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REVIEW

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Translational Animal Models of Atopic Dermatitis for Preclinical Studies

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There is a medical need to develop new treatments for patients suffering from atopic dermatitis (AD†). To improve the discovery and testing of novel treatments, relevant animal models for AD are needed. Generally, these animal models mimic different aspects of the pathophysiology of human AD, such as skin barrier defects and Th2 immune bias with additional Th1 and Th22, and in some populations Th17, activation. However, the pathomechanistic characterization and pharmacological validation of these animal models are generally incomplete. In this paper, we review animal models of AD in the context of preclinical use and their possible translation to the human disease. Most of these models use mice, but we will also critically evaluate dog models of AD, as increasing information on disease mechanism show their likely relevance for the human disease.

INTRODUCTION

Atopic dermatitis (AD) is characterized by highly pruritic inflamed skin lesions and dry skin (xerosis). The prevalence of human AD is 10 to 20 percent of the population in developed countries, and its onset is most common in early childhood; the disease has a severe impact on quality of life [1]. The first-line treatments for human AD include topical glucocorticoids or calcineurin inhibitors, whereas systemic immunosuppressants are used for more severe cases [2]. The efficacy and adverse event profiles of the currently available treatments are not always favorable, and this should prompt us to develop safer and/or more effective interventions [1].

In general, drug development is impaired by the failure to replicate preclinical *in vivo* studies in human clinical trials, thus emphasizing a problem in the translation from animals to humans [3]. At this time, there is an overestimation of the potential efficacy of new treatments following encouraging results of preclinical *in vivo* studies; current recommendations to alleviate this problem include the performance of power calculation, randomization and blinding, which are relatively simple to implement in a preclinical setting [4]. In contrast, the recommendations to "*match the models to human manifestation of the disease*" and "*to replicate in different models of the same disease*" are far more difficult to satisfy [4].

Our objectives are to review the most commonly

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†Abbreviations: ACD, allergic contact dermatitis; AD, Atopic dermatitis; DNCB, dinitrochlorobenzene; DNFB, dinitrofluorobenzene; *FLG*, *filaggrin*; HDM, house dust mites; HOME, Harmonising Outcomes Measures for Eczema; ILC, innate lymphoid cells; KO, knockout; Kx, keratin x; *ma*, *matted*; OVA, ovalbumin; OXA, oxazolone; PK/PD, pharmacokinetics/pharmacodynamics; rDer, recombinant *Dermatophagoides* HDM allergens; SDS, sodium dodecyl sulphate; SEB, Staphylococcal enterotoxin B; TDI, toluene diisocyanate; TNCB, trinitrochlorobenzene; TS, tape-stripping; TSLP, thymic stromal lymphopoietin.

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reported animal models for AD and to match their characteristics to the human disease. Furthermore, we will discuss the strength and limitation of each model with respect to its use in preclinical studies.

HUMAN ATOPIC DERMATITIS

The diagnosis of human AD usually relies on clinical features matched with diagnostic criteria [1]. These major criteria are pruritus, age-associated typical morphology, distribution of lesions, chronic or chronically relapsing dermatitis, and a personal or family history of atopic diseases. Even though the diagnosis of AD depends on certain clinical characteristics, both the clinical presentation and the intra- and inter-personal inflammatory profiles underlying the lesional and non-lesional skin seem highly heterogeneous [5]. Patients with AD can be stratified into groups (i.e. "endotypes") based on a normal or elevated serum total IgE levels (i.e. intrinsic or extrinsic forms of AD), the presence of filaggrin gene mutations, race, or presence of persistent secondary bacterial and viral infections. Disease flares can be caused by various triggers that include aeroallergens, food allergens, climate changes, hormonal changes, stress, and other irritants [6]. Major triggering allergens are those of the *Dermatophagoides farinae* and *pteronyssinus* house dust mites (HDM) [7], with 95 percent of human patients with moderate-to-severe AD having detectable serum levels of HDM-specific IgE [8].

The Harmonising Outcomes Measures for Eczema (HOME) working group recently defined excoriation, erythema, edema/papulation, and lichenification as the minimum clinical signs that should be measured in clinical trials for AD [9]. Erythema and edema/papulation are characteristic of acute stages while excoriation and lichenification represent more chronic lesions [1]. In patients with AD, the epidermal barrier integrity is altered due to the reduced expression of epidermal structural protein and a deregulation of lipid composition and organization, resulting in impaired protective function of the skin barrier. A disrupted skin barrier can be caused by genetic mutations and, in patients with moderate-to-severe AD, the expression of the epidermal proteins filaggrin and loricrin is typically reduced in non-lesional as well as lesional skin [10]. An impaired skin barrier function allows for the increased penetration of antigens into the skin leading to the activation of local immune responses and increased transepidermal water loss across the skin barrier promoting xerosis. Furthermore, the clinically unaffected skin of patients with AD is typically characterized by higher numbers of resident immune cells compared to healthy control, especially Th2 and Th22 cells, which quickly secrete pro-inflammatory cytokines upon local stimulation [1]. Antigens penetrating the skin stimulate the cellular interplay between skin immune cells and keratinocytes, further promoting inflammatory responses, increased disruption of the skin barrier, and the additional stimulation of a neurogenic itch response that causes scratching and thereby mechanical damage of the skin barrier. Thus, the progression of chronic skin lesions is driven by a vicious circle of mutually reinforcing processes promoting disruption of the skin barrier function, itch-scratch cycles, and cutaneous inflammation.

Histologically, skin lesions of AD include spongiosis in acute lesions and epidermal hyperplasia with developing chronicity; the latter is associated with an increased expression of the proliferation-associated markers keratin 16 (K16) and Ki67 [11]. Immunologically, skin lesions are characterized by infiltrating T cells, predominantly $CD4^+$, group 2 innate lymphoid cells (ILC2s), and dendritic cells along with an increased number of dermal mast cells and eosinophils [11]. Lesion development is associated with an upregulated expression of thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 by keratinocytes [1]. Acute lesions are dominated by Th2-associated cytokines (IL-4, IL-13, and IL-31) as well as IL-22, with the added presence of IL-17, especially in Asian populations [1,12-14]. During the shift from acute to chronic lesions, the Th2- and Th22-associated inflammatory response amplifies further, and there is an additional increase in the expression of Th1-associated cytokines (IFN-γ and IL-12) [12,13].

Recently, clinical studies have included biomarkers that could be used to assess treatment efficacy for human patients with AD. Some of the best described treatment-responsive biomarkers found in the skin are a decreased skin thickness and the downregulation of the proliferation-associated marker Ki-67. Inflammatory markers such as the wound-inducible keratin-16, which is upregulated in keratinocytes under inflammatory conditions, the metalloproteinase MMP12 that degrades primarily elastin and hence further impairs epidermal barrier function, the antimicrobial proteins S100A7-9, and S100A12 are recognized as alarmins and contribute to chronic inflammation. To these are added the chemokines MCP-4/CCL13, TARC/CCL17, PARC/CCL18, and MDC/CCL22 that promote local inflammation and the recruitment of immune cells. The cytokines IL-13, an amplifier of the inflammatory function of Th2 cells, IL-22, which induces keratinocyte proliferation and alters their differentiation and the pruritogenic IL-31 [15]. Furthermore, IL-31 and TARC/CCL17 can also be measured in serum where total IgE levels can also be used as a treatment biomarker [15]. Ideally, biomarkers found to be most relevant for human AD should be investigated also in animal models mimicking the human disease to establish a panel that could be used to assess the efficacy of treatment across species. For the models described in

Table 1. Characteristics spontaneous mouse models for atopic dermatitis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Spontaneous mice																	**References**
Flaky Tail	+	+	+	+				+			+						[75,96-98]
NC/Nga	+	+	+	+	+							+	+			+	[47,99,100]

A) Acute (erythema, edema, papules, spongiosis), B) Chronic (lichenification, epidermal hyperplasia), C) Itch manifestation (excoriation, alopecia), D) ↑Th2 (IL-4, IL-5, IL-13), E) ↑total or specific IgE, F) ↑Th22 (IL-22), G) ↑Th1 (IFN-γ, IL-12), H) ↑Th17 (IL-17), I) ↑TNF-α, J) Epidermal hyperplasia ↑K16 and/or Ki67, K) Disturbed skin barrier ↑FLG and/or LOR, L) ↑TARC/CCL17, M) ↑MDC/CCL22, N) ↑IL-31, O) ↑S100A7-9 or -12, P) Response to glucocorticoids.

Changes in expression of specific cytokines or chemokines are only included for the skin. Included references have reported that the control mice were housed under specific pathogen-free conditions while diseased mice were kept under either specific pathogen-free conditions or a conventional environment without additional application of living mites, allergen, or hapten. "+": data are available to show that this change is present in the model; "-": studies have shown that this characteristic is not present in the model; "blank field": indicates that we could find no data for this parameter.

the following sections, we will discuss molecular features including treatment biomarkers, when available.

GENERAL CONSIDERATIONS FOR PRECLINICAL STUDIES

In drug discovery, *in vivo* pharmacology studies are used for several purposes, e.g. for target validation and assessment of drug candidates in relation to pharmacokinetics/pharmacodynamic (PK/PD) parameters, preclinical efficacy as well as dose-to-man prediction. To be useful for preclinical efficacy studies, animal models of AD should be reproducible in regard to the onset and severity of skin lesions. As a result, inducible models are generally preferred to spontaneous ones that are generally less predictable in sign onset. To assess *in vivo* target activity and investigate the PK/PD of drug candidates, an animal model that simply represents the specific pathway being targeted might be sufficient. However, to increase confidence that a drug candidate would be effective in humans, the selected models should possess as many characteristics of the human disease as possible. This requires an extensive knowledge of the pathophysiology of human AD to evaluate the features that are mimicked in the existing animal models and to understand the ones that are not. Furthermore, differences in skin architecture and immunology between animals and humans should be taken into account [16-18]. The divergent immune responses of inbred laboratory mouse strains should also be carefully considered [19]. The thickness and composition of the skin also vary between species and genders [20]. In general, the human epidermis is considerably thicker than that of mice and dogs, likely due to the absence of a protective hair coat [21]. With regard to mimicking the immune response of human AD, it is imperative to remember that the immune response of C57BL/6 mice is generally Th1-biased, whereas that of BALB/c mice is more oriented toward a Th2 response [19]. Therefore, one should always carefully consider which mouse model to use for preclinical efficacy studies and for their predictability for human AD.

Most models will use some procedures to better reproduce the changes seen in the human disease and to enhance the absorption of AD-causing substances. To mimic the genetic or inflammation-induced epidermal barrier that exists in human AD skin, a tape-stripping method is often used: this procedure consists of repeated applications of an adhesive tape to the same area of the skin surface to remove successive layers of corneocytes, thereby altering the skin-protecting stratum corneum, its outermost layer. To increase the penetration of exogenous substances into this stratum corneum, skin occlusion with patches is often used. Occluding patches prevent water loss and thereby allow water retention within the skin causing damage to the epidermal barrier and, consequently, increasing the penetration of the applied substances.

Ultimately, when present, the severity of AD-like lesions will be generally graded with *ad hoc* scales rating the presence of several cardinal lesions of AD, such as acute erythema and edema, the excoriations that highlight the presence of associated pruritus, and, for chronic models, ulceration, crusting, and epidermal thickening (lichenification).

SPONTANEOUS ATOPIC DERMATITIS IN ANIMALS

Atopic dermatitis spontaneously develops in dogs

Table 2. Characteristics of genetically engineered mouse models for atopic dermatitis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Transgenic mice																	**References**
KIL-4 (CByB6)	+	+	+	+	+		+		+			-	-				[101-105]
KIL-4 (SKH1)		+		+	-		+		-								[105]
KIL-13	+	+	+	+	+							(+)	(+)				[32]
KIL-18		+	+	+	+		-										[47]
IL-31		+	+		-									+			[47]
KIL-33	+	+	+	+	+		-		-								[106]
ApoC1	+	+	+		+											+	[47]
KCASP1		+	+	+	+		-										[47]
CTSS	+	+	+	+	+		+		+	(+)							[107]
SCCE		+	+														[47]
Stat6CVT	+	+	+	+	+		+	+	+		+					+	[108-110]
KTSLP	+	+		(+)	+		(-)		(+)	(+)		(+)	(-)				[31,33]
Knockout mice																	**References**
RelB $^{-/-}$	+	+	+	(+)	+		+		(+)								[111,112]
CatE $^{-/-}$	+	+	+	+	+		-										[47]
KN1N2 $^{-/-}$		+		+	+	-	-	-	+	(+)	(-)				+		[31]
PLC-β3 $^{-/-}$		+			+												[113]
KCtip2$^{ep-/-}$		+		+	+		+	-	+	+	-	+	-				[114]

A) Acute (erythema, edema, papules, spongiosis), B) Chronic (lichenification, epidermal hyperplasia), C) Itch manifestation (excoriation, alopecia), D) ↑Th2 (IL-4, IL-5, IL-13), E) ↑total or specific IgE, F) ↑Th22 (IL-22), G) ↑Th1 (IFN-γ, IL-12), H) ↑Th17 (IL-17), I) ↑TNF-α, J) Epidermal hyperplasia ↑K16 and/or Ki67, K) Disturbed skin barrier ↑FLG and/or LOR, L) ↑TARC/CCL17, M) ↑MDC/CCL22, N) ↑IL-31, O) ↑S100A7-9 or -12, P) Response to glucocorticoids.

Changes in expression of specific cytokines or chemokines were detected in either skin, serum, or in supernatant from isolated lymph node or spleen T cells. If one transgene model has been established in more than one mouse strain, data from several strains are reported as one model. K in front of name: keratinocyte-specific expression; $^{-/-}$: homozygous knockout; $^{ep-/-}$: knockout keratinocyte-specific. "+": data are available to show that this change is present in the model; "-": studies have shown that this characteristic is not present in the model; "blank field": indicates that we could find no data for this parameter. Parentheses around "+" or "–" indicate a low level of evidence due to either absent quantification and/or statistical analyses. Abbreviations: RelB: NF-κB family member; CASP1: caspace 1 CatE: Cathepsin E; CTSS: Cathepsin S; N1N2: Notch1Notch2; PLC-β3: phospholipase C-β3; ApoC1: Apolipoprotein; SCCE: *stratum corneum* chymotryptic enzyme (also named kallikrein 7); Ctip2: chicken ovalbumin upstream promotor transcription factor (COUP-TF)-interacting protein 2 (also named BCL11B).

[22] that are exposed to the same environment as humans, and it is more common in dogs living indoors [23]. Canine AD resembles human AD with regards to clinical features [24] and treatment response [25]. Preclinical studies in privately-owned animals require comprehensive toxicology data and a high number of animals and, consequently, they are generally not the first choice to test the efficacy of new drugs. However, experimental canine models for AD have been developed in atopy-predisposed HDM-sensitized dogs [26,27].

Several mouse strains also have been described to naturally develop AD-like lesions. The most well-known are the NC/Nga mice, in which pruritic skin lesions develop when they are housed under conventional conditions; AD-like signs also spontaneously occur in Flaky Tail (*ft/ft)* mice (Table 1). The Flaky Tail mouse has a frameshift

mutation in both *filaggrin* (*flg*) and *matted* (*ma*) genes, the latter being responsible for the natural development of skin lesions [28]. The specific mutation underlying the NC/Nga phenotype has not been identified, but it is thought to involve the T-cell receptor [29].

GENETICALLY-ENGINEERED MODELS

Transgenic and knockout (KO) mice are highly valuable to elucidate the biological function of a single protein or pathway for target validation, as well as to model human diseases caused by specific mutations. However, these models might not always be relevant for preclinical efficacy studies due to their non-physiological inhibition or the activation of a single pathway with a resulting lack of complexity compared to that of human AD. For the mice presented in Table 2, the expression of the transgene is under the control of a basal keratinocyte keratin (K5 or K14) promoter that permits a constitutive epidermal-specific expression. All the models in Table 2 have been reported to exhibit varying degrees of dermal leukocytosis, which consists generally of T cells, macrophages, eosinophils, or neutrophils, and an increased number of dermal mast cells.

The use of knockout models for AD is limited. *Filaggrin*$^{-/-}$ KO mice have been generated in both C57BL/6 and BALB/c strains, but these mice do not develop dermatitis under specific pathogen free conditions [30].

Conditional models, such as the tamoxifen-inducible *Notch1*/*Notch2* KO [31], and the IL-13 [32] and TSLP [33] transgenic models were generated to offer the advantage of controlling the onset of skin lesions. An important disadvantage of these models is the added variability of the agent inducing the transgene expression, as too little could cause an insufficient protein expression whereas too much could lead to the development of toxic side effects [34]. Furthermore, the inducing agent could potentially affect the disease phenotype and the efficacy of any compound being tested as, for example, tetracyclines could be both neuroprotective [35], have an effect against proteases [36], and they are well-known antibiotics that could affect the surface microbiota [37]. Finally, the penetrance of the disease phenotype might also be variable in these models [38], they are time-intensive to generate, and their commercial availability is therefore limited [39,40].

HAPTEN-INDUCED MODELS

Haptens are small molecules that easily penetrate the epidermis and can provoke an immune response when they bind to tissue proteins, thereby leading to the development of allergic contact dermatitis (ACD). In contrast to humans in whom ACD can be induced by weak haptens [41], strong sensitizers such as oxazolone (OXA) or dinitrofluorobenzene (DNFB) have to be used in mice [42]. Because of their small size, haptens more easily penetrate healthy intact skin than protein allergens [43]; the induced immune responses are generally reproducible and predictive, and the cost of hapten-induced ACD in mice is generally low [39].

In C57BL/6 mice, DNFB, dinitrochlorobenzene (DNCB), trinitrochlorobenzene (TNCB), and OXA initially induce a Th1 response while toluene diisocyanate (TDI) has a high IL-4 expression; it is harder to discriminate between helper T cell responses induced by the same agents in BALB/c mice [44]. This simplified division of haptens as "*Th1 or Th2-inducers*" has been challenged by a study showing that the "*Th1-promoting*" TNCB-induced ear swelling 24 h after challenge was abolished in IL-4 KO C57BL/6 mice, while it could be restored with intravenous IL-4 or IL-13 injections [45]. In contrast, the ear swelling after 24 h induced by OXA was not compromised in IL-4 KO mice [45]. Acute hapten-induced dermatitis models are used to mimic ACD [46], whereas models based on repeated hapten challenges will lead to alterations in the skin barrier and a Th2-biased immune response that can then be used to model AD [47]. The models described in Table 3 have used comparable protocols for lesion induction and mice being housed under controlled conditions; for the TNCB models, regimens for lesion induction were not found. Although these models share some similarities with human AD, several of these—in particular the DNFB-induced models [48-51]—are described to exhibit crusting and desquamation that are not commonly present with human AD, unless the skin is infected secondarily with *S. aureus* (i.e. lesions are impetiginized).

In most of these hapten-induced models, the skin-infiltrating immune cells are not well-characterized, except for a common increased number of dermal mast cells and infiltrating T cells compared to normal skin. The T cell subsets in FITC-induced skin lesions are predominantly $CD4^+$ cells in BALB/c mice and $CD8^+$ cells in NC/Nga mice [52]; this observation highlights that the hapten-induced immune response also could be influenced by the chosen mouse strains. This finding was described also in the chronic OXA-induced models in which the hairless mice seem to have a more restricted Th2 response than in the BALB/c mice where the chronic response seems more Th1-dominated [53,54].

In most hapten-induced models, lesions have been found to respond to topical and/or oral treatment with glucocorticoids. Both JAK and PDE4 inhibitors have been tested in the acute TDI model, using a prophylactic (i.e. preventive) protocol design [55,56]. Similarly, to the situation seen with human AD [57], the non-sedative H1R antagonist fexofenadine did not reduce pruritus in the OXA BALB/c model [58]; this was also the case for

Table 3. Characteristics of hapten and MC903 induced models for atopic dermatitis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Hapten-induced models																	**References**
Acute TDI	+		+	+	+							+				+	[55,56,115]
Chr. DNCB		+	+	+	+		+									+	[116,117]
Chr. DNCB + SDS		+		+	+				+								[118]
Chr. DNCB + patch		+		+	+												[117,119]
Chr. DNCB Nc/Nga	+	+	+	+	+		+	+	+			+				+	[120-123]
Chr. DNFB	+	+	+		+		-	+	-		+						[48,49,124]
Chr. DNFB Nc/Nga	+	+	+	+	+		+		+			+				+	[50,51,125, 126]
Chr. FITC		+															[52]
Chr. FITC Nc/Nga	+	+		+		+	+	+	+							+	[52,127]
Chr. OXA BALB/c	+	+	+	+	+	-	+	+	+			-			+	+	[58,128-132]
Chr. OXA hairless	+	+	+	+	+		-									+	[47,53,133]
Chr. OXA Flaky tail	+	+	+		+												[134]
Chr. TNCB		+	+	+												+	[135,136]
Chr. TNCB hairless	+	+	+	+	+											+	[47,59,137]
Chr. TNCB Nc/Nga		+	-	+			+							-			[138,139]
MC903-induced model																	**References**
MC903 chronic BALB/c	+	+	+	+	+				+								[62-64,140]

A) Acute (erythema, edema, papules, spongiosis), B) Chronic (lichenification, epidermal hyperplasia), C) Itch manifestation (excoriation, alopecia), D) ↑Th2 (IL-4, IL-5, IL-13), E) ↑total or specific IgE, F) ↑Th22 (IL-22), G) ↑Th1 (IFN-γ, IL-12), H) ↑Th17 (IL-17), I) ↑TNF-α, J) Epidermal hyperplasia ↑K16 and/or Ki67, K) Disturbed skin barrier ↑FLG and/or LOR, L) ↑TARC/CCL17, M) ↑MDC/CCL22, N) ↑IL-31, O) ↑S100A7-9 or -12, P) Response to glucocorticoids.

Changes in expression of specific cytokines or chemokines are only included for skin, and all included references have reported that the mice were housed under specific pathogen free or controlled conditions, except for the TNCB model where no such publications were found. "+": data are available to show that this change is present in the model; "-": studies have shown that this characteristic is not present in the model; "blank field": indicates that we could find no data for this parameter. Abbreviations: chr.: chronic; DNCB: dinitrochlorobenzene; DNFB: dinitrophenylbenzene; FITC: fluorescein isothiocyanate; OXA: oxazolone; SDS: sodium dodecyl sulfate; TDI: toluene diisocyanate; TNCB: trinitrochlorobenzene.

chlorpheniramine treatment of hairless mice challenged with TNCB [59].

VITAMIN D- AND VITAMIN D ANALOGUES-INDUCED MODEL

The topical application of vitamin D3 or its synthetic

analogues induces AD-like inflammation in mouse skin [60,61]. More specifically, the skin inflammation model induced by the vitamin D analog calcipotriol (MC903), has recently gained an increased attention (Table 3). The repeated topical application of MC903 induces a high levels of TSLP and the infiltration of group 2 (IL-5$^+$ and IL-13$^+$) ILCs to the skin, thereby resembling some immune perturbations observed in skin lesions of humans with AD [62,63]. MC903-induced inflammation is TSLP-dependent in C57BL/6 mice [60,62], but TSLP-independent in BALB/c mice since the knockout of the IL-25R and to a lesser degree of ST2 (IL-33 receptor) decreases MC903-induced inflammation more than the removal of the TSLPR [64]. These results again indicate that differing genetic backgrounds could affect the cytokine cascade initiating the inflammatory responses in the skin of different strains of mice. However, the use of this model is only mechanistically addressing the infiltration of ILC2s, as MC903 has been shown not to induce the expression of TLSP in either healthy human skin, nonlesional AD skin, or skin from non-human primates [65].

ALLERGEN-INDUCED AND MIXED MODELS

Most allergen-induced animal models for human AD involve sensitizing mice to HDM or ovalbumin (OVA), (Table 4). Currently, 48 HDM and 10 egg white allergens have been recognized [66]. Commercially available HDM and OVA allergen extracts are likely to vary in their allergen composition and concentration, and this may account for differences seen when comparing results across *in vivo* studies [67,68]. The epicutaneous application of OVA or HDM to intact skin does not easily sensitize and initiate lesion development in BALB/c or C57BL/6 mice [69-71]. In contrast, the repeated application of HDM to a compromised skin barrier easily elicits dermatitis [72], while an additional occlusion is needed for OVA-induced skin lesions [73,74]. Both the NC/Nga and Flaky Tail mice exhibit spontaneous skin barrier deficiencies that facilitate their easier sensitization and the induction of lesions by epicutaneous application of HDM without prior barrier disruption [71,75,76].

In these mice, skin lesions are infiltrated by lymphocytes and a high number of dermal mast cells. The dermis of NC/Nga mice also contains numerous eosinophils [76], while that of Flaky Tail mice is more neutrophilic [75]. In NC/Nga mice, treatment with tacrolimus reduced both severity scores and TARC/CCL17 levels in the skin [76]. The high inter-individual variability in lesion severity scores [71] can be reduced by the application of sodium dodecyl sulphate (SDS) or mild tape-stripping (TS) (WB: personal observation). In the NC/Nga SDS+HDM model, an increased number of intra-epidermal nerve fibers has been reported recently [77], a characteristic of human AD skin lesions [78]. In contrast to HDM, OVA does not induce epidermal hyperplasia in NC/Nga mice, likely due to its less complex allergen content that does not contain proteases, as do HDMs [71]. The inflammatory response in the BALB/c tape-stripped OVA patch tests is dependent on αβ and independent of γδ T-cells [74]; an increased number of dermal mast cells, eosinophils, and dendritic cells are also present [79]. After three patch periods, the skin inflammation and IL-4 levels normally will subside [47]. As a result, this OVA patch model may not be optimal for preclinical studies of topical compounds, as the applications of tested products in the patch period would be complicated, and the occlusion would likely enhance the epidermal penetration of the tested drugs [80,81]. However, this model may be useful and valid for testing the efficacy of oral and injectable compounds.

The co-administration of HDM and staphylococcal enterotoxin B (SEB) has been found to increase the severity of dermatitis in NC/Nga mice and to induce mild lesions in BALB/c mice [82]. In this model, SEB not only functions as a superantigen, but it also serves as an allergen that induces the production of specific IgE, as seen in human AD [83,84]. To decrease the variability in HDM-induced models, the application of the recombinant HDM allergens Der (rDer) p 1 and rDer p 2 was tested on BALB/c mouse skin [85]. This model was found to exhibit epidermal thickening and dermal infiltration with leucocytes and eosinophils, while there were no detectable increases in serum IgE or dermal mast cells.

A recent study by Ewald and colleagues [54], compared the transcriptomic profile of several AD mouse models with that of human AD. Models with the highest overall similarity to the human disease homologue were IL-23-injected mice followed by the HDM-induced NC/Nga, the chronic OXA and the OVA-challenged mouse-models. Although the IL-23 model exhibited the highest overall resemblance with human AD at the transcriptomic level, the expression of the treatment biomarkers TARC/CCL17 and MAD/CCL22 was not increased. Furthermore, the IL-23 model shared the highest resemblance with human psoriasis, and it had more than twice as many differentially expressed genes as any of the other models, thereby underlining the concept that it is a broadly inflamed model that likely shares a high similarity with multiple human inflammatory diseases (i.e. a broad skin inflammation model). Beside the inflammatory aspect, the IL-23 model also seems to display some of the down-regulation of genes involved in epidermal barrier function, changes that are not detected in the other models. Nevertheless, when looking at the clinical and histological characteristics of all these models, the HDM-induced NC/Nga and the chronic OXA models reproduce most of the key characteristics of human AD. These include: an epidermal hyperplasia, an increased transepidermal

Table 4. Characteristics of allergen induced models for atopic dermatitis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Allergen-induced models																	**References**
Flaky Tail HDM	+	+	+		+												[75]
Flaky Tail OVA patch	+	+		+	+		+	+									[141]
NC/Nga HDM	+	+	+	+	+				+			+	+	+		+	[76,133] (WB: personal observation)
Nc/Nga SDS HDM	+	+	+	+	+		+	+			(+)						[77,142, 143]
Der p 1/Der p 2		+	+		+												[85]
TS OVA patch	+	+		+	+		+	+	+			(-)		+			[47,79, 119,144]
TS SEB patch	+	+		+	+		+							+		+	[83,84,144]
Dog epi HDM	+		+	+	+	+	(-)	(-)	+			+	+	+	+	+	[26,86,88, 89,45,146]
Dog env HDM	+															+	[87,90]
Mixed models																	**References**
TS DNCB HDM BALB/c	+	+	(+)	+	+	+	+	+	+					+		+	[147-150]
TS OVA SEB patch	+			+	+		+									+	[83]

A) Acute (erythema, edema, papules, spongiosis), B) Chronic (lichenification, epidermal hyperplasia), C) Itch manifestation (excoriation, alopecia), D) ↑Th2 (IL-4, IL-5, IL-13), E) ↑total or specific IgE, F) ↑Th22 (IL-22), G) ↑Th1 (IFN-γ, IL-12), H) ↑Th17 (IL-17), I) ↑TNF-α, J) Epidermal hyperplasia ↑K16 and/or Ki67, K) Disturbed skin barrier ↑FLG and/or LOR, L) ↑TARC/CCL17, M) ↑MDC/CCL22, N) ↑IL-31, O) ↑S100A7-9 or -12, P) Response to glucocorticoids.

Changes in expression of specific cytokines or chemokines are only included for skin, and all included references have reported that the mice were housed under specific pathogen free or pathogen controlled conditions. "h": elevated; "i": decreased; "+": data are available to show that this change is present in the model; "-": studies have shown that this characteristic is not present in the model; "blank field": indicates that we could find no data for this parameter. Parentheses around "+" or "–" indicate a low level of evidence due to either absent quantification and/or statistical analyses. Abbreviations: DNCB: dinitrochlorobenzene; epiHDM: epicutaneous HDM; envHDM: environmental HDM exposure; SDS: sodium dodecyl sulfate; TDI: toluene diisocyanate; TS: tape-stripping.

water loss with a decreased *stratum corneum* water content, parameters that all converge to highlight a disturbed epidermal function. These observations corroborate that microarray analyses cannot stand alone and must be examined in the context of other changes. Because microarray analyses are generally done on full thickness biopsies, mRNA expressed by small cell subsets in the biopsies are often below the detection threshold (e.g. mRNA encoding for the cytokines IL-4 and IL-13), thus excluding many of the biomarkers that are used for AD molecular profiling from the overall comparison analysis. Additional studies like the one done by Ewald [54], including follow-up studies with a more detailed analysis of biomarkers of interest, are necessary to gain a deeper understanding of the strengths and limitations of the various mouse models.

In the experimental canine models of HDM-induced AD, allergen challenges can be done in the environment or after epicutaneous applications, the latter being done either with (patch) or without occlusion. In these models, acute AD lesions (i.e. erythema, edema, papules) develop [86,87] and pruritus manifestations (e.g. excoriations) are seen more often after widespread rather than localized allergen challenges. Skin lesions are infiltrated with T cells, dendritic cells, and eosinophils [86,87]. A recent

microarray study of early HDM patch test-induced skin lesions revealed a Th2 and Th22 associated cytokines and chemokines dominating profile that is similar to that seen in human AD [88]. Finally, these HDM-induced acute skin lesions are responsive to the preventive treatment with topical or oral glucocorticoids. As the lesions spontaneously resolve within a week or two, these models are therefore best suited to the testing of drugs in a preventive rather than therapeutic manner. Examples of usage of these models for preclinical testing of both topically- and orally-administered compounds have been published recently [89,90].

CONCLUSIONS

Published studies on the characterization and pharmacological validation of animal models for human AD are incomplete. Information from several microarray studies of comparing gene expression before and after treatment in humans with AD are available [91-95]. These datasets provide a great opportunity for comparing results from pharmacological validation studies on animal models with those of human AD. A thorough comparison of detailed transcriptomic data in the animal models compared to those of human AD, such as that done recently by Ewald and colleagues [54], would help elucidating which one(s) of the treatment biomarkers identified in human studies would also be of interest in the various animal models. This, together with an increasing understanding of the various human AD endotypes, would provide a guide for better choosing the most optimal models to investigate a specific target as well as to select the most relevant outcome measures in preclinical efficacy studies.

In conclusion, we believe that the increased knowledge of animal model characteristics will help in selecting the proper model for a specific study purpose. Ultimately, this will likely lead to a better predictability and translatability of results to human clinical studies.

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