OVA-challenged epicutaneously sensitized WT mice, but not in CD1d$^{-/-}$ mice, and EC-sensitized CD1d$^{-/-}$ mice had decreased expression of IL-4, IL-5, and IL-13 mRNA in the lung and impaired airway hyperresponsiveness in response to airway challenge with OVA (ElKhal *et al.*, 2006).

We have used the EC sensitization model to examine the role of a number of molecules, cytokines, chemokines, and molecules of innate immunity in the development of allergic skin inflammation elicited by EC exposure to allergens (Spergel *et al.*, 1999; Ma *et al.*, 2002; Kawamoto *et al.*, 2004; Laouini *et al.*, 2005). Both the Th2 cytokines IL-4 and IL-5 and the Th1 cytokine IFN-γ play important roles in the inflammation and hypertrophy of the skin in AD. Eosinophils are virtually absent in OVA-sensitized skin sites of IL-5$^{-/-}$ mice. OVA-sensitized skin sites of IL-4$^{-/-}$ mice have increased inflammatory cells but decreased eosinophils, and those of IFN-γ$^{-/-}$ mice have decreased thickening of the dermal layer (Spergel *et al.*, 1999).

IL-10 plays an important role in the Th2 response to antigen and in the development of skin eosinophilia in our model (Laouini *et al.*, 2003a). Skin infiltration by eosinophils and expression of eotaxin, IL-4, and IL-5 mRNA in OVA-sensitized skin sites were all severely diminished in IL-$10^{-/-}$ mice. After *in vitro* re-stimulation with OVA, splenocytes from EC-sensitized IL-$10^{-/-}$ mice secreted significantly less IL-4, but significantly more IFN-γ than splenocytes from WT controls. IL-$10^{-/-}$ APCs skewed the *in vitro* response of OVA TCR transgenic T cells toward Th1. Examination of the Th response of WT and IL-$10^{-/-}$ mice immunized with OVA-pulsed WT or IL-$10^{-/-}$ DCs revealed that both DCs and T cells participate in IL-10-mediated skewing of the Th2 response *in vivo*. Current experiments are addressing the hypothesis that IL-10 released by keratinocytes after mechanical injury might promote the Th2 response to EC sensitization through polarizing skin DCs to support Th2 differentiation.

CCR3 is expressed by eosinophils, mast cells, and Th2 cells. Recruitment of eosinophils to OVA-sensitized skin was severely impaired in CCR$3^{-/-}$ mice. These mice also have impaired recruitment of eosinophils in their lung parenchyma and bronchoalveolar lavage fluid, and fail to develop airway hyperresponsiveness to methacholine following antigen inhalation. These results suggest that CCR3 plays an essential role in eosinophil recruitment to the skin and the lung and in the development of airway hyperresponsiveness (Ma *et al.*, 2002). Skin-homing T cells express the chemokine receptor CCR4. The CCR4 ligand thymus and activation-regulation chemokine is highly expressed in most AD skin lesions. Experiments with CCR$4^{-/-}$ mice have revealed decreased CD4^+ cell infiltration in OVA-sensitized sites as well as decreased expression of IL-4 and IL-13 mRNA levels (our unpublished observations). CCR10 is also expressed on a subset of skin-homing cells. Anti-CCR10 was reported to inhibit skin inflammation in response to EC sensitization with OVA (Homey *et al.*, 2002). However, we have found that CCR$10^{-/-}$ mice develop normal allergic skin inflammation (our unpublished observations).

Recently, we found that EC sensitization with OVA drives the generation of IL-17-producing T cells in DLNs and spleen and in a local and systemic Th17 response (He *et al.*, 2007). OVA inhalation by EC-sensitized mice induced IL-17 and CXCL2 expression and neutrophil influx in the lung along with bronchial hyperreactivity, which were reversed by IL-17 blockade. This is in contrast to the eosinophil-dominated response of intraperitoneally immunized mice to airway challenge. Although IL-17 was expressed in EC-sensitized skin, there was little expression of CXCL2 and little infiltration of neutrophils at EC-sensitized skin sites. However, mechanical injury upregulated the expression of IL-6 and IL-23 in skin. IL-6, like transforming growth factor-β, is an inducer of Th17 cells (Veldhoen *et al.*, 2006), whereas IL-23 promotes the growth of these cells (Langrish *et al.*, 2005). DCs trafficking from skin to lymph nodes expressed more IL-23 and induced more IL-17 secretion by naive T cells than splenic DCs. This was inhibited by neutralizing IL-23 *in vitro* and by intradermal injection of anti-transforming growth factor-β-neutralizing antibody *in vivo*. These findings suggest that initial cutaneous exposure to antigens in patients with AD may selectively induce the generation of IL-17 producing cells. Upon antigen inhalation, these cells are recruited to the lungs where they are activated to secrete IL-17, which drives a neutrophil-rich inflammation in the airways. These findings should prompt a search for airway and lung neutrophils in AD patients who develop asthma in response to inhalation of EC sensitizers. This could have important therapeutic implications.

We have identified a number of negative regulators of allergic skin inflammation in our model. C3a$R^{-/-}$ mice exhibited an exaggerated Th2 response to EC sensitization with OVA. Presentation of OVA peptide by C3a$R^{-/-}$ APCs caused significantly more IL-4 and IL-5 secretion by T cells from TCR-OVA DO11.10 transgenic mice compared with the presentation by WT APCs. C3a inhibited the ability of splenocytes, but not of highly purified T cells, to secrete Th2 cytokines in response to TCR ligation. This inhibition was mediated by IL-12 secreted by APCs in response to C3a. These results suggest that C3a–C3aR interactions inhibit the ability of APCs to drive Th2 cell differentiation in response to epicutaneously introduced antigen (Kawamoto *et al.*, 2004). Cyclooxygenase 2 was also shown to limit the Th2 response to EC sensitization. Infiltration by eosinophils and expression of IL-4 mRNA in OVA-sensitized skin sites, OVA-specific IgE and IgG1 antibody responses, and IL-4 secretion by splenocytes after OVA re-stimulation were all significantly increased in EC-sensitized mice that received NS-398, a cyclooxygenase 2 inhibitor. In contrast, OVA-specific IgG2a antibody response and IFN-γ secretion by splenocytes after OVA re-stimulation were significantly decreased in these mice. Cyclooxygenase 2-deficient mice also exhibited an enhanced systemic Th2 response to EC sensitization. These findings are important, as they suggest that cyclooxygenase inhibitors may worsen allergic skin inflammation in patients with AD (Laouini *et al.*, 2005). The complement component C3 is synthesized by keratinocytes and is activated after skin injury. Skin infiltration by eosinophils and the expression of Th2 cytokines in OVA-sensitized skin sites were impaired in C$3^{-/-}$ mice. Splenocytes from epicutaneously sensitized C$3^{-/-}$ mice secreted less IL-4, IL-5, IL-13, and IFN-γ in response to OVA re-stimulation than splenocytes from WT control animals. C$3^{-/-}$ mice also had impaired IgG1, IgG2a, and IgE antibody responses after both EC immunizations. These results suggest that C3 plays an important role in both the Th1 and Th2 response to antigen in AD (Yalcindag *et al.*, 2006). The opposing consequence of C3aR and C3 deficiency in our model suggests that C3 degradation products other than C3a may promote allergic skin inflammation. C3b is a good candidate, as its receptor is expressed on DCs.

Mechanical injury is critical in our model, because application of OVA to the skin of hairless mice does not result in the development of an immune response to OVA. Recently, we have begun to test the hypothesis that mechanical injury not only allows the breaching of the skin barrier and the entry of antigen which is then captured by skin DCs, but also releases mediators that may play critical roles in polarizing the DCs to drive the differentiation of Th2 cells in DLN. Gene array analysis of mouse skin 12 hours after skin injury reveals the upregulation of a number of cytokines with a remarkable increase in IL-6, a cytokine which is important for both Th2 and Th17 differentiation (our unpublished data). There is also an increase in IL-23, IL-1, and IL-10 gene expression. In addition, a number of chemokine genes, as well as genes for metalloproteinases and kallikreins, are highly upregulated. These injury-induced molecules are likely to play important roles in determining the polarity of the immune response to EC sensitization. In this regard, neutralization of IL-23 blocks the Th17 response (Langrish *et al.*, 2005; Chen *et al.*, 2006), and blocking IL-10 impairs the Th2 response (Oh *et al.*, 2002). We are using FITC painting of shaved versus shaved and tape-stripped skin to track DCs that have emigrated from skin to DLN to test the hypothesis that this polarization effect is exerted at the level of the DCs that carry antigen from skin to DLN (Figure 3). Preliminary data suggest that $FITC^{hi}$ DCs isolated from DLN of shaved tape-stripped skin induce significantly more Th2 cytokine secretion in TCR-OVA transgenic D011.10 cells than $FITC^{hi}$ DCs isolated from DLN of shaved skin that has not been tape stripped. Comparative analysis of the genes differentially expressed by these two populations of DCs should help elucidate the nature of the ''danger signal'' elicited by mechanical skin injury that results in the generation of a predominantly Th2 response to EC sensitization.

AD model induced by EC application of house dust mite allergen. Clinical studies have provided evidence that house dust mite allergen is associated with human AD (Kimura *et al.*, 1998). BALB/c mice subjected to EC application of the recombinant mite allergen Der p8 exhibited features of dermatitis with epidermal hyperplasia and spongiosis, skin infiltration with $CD4^+$ and $CD8^+$ cells, and a skewed Th2 response locally and systemically (Huang *et al.*, 2003). These findings are similar to those observed with EC sensitization with OVA. Immunohistochemistry revealed the expression of neuropeptides only in Der p8-treated skin. Nerve fibers were observed in close proximity of mast cells in the dermis. These findings may suggest an interaction between the nervous and immune systems in the skin lesions of AD.

Hapten-induced mouse models of AD. Haptens such as oxazolone (Ox) and trinitrochlorobenzene are commonly used to induce allergic contact dermatitis and have been thought to evoke primarily a Th1-dominated response. However, it has been recently reported that multiple challenges with Ox or trinitrochlorobenzene to the skin of hairless mice over an extended period cause the skin inflammation to shift from a typical Th1-dominated delayed-type hypersensitivity response to a chronic Th2-dominated inflammatory response that is similar to human AD (Matsumoto *et al.*, 2004) (Man *et al.*, 2008). Indeed, 9–10 challenges with Ox to hairless mice produced a chronic Th2-like skin inflammation. The inflammation was characterized by dermal infiltration of Th2 lymphocytes that express the PGD2 receptor CRTH2, mast cells, and eosinophils, increased expression of IL-4 in the dermis, and highly elevated IgE levels. Repeated challenge with Ox led to increased epidermal hyperplasia and decreased expression of the skin differentiation proteins filaggrin, loricrin, and involucrin. A skin barrier abnormality became evident and was associated with decreased SC ceramide content and hydration, increased transepidermal water loss and impaired lamellar body secretion, resulting in reduced lamellar membranes, as observed in AD patients. Furthermore, as in human AD, epidermal serine protease activity in SC increased and the expression of two lamellar body-derived antimicrobial peptides, CRAMP and mBD3, declined after Ox challenges, paralleling the decrease of their human homologs in AD skin lesions. These changes were not observed after a single challenge with hapten, the classical way to elicit hapten-delayed hypersensitivity reaction.

Although the hapten repeated sensitization model is not a genetically driven model, many of its aspects may be applicable to extrinsic allergen-driven AD. Indeed, it particularly illustrates the notion that once allergen is introduced via a breach in the barrier, the resulting allergen-driven inflammation further damages the skin barrier. This amplificatory cycle may play an important role in the perpetuation and exacerbation of human AD. This model needs to be compared head to head with a protein (OVA and house dust mite) repeated sensitization model. Because of its reproducibility, predictability, low cost, and relative rapidity, the hapten repeated sensitization model could prove useful for evaluating pathogenic mechanisms and potential therapies for AD.

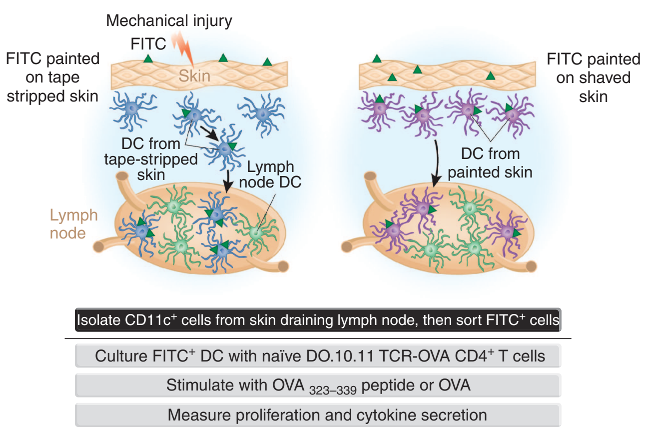

Figure 3. Scheme for testing the effect of tape stripping on DC polarity.

Superantigen-induced mouse models of AD. *Staphylococcus aureus* colonization or infection, the most common cause of AD exacerbates. Of all *S. aureus* strains isolated from lesional skin, up to 65% produce exotoxins with superantigenic properties. We have shown that application of SEB instead of OVA by repeated EC sensitization to tape-stripped skin was able to elicit Th2-dominated allergic skin inflammation accompanied by a systemic Th2 response to the superantigen (Laouini *et al.*, 2003b).

A murine model of AD in mice with food hypersensitivity. The pathogenic role of food allergy in a subset of AD patients has been supported by clinical studies (Sampson and McCaskill, 1985). Repeated intragastric sensitization of C3H/HeJ mice with cow's milk or peanut, with cholera toxin as adjuvant, caused hair loss, scratching, and chronic relapsing AD-like skin lesions in up to 35% mice. This was accompanied by elevated serum levels of specific IgE and blood eosinophilia (Li *et al.*, 2001). Our recent findings show that mice orally sensitized with OVA in the presence of the adjuvant cholera toxin develop allergic skin inflammation at skin sites challenged with OVA (M Oyoshi *et al.*, unpublished observations). These results raise the possibility that flare-ups of AD lesions may occur in orally sensitized individuals following the introduction of food allergens into the skin.

Genetically engineered mouse models of AD

IL-4 transgenic mice. Transgenic mice overexpressing IL-4 in the skin develop spontaneous pruritus and chronic dermatitis at the age of 4 months (Chan *et al.*, 2001). The onset and early progression of skin inflammation was found to correlate with the elevation of IgE and IgG1. The early skin lesions are characterized by prominent infiltration of T cells in the epidermis and dermis, whereas chronic lesions showed T-cell accumulation in the dermis. The chronic lesions also showed features of human AD, including acanthosis of the epidermis with mild spongiosis, hyperkeratosis, and dermal eosinophils.

IL-31 transgenic mice. IL-31 is a previously unidentified cytokine produced by activated T cells. IL-31 expression is upregulated in pruritic AD skin lesions but not in nonpruritic psoriatic skin inflammation (Dillon *et al.*, 2004). The expression level of IL-31 is associated with the magnitude of skin pruritus. Transgenic mice overexpressing IL-31, driven by the lymphocyte-specific promoter Lck or by the ubiquitous elongation factor-1α promoter, exhibited signs of dermatitis at the age of 2 months, including pruritus, mild-to-moderate hair loss, and considerable thickening of ear skin. These symptoms progressed with age, and reached a peak at the age of 6 months. Histological examination of skin lesions revealed hyperkeratosis, acanthosis, inflammatory cell infiltration, and an increase in mast cells, which resemble the skin lesions of human AD. However, these mice exhibited normal serum concentrations of IgE. The evaluation of local or systemic Th2 response was not reported.

TSLP transgenic mice. TSLP is expressed primarily by epithelial cells including epidermal keratinocytes. TSLP is highly expressed in the skin lesions of patients with AD, where it is associated with the activation and migration of DCs within the dermis (Soumelis *et al.*, 2002). Mice on BALB/c background were made to overexpress a tetracycline-inducible, skin-specific TSLP under the control of the keratin 5 promoter (Yoo *et al.*, 2005). Skin erythema occurred at $\sim$2–3 weeks of doxycycline treatment, and progressed to AD-like changes, including persistent erythema, mild xerosis, crusting, and erosions at 3–4 weeks. Histological examination of skin lesions showed changes similar to those observed in human AD, including acanthosis, spongiosis, hyperkeratosis, and dermal infiltration, characterized by a predominance of lymphocytes and macrophages, and an abundance of mast cells and eosinophils. Skin lesions of TSLP transgenic mice exhibited a Th2 cell profile with the upregulation of IL-4, IL-5, and tumor necrosis factor-α. These mice also showed elevated serum levels of IgE and IgG1, and decreased IgG2a. When crossed with TCR$\beta^{-/-}$ mice that lack T cells, TSLP transgenic mice still developed AD-like skin changes with a dense accumulation of mast cells and eosinophils in the dermis, suggesting that skin inflammation in these mice is T-cell independent.

Caspase-1 and IL-18 transgenic mice. IL-18 is a unique proinflammatory cytokine capable of strongly stimulating both IFN-γ and IL-4 production when it acts on freshly isolated T cells with IL-12 and IL-2, even in the absence of T-cell antigen receptor engagement. *In vitro* IL-18 in the presence of IL-3 directly stimulates basophils and mast cells to produce IL-4, IL-5, and IL-13 cytokines in an IgE-independent manner (Nakanishi *et al.*, 2001). This innate style of T cell and mast cell activation is one of the outstanding properties of IL-18. Administration of IL-18 to normal BALB/c or C57BL/6 mice induces polyclonal IgE production in a CD4 T cell-, signal transducer and activator of transcription factor 6 (STAT6)-, and IL-4-dependent manner (Okamura *et al.*, 1995). IL-18 is expressed in AD skin and single nucleotide polymorphisms in the IL-18 gene are associated with AD (Kim *et al.*, 2007). Similar to IL-1β, IL-18 is stored as a biologically inactive precursor in various cell types, including macrophages and keratinocytes, and becomes active after cleavage with caspase-1 (CASP1). Transgenic mice overexpressing the human CASP1 precursor gene in epidermal keratinocytes under the control of the human keratin 14 promoter (CASP1 transgenic mice) showed elevated serum levels of IgE and IgG1 at the age of 8 weeks, and mild pruritic dermatitis around the eyes and ears at the age of 16 weeks (Yamanaka *et al.*, 2000). Histological examination showed prominent acanthosis, papillomatosis, parakeratosis, and intracellular edema with dense infiltration of lymphocytes, neutrophils, and mast cells, but not eosinophils in the skin lesion. CASP1 transgenic mice on STAT6-deficient background still suffered from chronic dermatitis similar to that observed in CASP1 transgenic littermates, but with no detectable IgE production, suggesting a dispensable role of IgE in the development of the AD-like dermatitis in CASP1 transgenic mice. A high concentration of mature IL-18 was found in the serum of the CASP1 transgenic mice. IL-18-deficient CASP1Tg evaded the dermatitis, confirming the critical role of IL-18 in the CASP1 mouse model of AD and suggesting that IL-18 causes the skin changes in the absence of IgE and STAT6. Because of this and because in this model, there is no obvious need for allergen exposure to develop dermatitis, and it has been proposed that the CASP1 mouse model may mimic intrinsic AD in which elevated levels of serum IL-18 can also be found. Transgenic mice overexpressing murine mature IL-18 under the control of human K14 promoters exhibited similar skin changes with delayed disease onset when compared with CASP1 transgenic mice (Konishi *et al.*, 2002). IL-1β-deficient CASP1 and IL-18 transgenic mice exhibited similar dermatitis but at a later stage (around 6 months), suggesting a role for IL-1β in accelerating the dermatitis initiated by IL-18 locally released in the skin. Both CASP1Tg and IL-18Tg mice had increased neutrophils in the spleen and spontaneous deviation of splenic T cells to Th2 and away from Th1, evident by the increased production of IL-4 and IL-5 and decreased IFN-γ production in response to anti-CD3 stimulation. In addition, these Tg mice had elevated serum IgG1 as well as IgE levels. Importantly, the number of skin mast cells, the levels of histamine in the plasma, and the frequency of skin scratching behavior were all elevated in CASP1Tg and IL-18Tg mice as in human AD.

RelB knockout mice. RelB belongs to the NF-κB/Rel family of transcription factors, which play critical roles in stress-induced, immune, and inflammatory responses. In adult mice, RelB expression is restricted to lymphoid tissues. $RelB^{-/-}$ mice exhibit hematopoietic abnormalities and mixed inflammatory cell infiltration in several organs, including skin (Weih *et al.*, 1997; Barton *et al.*, 2000). These mice developed spontaneous dermatitis, hyperkeratosis, acanthosis, skin infiltration with $CD4^+$ T cells and eosinophils, and elevated serum IgE, all features of human AD, although pruritus was not reported. When crossed with nur77 transgenic mice in which the peripheral T cells are absent, $RelB^{-/-}$ mice exhibited an apparently alleviated dermatitis characterized by reduced epidermal hyperplasia and keratinocyte proliferation, suggesting that skin inflammation in these mice is T-cell dependent.

Cathepsin E knockout mice. The aspartic proteinase cathepsin E (Cat E) is localized mainly in the endosomal structures of APCs and has been implicated in a variety of immune responses. Under conventional conditions, Cat $E^{-/-}$ mice on C57BL/6 background developed pruritic and erosive skin lesions, from which *S. aureus.* was identified (Tsukuba *et al.*, 2003). Serum levels of total IgE were increased and the secretion of Th2 cytokines by splenocyte *in vitro* was also increased, whereas the production of IFN-γ and IL-2 was normal. Histological examination showed epidermal hyperplasia and dermal infiltration with eosinophils, lymphocytes, and macrophages. The function of skin DCs in the Cat $E^{-/-}$ mice was not reported. Given the recent findings that cathepsin E differentially regulated the nature and function of DCs and macrophages, these mice could be a good model for studying the role of APCs in AD pathogenesis.

SC chymotryptic enzyme transgenic mice. SC chymotryptic enzyme, which belongs to the kallikrein group of serine protease, is preferentially expressed in cornifying epithelia. Its expression is further increased in chronic lesions of AD as well as in psoriasis (Ekholm and Egelrud, 1999). Overexpression of a human SC chymotryptic enzyme transgene in suprabasal epidermal keratinocytes of mice led to the development of AD-like skin inflammation characterized by increased epidermal thickness, hyperkeratosis, and dermal inflammation starting at the age of 7–8 weeks or older (Hansson *et al.*, 2002). Transgenic mice showed signs of itching at the age of 10–11 weeks. The frequency of scratching increased with age. The fact that signs of itching occurred after epidermal thickening suggested that the pruritus was secondary to the changes in the skin, rather than a direct effect of SC chymotryptic enzyme. Histamine antagonists failed to alleviate the scratching behavior in SSCE transgenic mice, suggesting that histamine was unlikely the cause of pruritus in these mice.

Apolipoprotein C1 transgenic mice. APOC1 is an apolipoprotein involved in lipoprotein metabolism (Jong *et al.*, 1998) In healthy individuals, the protein is predominantly expressed in liver, skin, and brain tissue with macrophages and keratinocytes as major cell types. The protein is highly conserved and a high degree of homology exists between APOC1 in mice and humans. Mice transgenic for human apolipoprotein C1 (APOC1Tg mice) in liver and skin have increased levels of free fatty acids, cholesterol, and triglycerides, but show a complete absence of subcutaneous fat and atrophic sebaceous glands. The composition of the SC is dependent on lipid homeostasis. APOC1Tg mice not only have disturbed serum levels of lipids but they also spontaneously develop with age severe dermatitis with moderate epidermal hyperplasia, and hyperkeratosis and parakeratosis, scaling, lichenification, excoriations, and pruritus. Histological analysis shows increased epidermal thickening and spongiosis in conjunction with elevated numbers of inflammatory cells, including eosinophils, neutrophils, mast cells, macrophages, and $CD4^+$ T cells in the dermis (Nagelkerken *et al.*, 2007). In addition, the mice have increased serum levels of IgE and show abundant numbers of mast cells in the dermis. Importantly, they display a disturbed skin barrier function, evident from increased transepidermal water loss. Partial inhibition of disease could be achieved by restoration of the skin barrier function with topical application of a lipophilic ointment. Furthermore, the development of dermatitis in these mice was suppressed by corticosteroid treatment. These findings underscore the role of skin barrier integrity in the pathogenesis of AD.

Spontaneous mouse models of AD

The Nc/Nga mouse. Nc/Nga mice, an inbred mouse strain, was the first mouse model of AD reported by Matsuda *et al*. (1997). Skin changes develop spontaneously in Nc/Nga mice secondary to the exposure to various environmental aeroallergens and closely mimic human AD. AD-like disease only develops when mice are kept under conventional conditions, particularly when the mice are infected with mites, but not under sun protection factor conditions. Scratching behavior, the first sign of the skin changes, occurs at 6–8 weeks, and is followed by rapidly developing erythematous, erosive lesions with edema, and hemorrhages on the face, ears, neck, and back. Histological examination shows dermal infiltration with eosinophils and mononuclear cells before the appearance of clinical skin manifestations. Hyperparakeratosis, hyperplasia, and spongiosis are observed in the skin lesions at the age of 17 weeks. Nc/Nga mice display mutations on chromosome 9, which is linked to increased IgE production as well as increased Th2 responses. Constitutive tyrosine phosphorylation of Janus kinase 3, a tyrosine kinase responsible for IL-4R-mediated signaling, is thought to be involved in the enhanced sensitivity of B cells to IL-4, leading to the elevation of total IgE levels (Matsumoto *et al*., 1999). Along with the skin changes, Nc/Nga mice exhibit preferential Th differentiation toward Th2 cells in the spleen, dense accumulation of eosinophils and mast cells in skin lesions, and an increased serum level of total IgE (Vestergaard *et al*., 1999; Kohara *et al*., 2001). Moreover, the Th2-specific chemokines, thymus and activation-regulation chemokine, and monocyte-derived chemotactic cytokine, and their receptor, CCR4, have been reported to be highly expressed in the lesions of the Nc/Nga mouse as in AD skin lesions (Vestergaard *et al*., 1999; Kohara *et al*., 2001). These findings strongly suggest the involvement of Th2 cells in the development of AD-like skin lesions in the Nc/Nga mouse.

However, STAT6-deficient Nc/Nga mice exhibit skin changes comparable to those of STAT6-positive Nc/Nga littermates, but undetectable IgE serum levels, suggesting that AD-like skin changes in Nc/Nga mice are IgE/Th2 independent (Yagi *et al*., 2002). The DLN of the skin lesions in STAT6-deficient Nc/Nga mice exhibited massive enlargement elicited by the accumulation of activated IFN-γ-secreting T cells. Moreover, caspase I, IL-18, IL-12, and IFN-γ are found to be highly expressed in skin lesions, with simultaneous elevation of eotaxin 2 and CCR3 expression. Therefore, the Th2-mediated immune response is not necessary for the development of AD-like skin disease in Nc/Nga mice. The skin microenvironment that favored IFN-γ production in STAT6-deficient Nc/Nga correlates with the skin disease and infiltration of eosinophils, possibly because IFN-γ induces eotaxin 2 and CCR3 expression. It is yet to be observed whether the increased IFN-γ production in lesions of STAT6-deficient Nc/Nga mice is driven by a defect in the innate immunity of Nc/Nga mice. In this regard, the IL-18 gene is near the locus responsible for skin disease in Nc/Nga mice on chromosome 9. It is possible that a non-immune reaction with increased caspase 1, IL-18, and IL-12 expression in the skin microenvironment may result in increased IFN-γ production, leading to the induction of eotaxin 2 expression that acts as a chemoattractant for CCR3-expressing eosinophils. Therefore, the IFN-γ-favored skin microenvironment is likely the cause of pathology in these mice. Generation of Rag2-, caspase 1-, IL-18-, and IFN-γ-deficient Nc/Nga mice is needed to answer these questions.

Nc/Nga mice show skin barrier abnormalities with increased transepidermal water loss and abnormal skin conductivity under conventional conditions, and impaired ceramide metabolism, all of which might predispose these mice to the development of dermatitis (Aioi *et al*., 2001).

Other mouse strains with spontaneous dermatitis. Other stains of mice that spontaneously develop dermatitis have been proposed as possible models of allergic dermatitis. Naruto Research Institute Otsuka (NOA) mice exhibit hair loss and pruritic ulcerative dermatitis with mast cell accumulation in the dermis, and high serum levels of IgE; however, they lack classical histological characteristics of human AD (Watanabe *et al*., 1999). DS-Ng mice, another inbred strain that was established 20 years ago, have been reported to develop spontaneous dermatitis only under conventional conditions. The severity of the dermatitis correlated with the total IgE serum levels (Hikita *et al*., 2002). Interestingly, heavy colonization of *S. aureus* was found in skin lesions. Moreover, skin application of heat-killed *S. aureus* to DS-Nh mice induced similar dermatitis. Therefore, these mice could be a good model for *S. aureus*-associated AD (Haraguchi *et al*., 1997).

Conclusion

Mouse models of AD have shed important light into the pathogenesis of allergic skin inflammation. They have highlighted the role of mechanical injury and of skin barrier disruption in allergic sensitization to epicutaneously introduced allergens. More importantly, they allow an in-depth dissection of the mediators and cells that are critical for the development of the allergic response to EC sensitization. A better insight into the mechanisms of the elicitation and effector phases of EC sensitization is made possible by these models and will ultimately lead to a wider array of therapeutic interventions in this common and potentially debilitating disease.

There is strong evidence that a genetically defective skin barrier function is an important predisposing factor for AD, as illustrated by the observation that ∼15% of AD patients have a defective filaggrin gene. Mice that have a genetic defect in barrier function will most likely provide a model of AD closer to the human disease than models provided by EC sensitization of WT mice with allergens or haptens or by transgenic overexpression of cytokines in the skin or disruption of immune genes and will have an advantage over Nc/Nga mice in which the genetic defect is not known. On the basis of observations that barrier disruption by skin injury in WT mice results in Th2-dominated skin inflammation and on the observation that chronic inflammation in normal mouse skin repeatedly sensitized with hapten disrupts barrier function, we predict that mice with genetically defective barrier function, for example, filaggrin-deficient mice, will be highly sensitive to the development of Th2-skewed skin inflammation in response to environmental antigens and that this inflammation will further exacerbate the skin barrier defect and result in the downregulation of the expression of antimicrobial genes in the skin and predisposition to bacterial growth and superinfection, all features of human AD. There is little doubt that the generation of mice deficient in filaggrin and other epidermal genes that are important for intact skin barrier function is forthcoming. Application of the knowledge gained from existing mouse models of AD to mice with genetic defects in skin barrier function should provide AD models that closely mimic human disease.

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