REVIEW

Preclinical Models of Atopic Dermatitis Suitable for Mechanistic and Therapeutic Investigations

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Abstract: Atopic dermatitis (AD) is a complex immune-mediated abnormality of the skin characterized by impaired barrier function, eczematous dermatitis, chronic pruritus and itch. The immunological response in AD is mediated by a Th2-dominated immune response in the early acute phase followed by a Th1/ Th2 mixed immune response in the chronic phase. AD is the first step of the "atopic march" that progresses into food allergy, allergic rhinitis, and asthma. Different models are indispensable for studying AD pathogenesis and for designing pre-clinical studies for therapeutic discovery. They reflect the characteristic morphological features of typical human AD with regard to epidermal thickening, hyperkeratosis, acanthosis, and spongiosis and help understand the immunopathogenesis of the disease with respect to IgE levels and cellular infiltration of eosinophils, mast cells, and lymphocytes. Although it is difficult to replicate all human AD clinical features in a model, several AD in vivo models comprising spontaneous, induced, transgenic, and humanized and in vitro models, including 2D, co-culture, and 3D, have been described previously. However, several questions remain regarding whether these models satisfactorily reflect the complexity of human AD. Therefore, this review comprehensively highlights the diversity of currently available models and provides insights into the selection of suitable models based on research questions. It also summarizes the diverse mechanisms associated with each model, which may be valuable for better study design to test new therapeutic options.

Keywords: atopic dermatitis, eczema, in vitro models, in vivo models, Th2 cytokine, microbiome

Introduction

Atopic dermatitis (AD) is an inflammatory condition that causes dry skin, rash, scaly patches, blisters, and inflammation. It is a non-contagious condition but is often painful and irritating, leading to recurrent eczematous lesions and changes in skin color. AD lesions are commonly distributed on the face, trunk, and limbs, particularly in the popliteal and cubital fossa. The prevalence of AD is high in many Western countries, and its incidence is rising in developing countries.[1] The disease is common in 50–60% of individuals within the first year of life and 90% in patients up to five years.[2] It can also begin during adolescence or adulthood. The clinical symptoms include erythema, papules, exudative lesions, and varying degrees of dryness as well as itch mediated by neuroinflammation. Drying and constant scratching also lead to skin thickening and lichenification. The pathogenesis of eczema is mediated by a complex interplay between multiple factors, such as altered skin barrier function, host genetics, immune cell abnormalities, and environmental factors, including exposure to specific pathogens such as *Staphylococcus aureus*. Patients diagnosed with eczema often show an increased incidence of other allergies such as food allergies, asthma, and allergic rhinitis.[3] Furthermore, the majority of patients in the severe disease category show IgE-mediated sensitization to common allergens.[4] This sequence of diseases is often called atopic or allergic diseases.

Pathological Mechanisms

The exact mechanism underlying the complex progression of AD is not yet well understood. However, considerable progress in understanding the disease mechanism has led to many findings related to genetic abnormalities, immunological

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dysfunction, and environmental factors as the prime drivers of AD exacerbation.[5] Any factor contributing to an impaired skin barrier, including environmental factors, allergens, or genetic predispositions, leads to rupture of skin integrity, allowing allergen access to the epidermis. Activated skin cells release thymic stromal lymphopoietin (TSLP), interleukin (IL)-25, and IL-33 from keratinocytes, leading to the activation of the Th2 immune axis through type 2 innate lymphoid cells (ILC2) and Th2 cells. In the acute phase of AD, Th2 cytokines (IL-4, IL-5, and IL-13) play major roles in barrier dysfunction. Subsequently, these cytokines are responsible for the increased production of immunoglobulin E (IgE) and eosinophil recruitment. Clinically, IL-4 and IL-13 contribute to skin barrier defects, cutaneous infections, inflammation, thickening, and itching.[6] Another cytokine, IL-31, accounts for the stimulation of itch receptors, ultimately leading to pruritus in AD (Figure 1). [7] Chronic AD is characterized by Th1/ Th2 mixed immune responses with intensification of Th2, Th1, and Th17 responses.[8] Increased levels of Th1 cytokine interferon-gamma (IFN-γ), IL-12, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are highly dominant in chronic AD. Furthermore, the increased activity of IL-36, which stimulates the maturation and function of DCs, drives T cell proliferation to amplify the skin immune response is observed.[9] Additionally, dendritic cell subclass, Langerhans Cells (LC), and inflammatory dendritic epidermal cells (IDECs), which normally reside beneath the tight junction in the epidermis, penetrate the tight junction, and probably increase allergen uptake to prime T cells.[10] Similarly, Th17 and Th22 (IL-17, IL-19, and IL-22) contribute to the formation of chronic skin lesions in AD. These cytokines are responsible for barrier function disruption, the elevation of anti-microbial proteins, and an increase in abnormal epidermal markers. Furthermore, IL-17 stimulates B cells to differentiate into IgE-producing plasma cells and promotes the release of IL-8, TNF-α, and TSLP, the combined actions of which trigger chronic AD (Figure 1).
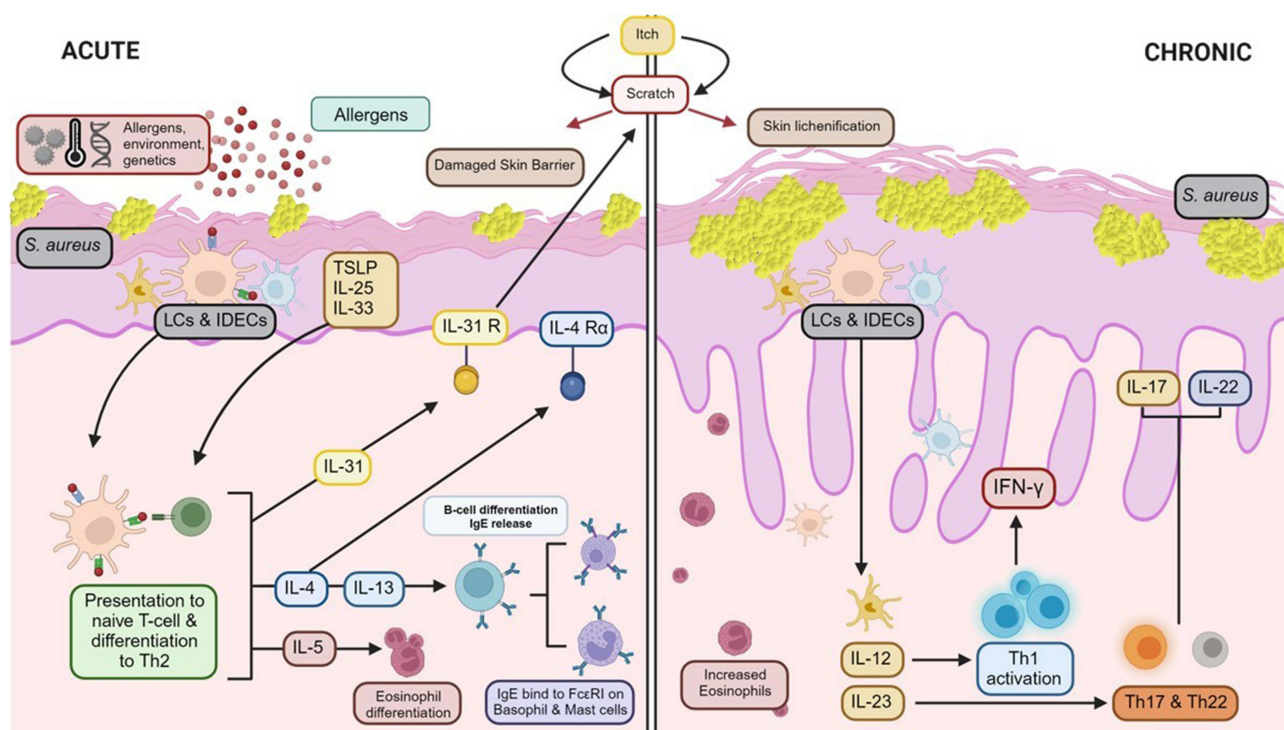

Figure 1 Mechanism of acute and chronic eczema. Genetic, environment and allergens lead to compromised skin barrier allowing allergen penetration along with impairment of skin microbiota (mostly Staphylococcus aureus) colonization. Activated keratinocytes release TSLP, IL-25 and IL-33 to activate skin LCs and IDECs to activate Th2 mediated immune response. In acute eczema, the Th2 immune response predominates with release of classical effector cytokines- IL-4, IL-5 and IL-13 causing further barrier dysfunction. B-cells differentiate to produce IgE which binds to basophils and mast cells. IL-31 binds to IL-31R and induces an itch response. In chronic eczema, Th1/ Th17 immune response dominates with production of IL-17, IL-22. Activate Th1 cells produce IFN-γ and combined action of all these cytokines modulate local immune response leading to epidermal hyperplasia and skin lichenification. The colonization of Staphylococcus aureus is highly dominated in chronic eczema (created with BioRender).

Abbreviations: LC, Langerhans cell; IDEC, Inflammatory dendritic epidermal cell; IL, Interleukin; ILC2, Innate lymphoid cell type 2; R, Receptor, TSLP, Thymic stromal lymphopoietin; IgE, Immunoglobulin E; *S. aureus, Staphylococcus aureus.*

Skin Barrier Dysfunction

Skin barrier dysfunction is considered the first step in the development of atopic march.[11] However, this remains controversial. The classical "atopic march" is but only one possibility with regards to progression of AD to atopic multimorbidity and that early life AD phenotypes, genotypes and environmental factors influence overall atopic disease trajectory and the relationships between and timing of onset of atopic comorbidities are more complex and many different patterns exist.[12] A normal skin is comprised of a stratum corneum that acts as a permeable barrier to prevent transcutaneous water loss and provides an antimicrobial barrier favoring the colonization of nonpathogenic bacteria flora.[13] Any factors contributing to the defect in this barrier, including environmental, allergens, or genetic predispositions, result in degradation of intracellular connections, higher protease activity, increased epidermal permeability, infiltration of antigens, and stimulation of proinflammatory cytokines.[2]

Genetic Susceptibility

Eczema has a strong heritability, as is evident from twin studies.[14] Genome-wide association studies have identified 34 different loci that account for approximately 20% of eczema heritability.[15] Most of which contain multiple genes with roles in immune response, T-cell activation, epidermal differentiation, and innate immunity.[15–17] The most important risk gene identified in eczema is filaggrin (FLG), a major component of the stratum corneum (SC). It contributes to SC hydration and maintains the acidic pH of the skin.[18] FLG mutation is associated with an increased risk of eczema, including early onset and persistent skin infection.[19] Besides, other immune pathway genes associated with alterations in the Th2 signaling pathway have been identified. A gain of functional mutations in genes IL-4R and IL-13R is believed to lower FLG expression and contribute to eczema pathogenesis.[20] Furthermore, other immune-related genes that have been reported in eczema include IL-31, IL-33, signal transducer and activator of transcription 6 (STAT-6), TSLP, toll-like receptor 2 (TLR-2), and FcRI-α.[21,22] Recent studies have demonstrated that the vitamin D receptor and the cytochrome P450 variant (CYP27A1) are associated with eczema. Vitamin D plays a role in immune modulation and CYP27A1 is involved in metabolism.[23]

Th2 Skewed Immune Responses

Another important factor contributing to eczema pathogenesis is a dysregulated Th2 immune response. Excessive production of cytokines IL-4, IL-5, and IL-13 stimulates IgE antibodies and eosinophils in the tissue, leading to inflammation.[24] These further damages the epidermal barrier and stimulates keratinocytes to produce proinflammatory cytokines such as TSLP, IL-25, and IL-33, which further activate the Th2-mediated immune response.[25]

Dysbiosis

The epidermis of the skin hosts various bacterial flora. These microorganisms participate in metabolic processes, maintain immune responses, protect against colonization by pathogenic microorganisms and support the epidermal barrier. Therefore, any disturbance by various microorganisms can lead to a number of diseases, including eczema. In eczema, a previous report indicated a reduction in commensal bacteria of the genera *Streptococcus, Corynebacterium, Cutibacterium* and *Proteobacteria* and an increase in the genus *Staphylococcus* (*S. aureus* in particular).[26] Changes in qualitative and quantitative colonization by *Staphylococcus aureus* (*S. aureus*) precede the clinical manifestation of eczema and further exacerbate the disease.[27] *S. aureus* has gained considerable attention for the development of eczema. It is a Gram-positive bacterium that appears on the skin of individuals with eczema. Skin lesion of 80–100% of patients with eczema are colonized by *S. aureus*. A correlation between eczema severity and colonization *with S. aureus* has been demonstrated, and it is believed that bacterial colonization is an important mechanism of aggravated skin lesions.[28] Several factors associated with *S. aureus* infection contribute to eczema pathogenesis. First, the compromised epithelial barrier that leads to water loss provides a suitable platform for S. aureus colonization of the skin with *S. aureus*. Second, the adhesion factors located on the surface of the cell wall, such as fibronectin-binding proteins, clumping factors A and B, and iron-regulated surface determinants, facilitate binding on the epidermis of the skin.[29] Through various virulence factors, *S. aureus* damages the epidermal layer and exacerbates the inflammation.[30] Staphylococcal α-toxin causes

membrane damage and lysis of keratinocytes, further enhancing biofilm formation, preventing bacterial clearance, damaging barrier components, and promoting the development of viral infection.[31,32] Staphylococcal δ-toxin induces mast cell degranulation[33] via PI3K and calcium influx, without IgE cross-linking.[34] *S. aureus* isolates from the skin of eczema patients also released staphylococcal enterotoxins A, B, and C, as well as toxic shock syndrome toxin 1 (TSST-1), also known as a superantigen, and triggered inflammation by inducing uncontrolled activation of lymphocytes and macrophages.[35] Superantigens are unconventional antigens that elicit a response by binding outside the complementarity-determining regions (CDRs) of their target immune receptor macromolecules (antibodies or T-cell receptors).[36] These superantigens can also generate IgE responses and further enhance mast cell degranulation.[37–39] Enterotoxin B increases the expression of IL-31, increasing itchiness, and inhibits filaggrin and AMP expression. Other virulence peptides, including phenol-soluble modulin (PSM-α), induce the release of keratinocyte IL-1α and IL-36α, which in turn induce IL-17 production and enhance neutrophil recruitment.[40] Furthermore, *S. aureus* triggers Th2 skewing by initiating B cell activation via TLR2-dependent mechanisms.[41] It also disrupts the proteolytic balance of the skin by producing proteases that disrupt the skin's barrier.[42] In addition to bacterial flora, fungi and viruses that colonize the skin are currently the focus of AD research. However, the role of fungi and viruses in patients with AD remains poorly understood. Shotgun metagenomic analysis could provide valuable insights into the species and subspecies levels in the whole picture, with the hope that the complex interplay between numerous microorganisms and allergic diseases, such as AD, can be delineated in a more accurate and precise manner.[43,44]

Murine Models to Study AD Pathophysiology

Mouse models have been valuable tools for studying and investigating mechanisms associated with complicated disease progression and optimizing the effect of drugs on multiple phenotypes and endotypes. These models are valuable for clinical interventional research involving novel therapeutics and preclinical platforms. There are a few challenges associated with selecting the right model to replicate human diseases in mice, as the majority of models documented to date cannot simulate the complex disease entity. It is clear that certain criteria need to be prioritized when selecting a murine model to replicate human AD. Although some of these defined models mimic the core clinical, immunological, histological, and neurophysiological characteristics of human AD, biomarkers that are of great therapeutic importance and can predict disease outcomes in human AD seem to be missing in these models.

To date, more than 20 AD mouse models have been reported. Although these models do not completely display all the characteristic features of human AD, several models that exhibit some AD-like symptoms but lack others have been developed and analyzed. These models can be broadly classified into four main categories: (1) mice induced by the application of exogenous agents, (2) genetically engineered mice in which certain genes of interest are overexpressed or deleted, (3) spontaneous mice, and (4) humanized mouse models. Based on the current understanding, selecting a system to answer specific questions that generate preclinical and clinical data as well as mechanistic and conceptual insights that are more clinically relevant to human AD using these mouse models is challenging. Therefore, it is necessary to have a deep understanding of how these models differ from one another, and which models best represent classical human AD. In this review, we discuss some of the existing models and provide a detailed comparison of how they differ from each other, and which best reflects human AD.

Induced Mice by Application of Exogenous Agents

AD induction using exogenous agents can be classified into different categories, depending on the agents used: haptens, antigens, and cytokines (Table 1). The advantage of using the induced model is that it allows the controlled application of different agents and provides the flexibility to monitor induction in a timely manner. This can be applied to many different strains of mice depending on what is intended to be achieved. The most common method of application of these agents to the skin is experimental disruption of the epidermal barrier and epicutaneous application. This can be achieved by tape stripping to disrupt the epidermal barrier, thereby allowing for increased allergen penetration.[45] These models allow for the exploration of the roles of different allergens in AD.[46] Moreover, studies have shown that these models contribute to a better understanding of mast cell activation and the role of IgE in AD[47,48] and further understand the role of different chemokines, such as CCL20,[49] TARC,[50] and eosinophil chemoattractant protein eotaxin.[51] One main

Table 1 Induced Murine Model of AD

Strain	Methods of induction	Agent (Haptens)	Findings	Immune Polarization	Ref
Hairless mice	Skin application	Oxazolone	Epidermal hyperplasia; Type 2 mediated immune response; severe itch response	Th2	[52]
CBA/J	Skin application	DNCB	Epidermal hyperplasia; Type 2 mediated immune response; severe itch response;	Th2	[53]
BALB/c	Skin application, repeated exposure	DNFB CMIT/MIT with ova	Epidermal hyperplasia; type 1, type 17, type 2 and itch, Type 2/Type 17 mixed inflammation	Mixed	[54, 55]
BALB/c	Epicutaneous	OVA	Thickening of epidermis and dermis. Infiltration of eosinophils, mast cells, inflammatory cells. Elevated total IgE and ova specific IgE, IgG2a	Th2	[56]
	Epicutaneous	DNFB and DfE	Severe erythema, erosion, scarring and excoriation on the entire back. Elevated Th2 cytokine in serum and skin	Th2	[57]
	Epicutaneous	DNCB	Severe eczema with hyperplasia, hyperkeratosis, and spongiosis. Elevated levels of IgE, IFN-γ and IL-4	Mixed	[58]
NC/Nga	Skin application	Crude extract of HDM	Dry, hemorrhagic skin, itch response. High mast cell accumulation. Total IgE and DfE specific IgE and IgG were elevated	Th2	[59]
	Epicutaneous	HDM+SEB	Absence of typical skin lesion. Histologically and immunologically similar to human AD. High total serum IgE. Infiltration of inflammatory immune cells.	Th2	[60]
DS-Nh	Epicutaneous	Staphylococcus exotoxin C (SEC)	Erythema and oedema dry skin were observed at the bacterial patched site IL-4 and IFN-γ were induced by Staphylococcal enterotoxin	Mixed	[61]
C57BL/6J	Epicutaneous	Staphylococcus epidermidis EcpA (extracellular cysteine protease)	Induced skin inflammation characterized by epidermal thickening, immune cell infiltration, and increased expression of cytokines, including IL-6, IL-4, and IL-17α	Mixed	[62]
C57BL/6J	Epicutaneous	Staphylococcus aureus	S.aureus developed AD-like skin inflammatory and induced production of the cytokine IL-13 and also had the potential to make higher levels of interleukin-17A (IL-17A)	Mixed	[63]
C3H/HeJ	Intragastric	Cow's milk or peanut with cholera toxin (CT)	Eczematous skin with elevated blood eosinophils, serum IgE	Th2	[64]
			Cellular infiltration of CD4+ lymphocytes, mast cells and increased expression of IL-4, IL13 in skin		
CCR2−/−	IL-23 injection in ear	IL-23	IL-22 dependent dermal inflammation and acanthosis. Elevated levels of IL-4, eosinophils and TSLP in the skin.	Mixed	[65]
C57BL/6J	Skin application	Vitamin D or its analog MC903 (calcipotriol)	TSLP induction leading to atopic dermatitis-like skin inflammatory infiltrate mainly composed of CD4+ T helper (Th) type 2 cells, dendritic cells, eosinophils, and mast cells and systemic abnormalities, including elevated serum IgE and IgG levels and blood and tissue eosinophilia.	Mixed	[66]

(*Continued*)

Table 1 (Continued).

Strain	Methods of induction	Agent (Haptens)	Findings	Immune Polarization	Ref
Hairless mice (hr/hr)	Skin application	Particulate matter (PM)	PM induced TNF-α causing FLG deficiency in the skin to induce skin barrier dysfunction, increased trans-epidermal water loss. Increased Th17 polarization were linked to TNF-α exposure	Th17	[67]

disadvantage of this method is the standardization of some key agents used. Furthermore, there are many documented variable protocols, and each investigator has developed their own dosing, concentration, and duration using these agents. The immune response substantially differs between mouse strains. Finally, this process is labor-intensive, as it requires daily application of the agents for a longer period of time.

Some widely used methods to induce AD in murine models using haptens have been documented. Haptens are small molecule irritants that elicit an immune response. Therefore, chronic skin surface application can lead to AD-like diseases in mice. Some of these include oxazolone, 2,4-dinitrochlorobenzene (DNCB), 2,4-dinitrofluorobenzene (DNFB), chloromethylisothiazolinone/methylisothiazolinone (CMIT/MIT), ovalbumin (OVA) and 2, 4, 6-trinitrochlorobenzene (TNCB). Oxazolone is the most commonly used hapten for AD induction.[68] Repeated exposure to DNCB and DNFB often leads to hypersensitivity reactions in the skin and is not considered an AD model.[69] Moreover, the majority of the immune responses induced by these haptens are Th1-dominant and often lead to keratinocyte necrosis.[70] The majority of studies have used repeated application of these agents to the surface of the skin to develop AD-like lesions. Some of the strains widely used to achieve this phenotype include hairless mice, CBA/J, BALB/c, and NC/Nga mice.

The use of antigens to induce AD in the mouse skin has also shown promising results in murine models. Antigens that have been used include ovalbumin, DNFB/DfE, crude extract of house dust mite (HDM), HDM in combination with Staphylococcus exotoxin B (SEB), and Staphylococcus exotoxin C (SEC). These antigens are then exposed to the external surface of the skin through repeated epicutaneous exposure. The three most common strains used in this study were BALB/c, NC/Nga, and DS-Nh. OVA, a protein allergen, is the most commonly used antigen to induce AD. This has led to murine models in where Th2 cytokines-IL-4, IL-5, and IL-13 predominate, followed by IgE synthesis.[69] Furthermore, these models showed activation of IL-17 immune response.[46] It is well known that *S. aureus* colonization is common in AD patients and more than 90% of the patients are colonized with *S. aureus*.[71] The application of both HDM and staphylococcal enterotoxin B (SEB) in NC/Nga mice induced severe dermatitis and relatively mild dermatitis in BALB/c mice.[60]

Another approach involves the use of cytokines to induce AD. This approach involves the use of agents such as IL-23, vitamin D or its analog MC903 (calcipotriol), and particulate matter (PM). Some strains widely used for cytokine-based induction of AD include chemokine receptor 2 deficient (CCR2$^{-/-}$), C57BL/6J, and hairless mice. The most common method of inducing AD using these agents is repeated application on the skin surface. MC903 is a well-characterized cytokine used to induce AD in mice. Atopic skin inflammation is dependent on TSLP and contributes to Th2 mediated cytokine expression via ILC2 mediated pathways.[72,73] Furthermore, MC903 causes severe eosinophilic inflammation and skin irritation, which are the features of human AD. Th2 inflammation appeared to be dominant in these mouse models, although mixed endotypes were present. What is more special is the PM mice model. In this model, PM2.5, which induces TNF-α expression, may account for the AD phenotypes, with increased Th17 polarization linked to TNF-α exposure[67] (Table 1).

Genetically Engineered Mice

The use of genetically engineered mice to study AD-related pathogenesis has been well-documented in the literature. These mice were genetically modified to alter the expression of AD-related genes in order to better understand the biological functions of each molecule. These genes were either overexpressed or ablated (Table 2). Some key genes that

Table 2 Genetically Engineered Mice

Transgenic Mice Overexpression	Highlight	Findings	Immune Polarization	Ref
IL-4	Th2 gene	Spontaneous pruritus and chronic dermatitis with involvement of Th2 cells, mast cells, eosinophils, and Staphylococcal aureus skin infection. Early skin inflammation correlates with elevation of IgE and IgG1	Mixed	[74]
IL-31	Activated T cell cytokine	Exhibited signs of dermatitis at age of 2 months, including pruritus, mild to moderate hair loss and considerable thickening of ear skin. Symptoms progressed with age and reached a peak at the age of 6 months. Normal serum IgE	Th2	[75]
TSLP	Epithelial cell derived factor	Exhibit AD like changes including persistent erythema, mild xerosis, crusting and erosions at 3–4 week. Histology reveals hyperkeratosis and dermal infiltration characterized by a predominance of lymphocytes and macrophages. Skin gene expression show Th2 cell profile with upregulation of IL-4, IL-5, and TNF-α with elevated serum IgE	Mixed	[76]
Caspase-1	IL-1β-conversing enzyme	Spontaneously developed recalcitrant dermatitis, skin ulcer, and keratinocyte apoptosis. Skin showed elevated levels of IL-18 and IL-1β.	Th1	[77]
IL-18	T cell stimulant	Spontaneous relapsing dermatitis with mastocytosis and Th2 cytokine infiltration. Elevation of serum IgE and histamine.	Th2	[78]
Stratum corneum chymotryptic enzyme (SCCE)	SCCE, kallikrein group of serine protease, is expressed in cornifying epithelia	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, signs of itching at the age of 10–11 weeks. The frequency of scratching increased with age	Immune responses were not measured	[79]
ADAM 17 (Adam 17 fl/fl Sox9Cre)	Cleaves TNF-α and E-cadherin	Spontaneous dysbiosis and eczematous skin inflammation. S. aureus drove prominent AD formation. Th1 and Th2 cells and prominent Th17 cells were increased.	Mixed	[80]
RelB	RelB belongs to NfkB/ Rel family	Developed spontaneous dermatitis, hyperkeratosis, acanthosis, skin infiltration with CD4$^+$ T cells and eosinophils and elevated serum IgE. Hematopoietic abnormalities and mixed inflammatory cell infiltration in several organs, including skin	Th2	[81, 82]
Cathepsin E	Cathepsin E (Cat E) is intracellular aspartic proteinase expressed in immune cells and skin	Cat E$^{-/-}$ mice on C57BL/6 background developed pruritic and erosive skin lesions. Elevated serum IgE, Th2 cytokines by spleen. Histological features show epidermal hyperplasia and dermal infiltration with eosinophils, lymphocytes, and macrophages	Th2	[83]
IL-36R	Keratinocyte release of IL-36α along with IL-4	Activation of IL-36R signaling directly triggered B cell IgE class-switching, plasma cell differentiation, and increased serum IgE levels—all of which were abrogated in IL-36R–deficient mice.	Mixed	[37]

(*Continued*)

Table 2 (Continued).

Transgenic Mice Overexpression	Highlight	Findings	Immune Polarization	Ref
Cathelicidin	Cathelicidin is an anti-microbial peptide in the skin.	Camp$^{-/-}$ mice on C57BL/6 background increased expression of IL4, IL13, IL22, TSLP and other cytokines associated with AD with the entry of Staphylococcus aureus	Mixed	[84]

play important roles in AD pathogenesis and are overexpressed in murine models include IL-4, IL-13, IL-31, TSLP, IL-18, IL-33, Caspase-1, Cathepsin E, SCCE, and apolipoprotein C1. Similarly, the two key genes ablated in murine models are ADAM17 and CARMA-1. Genetically engineered mice provide detailed information on the gene-specific mechanisms of AD progression and can be valuable if these strains are crossed with other strains. In contrast, these mice are very expensive to maintain, as if required, for regular breeding, genotyping, and selecting the one with the desired alteration in the gene of interest. Furthermore, the generation of these mice is labor-intensive, and there is always a high chance of undesirable effects from unwanted or expected gene expression or alterations.

Spontaneous Mice

Spontaneous mice are often referred to as inbred mice. They show characteristics of AD-like lesions under normal housing conditions. The phenotypes observed in these mice arise from spontaneous genetic mutations caused by environmental factors or defects in DNA replication. Some of the well-defined stains used to study AD pathogenesis include Nc/Nga, Flaky tail, STAT-6 deficient, Otsuka, Sharpin^{cpdm}, Ds-Nh, and Naruto Research Institute (Table 3). The Nc/Ng inbred mouse is the first mouse model of AD reported.[85] Flaky-tail mice have filaggrin gene defects and provide a better understanding of the role of filaggrin, as well as the role of Th2 related cytokines and chemokines in AD.[86,87] This is the most widely studied spontaneous model and provides detailed information on the involvement of microbes and other environmental factors in the pathogenesis of AD. All mice showed multiple mixed immune responses (Table 3) and developed skin lesions beginning with itching, erythema, deep tissue erosion, excoriation, skin dryness, and hair loss.[88–90]

Table 3 Spontaneous AD Mice

Strain	Highlight	Findings	Immune Polarization	Ref
Nc/Nga mouse	Mutation on chromosome 9 which is linked to increased IgE production as well as Th2 responses	Skin changes develop spontaneously in Nc/Nga mice under conventional conditions. The scratching behavior, as the first sign of the skin changes, occurs at 6–8 weeks, and is followed by rapidly developing erythematous, erosive lesions with edema and hemorrhage on the face, ears, neck and back. Histological examination shows dermal infiltration with eosinophils and mononuclear cells prior to the appearance of clinical skin manifestations. Showed involvement of Th2 cells in development of AD-like skin lesion	Th2	[85]
STAT-6 deficient mice	STAT6-/-	Comparable skin lesion to Nc/Nga mice but indetectable serum IgE levels. Splenocyte show massive accumulation of activated IFN-γ secreting T cells. The Th2-mediated immune response is not necessary for the development of AD in this model.	Th1	[89]

(*Continued*)

Table 3 (Continued).

Strain	Highlight	Findings	Immune Polarization	Ref
Naruto Research Institute Otsuka (NOA) mice	Spontaneous	Exhibit hair loss and pruritic ulcerative dermatitis with mast cell accumulation in the dermis and high serum IgE levels, lack classical histological features comparable to humans.	Mixed	[91]
Flaky tail	Deficient in filaggrin	Develop clinical and histological eczematous skin lesions similar to human AD with skin barrier dysfunction. Specific Th2 (IL-4, IL-5, IL-13), Th1 (IFN-γ), regulatory (IL-10) and Th17 (IL-17) cytokines were increased.	Mixed	[92–94]
Cpdm mice	Sharpin-/-	Epidermal hyperplasia, apoptosis of keratinocytes, cutaneous and systemic eosinophilic inflammation. TSLP and IL-33 were both increased.	Mixed	[95]
DS-Nh mice	(DS Hh/+)	Spontaneous dermatitis under conventional housing conditions is characterized by erythema, edema, and erosion on the face, neck, chest, and flexor surfaces of their forelegs with marked scratching behavior. Severe infiltration of mast cells, eosinophils, CD4+ T lymphocytes and CD11b positive macrophages in the skin.	Mixed	[107]

The primary advantage of using these models is that they provide a natural course of human AD under normal conditions, without additional sensitization or manipulation. They can be enhanced by percutaneous sensitization to different haptens and allergens to exacerbate preexisting AD lesions. Similarly, these models allow the investigation of genetic abnormalities associated with AD. The major pitfalls associated with these strains are the lack of genetic information in some strains, development of disease only in an uncontrolled environment, disease progression in a very short period of time, and variable induction protocols when combined with other haptens or allergens. Other disadvantages of using this model include large variations among the groups, as some models do not spontaneously develop dermatitis under specific pathogen-free conditions.

Humanized AD Mouse Models
In addition to these three models, the skin explant mouse model may provide a new and promising approach. For example, human skin xenotransplants on severe combined immunodeficiency (SCID) mice (humanized AD mouse models) offer a particularly promising preclinical research alternative to the currently available "AD" mouse models. These include the generation of entirely genetically engineered human skin on the back of NOC/SCID mice,[96] bioengineering based humanized mouse models using samples derived from patients or healthy donors.[97] And recent model where skin from health donors xenotransplanted on SCID mice followed by in-vivo intradermal injection of pre-activated, autologous human lymphocytes, CD4+ and CD8+ T cells was sufficient to induce AD, indistinguishable from spontaneous AD.[98] These newer humanized models may be valuable for studying AD, as they mimic many spontaneous AD features with respect to lipid metabolism abnormalities, response to clinical AD treatment, and relapse upon exposure to insults. Furthermore, these SCID skin mouse models allow analysis of various steps in the migration of human T cells into the skin in response to selective chemokines.[29]

In vitro Models to Study AD Pathogenesis
Despite animal models serving as valuable tools for studying AD pathogenesis, strict implementation of ethical grounds limits the use of animals. Therefore, many alternative in vitro models that recapitulate AD pathogenesis of AD have been developed. These include 2D monolayers and co-culture models, 3D-skin models, and skin-on-a-chip systems.

Monolayers are easily generated by culturing HaCaT cells or primary human keratinocytes (KC) with cytokines IL-4, IL-13[99] and TSLP.[100] Co-culture modalities have been widely used to understand the roles of cytokines and chemokines in AD. These models are generated by co-culturing fibroblasts (FB) and KC with basophils, eosinophils, and activated T-cells to study the expression and release of chemokines (CXCL8, CLL2, CCL5, CXCL8, and CCL4).[101,102] Similarly, different 3D organoid models have been implemented to study the pathomechanisms and uncover therapeutic approaches. The two most common 3D models used to study epidermal properties are reconstructed human epidermis (RHE) and full-thickness human skin equivalents (HSE). Both models closely mimicked the in vivo characteristics of the human epidermis in terms of barrier function, differentiation, and morphology, as depicted by histology. Most RHE models rely on external challenges involving a variety of cytokines including IL-4, IL-13, TNF-α, IL-22, IL-25, and poly I:C.[103–108] Lastly, adding to the complexity of AD, a relatively new technology that combines the concept of 2D and 3D cultures is developed as an alternative to current AD in vitro models. These models have used robust tissue engineering to generate a range of models that increase the movement of signaling molecules and cell-cell communication,[109,110] increase vascularization for long-term survival, functionality, viable engraftment,[111] and cell transmigration.[112]

Discussion

The progression of AD is very complex and consists of several different stages ranging from acute to chronic, with other phenotypes that are prominently associated with ichthyosis, keratosis pilaris, distinct variants such as nummular eczema and atopic prurigo.[113] Furthermore, AD exhibits both racial and ethnic variation.[114] It is evident that no single in vitro or murine model can stimulate all the phenotypes and endotypes that exist in human AD. Furthermore, the claims that these existing models emulate based on the data generated do not precisely define which phenotype or endotype of AD they are referring to, leading to a generalized claim that only a universal AD phenotype exists. It is very evident that researchers should consider minimal criteria to best reflect human AD to replicate more convincing, clinically relevant, and therapeutically equipped mouse models of AD.

Based on information extracted from the literature, it appears that their needs are a set of defined basic parameters considered to replicate human AD in mice. Impairment of skin barrier integrity is considered the most important factor contributing to AD; therefore, genetic abnormalities in the structural proteins filaggrin,[5] TSLP,[115] and claudin-1[116] seem to be of utmost importance. These factors are associated with epidermal hyperplasia and hyperproliferation with increase in expression of keratin-16 ultimately leading to excessive trans epidermal water loss (TEWL) and increased levels PAR2 in keratinocytes driving barrier dysfunction, inflammation and sensitize skin to drive several levels of neuro-epidermal communication to drive itch response.[117] Recent studies show IL-31 mediated signaling pathway is involved in inducing the itch response and orchestrating the release of a series of chemokines and cytokines from the skin.[118] It is still not well established whether stress can trigger AD without previous sensitization and requires further investigation. Therefore, monitoring the itch response by scratching behavior is an important consideration in murine models. These findings suggest that neurogenic factors are highly relevant to the worsening of AD, and their interaction with immune pathways should be considered in AD studies.[119,120] Other important factor contributing to AD and needs to be considered include dysbiosis of the skin microbiome particularly the overgrowth of Staphylococcus aureus and progressive increase in terminally differentiated proteins and antimicrobial peptides S100A7, S100A8, and S100A.[121] Likewise, T-cell infiltration, Th2 cells, Th17, Th22, and type 2 innate lymphoid cells equally contribute to clinical manifestations and further favor epidermal barrier disruption.[121,122] In addition, the presence of other inflammatory cells, including mast cells and eosinophils, should be considered in AD pathogenesis.[5] A larger number of biomarkers have been identified in AD that correlate with disease severity and need attention when considering murine models of AD. Biomarkers include TARC, CCL17, and CCL27.[123,124]

Besides these findings, responses to the use of well-standardized clinical care for human patients that are available for the treatment of AD, such as glucocorticoids, calcineurin inhibitors, specific Th2 inhibitors against IL-4, IL-13 mediated signaling must be included to determine the effectiveness of these models in the replication of human AD.[125] Furthermore, heterogeneity across different age groups and races/ethnicities, specifically focusing on different endotypes on how AD varies in these backgrounds with respect to their IgE levels, epidermal barrier genes, cytokines, and

inflammatory pathways, may provide important frameworks for selecting murine models for more robust targeted therapy design in this era of personalized medicine.[126]

Considering these important factors, no single model can fully recapitulate the pathogenesis of AD. Moreover, none of the existing models reproduce a sufficient number of characteristics observed in human AD. This is because the anatomy of human and mouse skin is very different. Moreover, transcriptome-profiling studies have demonstrated that a full-blown AD mouse model shares approximately 4%–37% homology with human AD.[53,127] Therefore, the selection of a murine model while considering AD studies should rely on outcome measures and should be restricted to a specific phenotype to make a credible claim. There needs to be a general consortium on minimal criteria that must be considered in any murine model, which is well defined in any endotype of AD, before selecting these murine models to make a big claim or design any therapeutic approaches. Furthermore, transplantation of human lesional skin in SCID mice may be an alternative approach to study mechanisms and therapeutic approaches and has shown fully developed human skin grafts expressing human adhesion molecules and selectins.[29] However, a human skin biopsy is a painful process that is not readily available. To overcome these challenges, intradermally injected stimulated PBMCs in vitro with appropriate cytokines that can robustly induce skin lesions have been developed.[69] Furthermore, the advancement in 3D models has significantly helped overcome some of the limitations, and it seems to be a reliable tool for studying AD at the cellular level. These in vitro models display various AD features, such as abnormal differentiation, loss of structural proteins, inflammation, hyperproliferation, disease-associated gene expression patterns, and apoptosis.[128] Further upgrades in technologies are constantly needed to gain a closer understanding of the signaling processes and pathogenesis of skin diseases such as AD.

Conclusion

Overall, we now have a better understanding of AD pathogenesis. Therefore, the use of existing murine models, as well as exploring newer alternative models, especially the humanized mouse model and 3D in vitro models to replicate unique AD endotypes, might be a suitable approach to maximize human AD needs, understand immunological signatures, identify novel prevention strategies, and design appropriate therapeutic studies.

Abbreviations

AD, Atopic dermatitis; Th, T helper; ESAI, Eczema Area and Severity Index; SCORAD, Scoring Atopic Dermatitis; TSLP, Thymic stromal lymphopoietin; IL, Interleukin; IgE, Immunoglobulin E; IFN, Interferon; GM-CSF, Granulocyte-macrophage colony-stimulating factor; TNF, Tumor necrosis factor; FLG, Filaggrin; SC, Stratum corneum; STAT, Signal Transducer and Activator of Transcription); TLR, Toll like receptor; CYP, Cytochrome; S. aureus, Staphylococcus aureus; PI3K, Phosphoinositide 3-kinase; TSST, Toxic shock syndrome toxin; AMP, Antimicrobial peptide; PSM, Phenol-soluble modulin; FDA, Food and Drug Administration; CCL, Chemokine; TARC, Thymus and activation-regulated chemokine C; DNCB, Dinitrochlorobenzene; DNFB, Dinitrofluorobenzene; CMIT/ MIT, chloromethylisothiazolinone/methylisothiazolinone; OVA, Ovalbumin; TNCB, 2, 4, 6-trinitrochlorobenzene; SEC, Staphylococcus exotoxin C; HDM, House dust mite; SEB, Staphylococcus enterotoxin B; DfE, *Dermatophagoides farina*; PM, Particulate matter; CT, Cholera toxin; ADAM, A Disintegrin And Metalloprotease; CARMA, Caspase recruitment domain family; SCCE, Stratum corneum chymotryptic enzyme; TEWL, Trans epidermal water loss; PAR, Protease-activated receptor; SCID, Severe combined immunodeficiency; PBMC, Peripheral blood mononuclear cells.

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