Human and computational models of atopic dermatitis: A review and perspectives by an expert panel of the International Eczema Council

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Atopic dermatitis (AD) is a prevalent disease worldwide and is associated with systemic comorbidities representing a significant burden on patients, their families, and society. Therapeutic options for AD remain limited, in part because of a lack of well-characterized animal models. There has been increasing interest in developing experimental approaches to study the pathogenesis of human AD *in vivo*, *in vitro*, and *in silico* to better define pathophysiologic mechanisms and identify novel therapeutic targets and biomarkers that predict therapeutic response. This review critically appraises a range of models, including genetic mutations relevant to AD, experimental challenge of human skin *in vivo*, tissue culture models,

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integration of "omics" data sets, and development of predictive computational models. Although no one individual model recapitulates the complex AD pathophysiology, our review highlights insights gained into key elements of cutaneous biology, molecular pathways, and therapeutic target identification through each approach. Recent developments in computational analysis, including application of machine learning and a systems approach to data integration and predictive modeling, highlight the applicability of these methods to AD subclassification (endotyping), therapy development, and precision medicine. Such predictive modeling will highlight knowledge gaps, further inform refinement of biological models, and support new experimental and systems approaches to AD. (J Allergy Clin Immunol 2019;143:36-45.)

Key words: *Atopic dermatitis, atopic eczema, endotype, human models, machine learning, mechanistic models, precision medicine, tissue culture models, skin equivalents, systems biology*

Atopic dermatitis (AD; synonym atopic eczema) has a complex etiology involving multiple genetic and environmental factors.[1,2] With its very high incidence in childhood, chronicity, devastating effect on quality of life for affected patients and their families, enormous socioeconomic costs, and limited therapeutic options to date, AD represents a major challenge. Furthermore, there is clear evidence that AD represents a systemic inflammatory disease with multiple comorbidities extending beyond the well-recognized atopic associations.[3] Consequently, a number of animal models have been developed and used by investigators and the pharmaceutical industry to better understand the disease and consider new pathways to target.[4] However, as recently reviewed, mouse models do not adequately reflect the transcriptomic and gene pathways activated in human AD skin,[5] and the intrinsic difference between mouse and human skin represents a barrier to direct translation of findings from animals into human disease. Consequently, there has been increasing interest in experimental studies in human subjects (in part facilitated by technological and "omics" developments), cell culture models using human tissue, and use of computational or mathematical models that are developed by integrating these data.

In this review article we have used the term *human AD model* to define representations of the disease state and interventions that enable scientific insight into disease pathogenesis, disease course, and response to therapy. We delineate and critically appraise these AD modeling approaches that range from experimental study of human skin *in vivo* (including challenge studies and detailed phenotyping and investigation of patients harboring specific genetic mutations) to generation of AD-relevant models by using immunologic, genetic, and molecular methods in 2-dimensional and 3-dimensional human tissue culture to development of *in silico* computational models using a systems biology approach. Although by definition a reductionist approach cannot recapitulate the full spectrum of AD, these models have greatly increased our understanding of the molecular drivers of AD and provide a powerful tool for preclinical drug development and target validation. However, just as the etiology, clinical expression, and severity of AD range broadly among patients, *in vitro* and *in silico* models of AD vary widely both in how the AD phenotype is induced and how the models are evaluated. Therefore we invited members of the International Eczema Council (www.eczemacouncil.org), a group of experts in AD, and associated authorities in the field to contribute to a scoping and development meeting and subsequently to evaluate and critically appraise the breadth of human AD and computational models to determine their strengths and weaknesses in how they recapitulate the pathophysiology of AD and enable therapeutics to be tested and validated.

Abbreviations used
ACD: Allergic contact dermatitis
AD: Atopic dermatitis
APT: Atopy patch test
FLG: Gene encoding filaggrin
LC: Langerhans cell

IN VIVO MODELS OF AD

Two general approaches using human *in vivo* models have been followed to dissect the pathogenesis of AD: (1) the study of rare genetic variants with AD-like phenotypes and (2) the experimental challenge of patients with or without AD with allergens or irritants. Regarding the first approach, numerous studies have characterized genetic disorders that display skin barrier function abnormalities. Most often, these studies characterized ichthyosis vulgaris, a disease that allowed insight into the function of the epidermal differentiation gene filaggrin *(FLG)*, in which mutations show the strongest association to AD development of all known genes (Fig 1).[6]

Other studies have focused on disorders characterized by systemic inflammation[3] and immunodeficiency with AD-like skin manifestations (Fig 1). One example is immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, which serves as a model to study how systemic imbalances in the regulatory T-cell population can drive cutaneous AD-like inflammation.[7] In addition, the link between type 2 immunity; transcription factors, such as Janus kinase or signal transducer and activator of transcription; and high levels of IgE was investigated in immunodeficiency syndromes, such as signal transducer and activator of transcription 3 and dedicator of cytokinesis 8 hyper-IgE syndromes or combined immunodeficiency disorders.[8,9]

Table E1 in this article's Online Repository at www.jacionline.org lists the main genetic conditions that have provided insight into AD pathogenesis to date. Although the study of rare variants offers the opportunity to delineate distinct molecular mechanisms and control pathways of a particular phenotype and thus can be regarded as "human models of AD," a limitation of this approach is that not all observed phenomena are relevant in patients with AD, which is more complex and heterogeneous than monogenic disorders.

The second *in vivo* approach to study the pathogenesis of AD is standardized challenge with allergens or other environmental factors. The most commonly used model is the atopy patch test (APT), an epicutaneous challenge of specific allergens dissolved in vehicle,[10] which has provided insight into the temporal development of immune phenomena in patients with AD (see Table E2 in this article's Online Repository at www.jacionline.org).[11] Although developed in part to define clinically relevant reactions to aeroallergens, food allergens, and autoantigens,[12-14] its validity and predictive value depend on a variety of factors

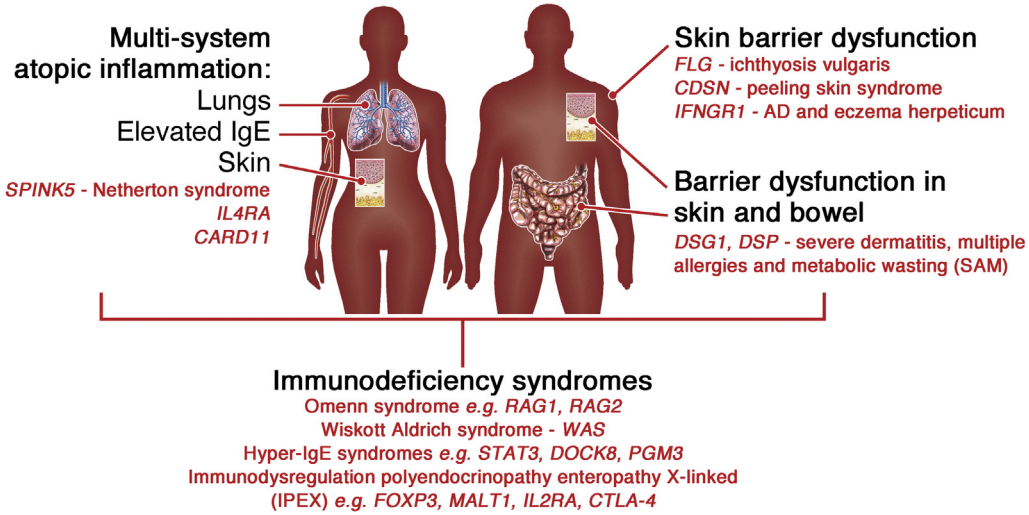

FIG 1. Diagrammatic representation of "human knockout" monogenic models, providing insight into the pathomechanisms of AD. Specific genetic variants affecting the structural and/or immune functions of skin or other organs recapitulate features but not the entire phenotype of atopic inflammation and AD. *CARD11*, Caspase recruitment domain-containing protein 11; *CDSN*, corneodesmosin; *CTLA4*, cytotoxic T lymphocyte–associated protein 4; *DOCK8*, dedicator of cytokinesis 8; *DSG1*, desmoglein 1; *DSP*, desmoplakin; *FLG*, filaggrin; *FOXP3*, forkhead box protein 3; *IL2RA*, IL-2 receptor α; *IL4RA*, IL-4 receptor α; *IFNGR1*, IFN-γ receptor 1; *MALT1*, mucosa-associated lymphoid tissue lymphoma translocation protein 1; *PGM3*, phosphoglucomutase 3; *RAG1*, Recombination-activating gene 1; *RAG2*, recombination-activating gene 2; *SPINK5*, serine protease inhibitor Kazal type 5; *STAT3*, signal transducer and activator of transcription 3.

in the protocol,[15] and the APT is not used routinely in clinical practice. Experimentally, the APT has provided insight into the temporal sequence of cutaneous cellular infiltrates. Acute skin lesions show a highly reproducible T_H2-dominant infiltrate,[16] although other cell types, including T_H17 cells, are also present.[17,18] This T_H2 dominance is in sharp contrast to other inflammatory skin diseases, such as psoriasis.[19,20] Time-course studies have shown that additional immune cell subsets, such as T_H1 and T_H22 cells, progressively infiltrate the skin during an ongoing APT reaction, mirroring the cellular composition of acute versus chronic human AD.[17,21] The APT has also been used to characterize dendritic cells within early lesional AD skin, such as inflammatory dendritic epidermal cells.[18] Furthermore, the APT has provided insight into the interaction of microbiota and our immune system, in particular the role of bacteria-derived superantigens acting as an amplifier of the allergen-specific cutaneous response in patients with AD.[21-23] In all these experimental APT studies, the population of patients with AD were well defined, with specific inclusion and exclusion criteria (although the precise definitions of AD varied); in most studies AD, together with specific IgE to the corresponding allergen used in the APT, was an inclusion criterion.

Hapten challenge to induce classical allergic contact dermatitis (ACD) in patients with AD has also broadened our understanding of AD pathogenesis (see Table E2). Whether patients with AD have an increased risk of ACD remains controversial and might depend on whether they harbor *FLG* mutations, which might have allowed increased allergen penetration. However, attenuated ACD reactions have been reported in patients with AD compared with control subjects in a severity-dependent manner.[24,25] This might be due to the fact that haptens induce distinct immune responses,[26] with fragrances mimicking the T_H2/T_H22 dominance of AD, whereas nickel, 2,4-Dinitrochlorobenzene (DNCB), or imiquimod[27] induced T_H1/T_H17-skewed immune responses. Of note, patients with AD show a T_H2-skewed ACD reaction,[28] and this immune deviation might account for the diminished ACD prevalence in patients with AD. A T_H2 immune reaction profile of patients with AD was also observed in an aero-challenge setup,[29] as well as when challenging patients with AD with physical factors, such as hard water.[30,31]

All current challenge models have some limitations (see Table E2) because they only represent acute reactions, and the small areas of application cannot reproduce the intense pruritus and sleep disturbances usually present in patients with AD. Furthermore, to date, they have not stratified for genetic differences/endotypes among patients with AD. For example, comparing APTs in patients with and without *FLG* mutations might be a useful line of future investigation. Moreover, in the future, molecular profiling of lesional skin from standardized challenge models adjusted according to AD endotype can be used in early clinical studies to evaluate the potential of new drugs to improve AD.[32]

IN VITRO MODELS

As shown in Table E3 in this article's Online Repository at www.jacionline.org, there are several 2-dimensional cell-culture and 3-dimensional organotypic models for AD that complement each other in addressing specific experimental questions. Although 2-dimensional cell culture models (by definition) do not duplicate the architecture of skin, they are amenable to high-throughput techniques for drug discovery and target validation (2-dimensional model section, see Table E3). Accordingly, Otsuka et al[33] used 2-dimensional cultures to screen a chemical library for compounds that enhance *FLG* transcriptional activation and mRNA expression, suggesting a

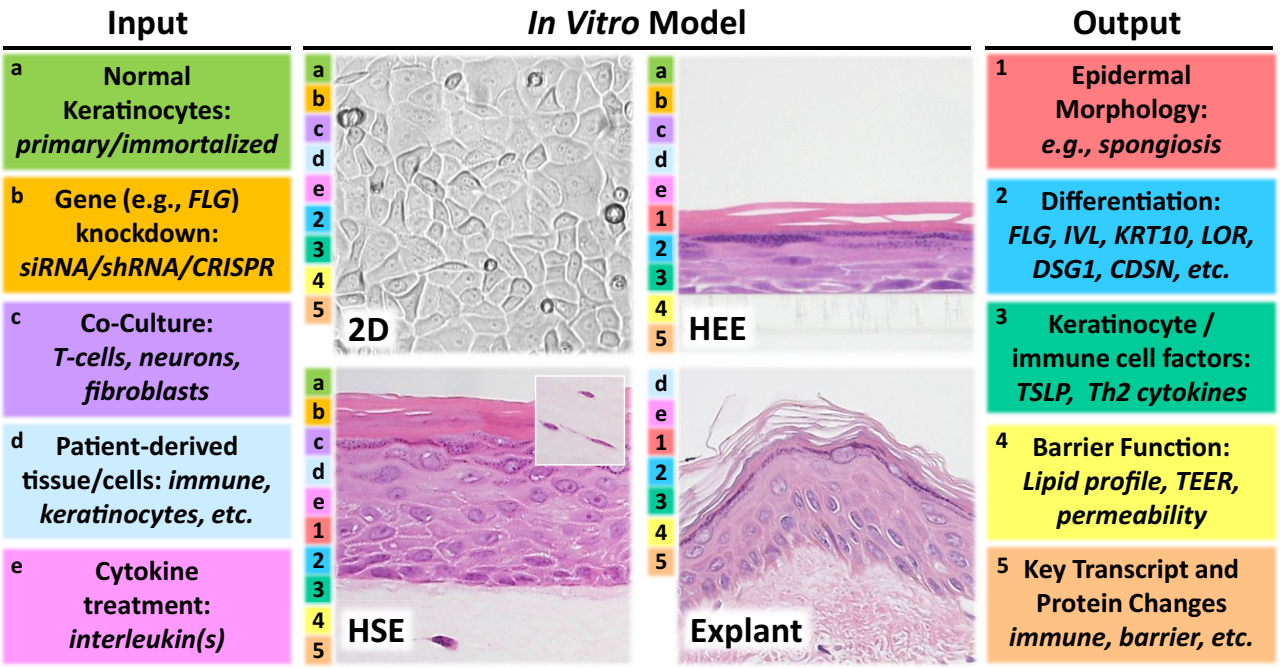

FIG 2. Human *in vitro* models of AD. *In vitro* models can be designed to address specific experimental questions based on the input materials of the cultures. Assessment of the cultures or output depends on the type of culture. *CDSN*, Corneodesmosin; *DSG1*, desmoglein 1; *FLG*, filaggrin; *HEE*, human epidermal equivalent; *HSE*, human skin equivalent (*inset*, fibroblasts in collagen); *IVL*, involucrin; *KRT10*, keratin 10; *shRNA*, short hairpin RNA; *siRNA*, small interfering RNA; *TEER*, transepithelial electrical resistance; *TSLP*, thymic stromal lymphopoietin.

potential novel therapeutic agent for AD. On the other hand, 3-dimensional models replicate the stratified squamous epithelium of epidermis but require specific expertise and are time consuming. Epidermal equivalents consist of keratinocytes without a dermal compartment, whereas skin equivalents have a dermis, such as fibroblast-embedded collagen (3-dimensional model section, see Table E3). Both 2-dimensional and 3-dimensional models are amenable to treatment with disease-relevant cytokines, gene knockdown, use of patient-derived cells, and/or coculture (Fig 2 and see Table E3).

The immune system is a major driver of AD, and *in vitro* immune modulation with disease-relevant cytokines, such as interleukins, can lead to AD-like phenotypes in normal primary keratinocytes[34] and 3-dimensional models (3-dimensional cytokine model section, see Table E3).[35-41] Knockdown of filaggrin (FLG) in culture systems can provide insight into the molecular and proteomic changes associated with its loss in patients with AD,[42] and combining FLG knockdown with other perturbations, such as cytokine treatment, can be used to study the multifactorial drivers of AD. For example, Honzke et al[43] reported that FLG knockdown exacerbated epidermal responses to IL-4 and IL-13, including increased proliferation and keratinocyte-released cytokines in 3-dimensional skin equivalents. Patient-derived cells for 2-dimensional and 3-dimensional culture or tissue for explant culture are limited by access and availability but might be the most relevant in terms of modeling AD.[44-47]

Furthermore, patients' biopsy specimens can be a source of skin cells other than keratinocytes, allowing for coculture models. Given that multiple systems contribute to AD, coculture models that include immune cells, dermal fibroblasts, and neurons can begin to address their interplay with keratinocytes. For example, Berroth et al[47] derived keratinocytes and fibroblasts from normal and AD skin and showed that AD-derived fibroblasts are sufficient to decrease *FLG* mRNA in normal-derived keratinocytes in 3-dimensional culture. Moreover, combining *FLG* knockdown with $CD4^+$ activated T cells uncovered direct cross-talk between keratinocytes and T cells that resulted in T-cell migration within the dermal compartment toward the epidermis.[48]

These studies highlight the levels of complexity that can be engineered into the 3-dimensional culture models. Three-dimensional culture systems have also been used to understand environmental influences on skin, including air pollution, UV radiation exposure, and bacterial infection.[49-51] Therefore these relevant environmental factors could be incorporated into *in vitro* models of AD. The 3-dimensional cultures and skin explants can also be used to assess the comparative efficacy and practical applicability of novel drug delivery systems.[52,53] Notably, despite the assorted methodologies applied in developing *in vitro* models of AD, there is overlap in the AD-like characteristics among the various models: most produce perturbed epidermal morphology, abnormal differentiation, and barrier dysfunction. Most often, disparities in reported phenotypes appear to stem, at least in part, from differences in the methodologies used in evaluating models (not necessarily because of the absence of the phenotype).

Although *in vitro* models might not mimic certain symptomatic and/or subjective aspects of the disease, such as pruritus and pain, they allow monitoring of changes in epidermal morphology and differentiation, gene and protein expression, lipid synthesis, and barrier function. Histologically, AD skin sections and most 3-dimensional models of AD show profound changes in the

epidermal compartment, including hypogranulosis, spongiosis, and increased cellularity because of hyperproliferation (3-dimensional model section, see Table E3). Changes in expression of genes (detected by using microarray, RNA-sequencing, or quantitative PCR) and protein (detected by using liquid chromatography–mass spectrometry, Western blotting, ELISA, or immunohistochemistry) can be used to evaluate disturbances in differentiation and immune response in 2-dimensional and 3-dimensional models. Lipid synthesis, which is required for optimal barrier function, can be monitored by expression of related enzymes or directly by using mass spectrometry. Epidermal barrier function can be monitored in 2-dimensional and 3-dimensional models, depending on the assay.

We recommend that the phenotype of any AD *in vitro* model should be extensively characterized and should include parallel analysis of epidermal morphology, differentiation status, loss or gain of key transcripts/proteins, analysis of immune components, and assessment of functional epidermal barrier parameters. Full characterization of any AD model can inform downstream evaluation of potential therapeutic agents with respect to reversing different aspects of the disease. Testing potential targets or drugs in several model types can add rigor and indicate whether a signaling pathway or protein is central to the diverse manifestations of AD.

IN SILICO COMPUTATIONAL MODELS

A core element of a systems biology approach is development of *in silico* computational models (mechanistic models) by means of integration of different types of experimental and clinical data from multiple studies, including those associated with disease conditions. *In silico* experiments (ie, computer simulations or mathematical analysis of *in silico* models) can test model-specific hypotheses, predict disease prognosis or treatment outcomes, and identify knowledge gaps, guiding future experiments and clinical trials that produce further data. This iterative process refines *in silico* models, providing holistic systems-level mechanistic insight into how perturbations (treatments or risk factors) lead to whole-organism phenotypes.

A mechanistic model describes causative interactions between the system's components involved in the phenomena of interest (eg, disease or treatment outcomes). Existing mechanistic models of AD vary widely, depending on the levels of interaction (tissue, cells, proteins, and genes) included in the model and mathematical methods used to describe the interactions.

Domínguez-Hüttinger et al[54] developed a multi-scale deterministic model that delineates interactions between the environment, skin barrier integrity, and immune activation using ordinary differential equations (Table I).[54-61] Two bistable "switches" are described: the first regulating the onset of AD flares and the second controlling progression to severe and persistent disease. The model predicts, for example, that genetic predisposition to barrier dysfunction (eg, *FLG* haploinsufficiency) predisposes to longer flares and more persistent disease and that prophylactic emollient use might be beneficial (Table I).

Application of optimal control theory to the hybrid mathematical model can inform the design of patient-specific optimal strategies for "proactive therapy" to prevent recurrent flares once the disease has been brought under initial control.[55] For example, this computational model supports the need for a greater topical steroid treatment dose after disease worsening and the potential need for more frequent than 2 to 3 days per week application of topical steroid treatment to maintain remission[62] in patients with *FLG* haploinsufficiency (Table I), presenting a readily testable stratification treatment regimen based on genotype.

Polak et al[56] developed a stochastic Petri net model that delineates genetic regulatory mechanisms responsible for immune responses in Langerhans cells (LCs; Table I). The model describes reported interactions between interferon regulatory factors, interferon regulatory factor transcription partners, and DNA sequences in a logic-based diagram. *In vitro* experiments validated model predictions that the ability of LCs to present a peptide is altered by cytokine milieu and that a phosphoinositide 3-kinase γ inhibitor reduces the ability of LCs to induce T_H1 responses. These smaller-scale and focused mechanistic models can describe detailed interactions that are difficult to include and validate in multiscale models. Inclusion of the detailed interactions would make the multiscale models too complex to interpret and validate because of the current lack of quantitative dynamic data measuring the variables across different scales simultaneously.

Subramanian et al[57] used a pathway model that included manually curated skin-specific pathways and relevant genes (Table I). Pathway enrichment analysis using transcriptomic data sets of patients with AD provided mechanistic insights into drug actions of topical betamethasone and pimecrolimus. The pathway model would allow *in silico* experiments once the kinetics parameters for pathways are identified to provide quantitative and dynamic predictions of disease progression and treatment outcomes.

Population pharmacokinetic and pharmacodynamic models have also been developed to describe differences and variability in pharmacologic effects observed in large clinical studies for AD treatment.[58,59] The authors identified the model parameters that best fit the effects of nemolizumab and dupilumab measured in terms of AD severity score or pharmacokinetics (Table I).[58,59] Population pharmacokinetic and pharmacodynamic models could help achieve mechanistic understanding of pharmacologic effects if combined with mechanistic models.

One of the challenges in developing mechanistic models is identification of the components and pathways relevant to the model-specific hypothesis to be tested. This can be achieved by using unbiased multivariate analyses of a collection of large-scale data, for example by using machine learning data analysis. Application of machine learning methods to AD-related data is relatively limited at present, but some relevant works have been already published. Thijs et al[60] developed a piecewise linear mixed model to predict AD severity scores after different treatments, and Kiiski et al[61] developed a multivariate logistic regression model to predict a "good treatment response." A sufficient level of cross-validation is crucial to reduce bias and ensure the general applicability of models with predictive power beyond mere data description.

All the models presented above were developed based on the published data derived from studies in which the inclusion and exclusion criteria for AD were specified. Although the majority of studies used the Hanifin and Rajka criteria and specified further clinical (including comorbidities) and demographic details, it is clear that patients with AD present with a wide spectrum of

TABLE I. *In silico* computational models of AD

Model type	Scientific merits	Clinical utility	Limitations	Key features	Key findings/ predictions	References
Multiscale mechanistic model	Mechanistic understanding of system-level effects of potential triggers and processes on disease state	Identification of therapeutic targets and their mechanisms for further clinical investigation Prediction of dynamic effects of therapeutics, leading to patient stratification	Models developed based on hypothesized relationships that were previously described experimentally	A hybrid ordinary differential equation model of the dynamic interplay between skin barrier function, immune responses, and environmental stressors that determines AD pathogenesis	Preventive effects of emollients against AD progression (shown by clinical trials) Synergistic effects of environmental (eg, microbiome) and genetic (eg, FLG) risk factors on AD progression (shown by mouse experiments with ovalbumin challenge or dose-dependent effects of FLG deficiency)	Dominguez-Huttinger et al[54]
				A hybrid model of treatment effects of corticosteroids and emollients on AD pathogenesis and exploration of optimal regimens for induction of remission and maintenance of remission	Poor adherence to the suggested optimal treatment schedule leads to higher treatment doses. Application of corticosteroids for 2 consecutive days per week is optimal for the maintenance period.	Christodoulides et al[55]
Gene regulatory network model	Understanding of gene regulatory mechanisms behind disease processes	Identification of therapeutic targets and their mechanisms at the gene regulation level	Models developed based on published genetic interactions	Stochastic Petri net model of interferon regulatory factor gene regulatory network in response to *in vitro* treatment of LCs with TNF-α and TSLP	*In vitro* experiments validated predictions that the ability of LCs to present a peptide is altered by cytokine milieu and that PI3Kγ inhibitor reduces the ability of LCs to induce T_H1 responses.	Polak et al[56]
Pathway models	Understanding of disease mechanisms	Identification of therapeutic targets and their mechanisms	Models developed based on published pathways	A pathway model including 35 manually curated skin-specific pathways and >2600 genes	Pathway enrichment analysis using transcriptomic data sets of 10 patients with AD treated with betamethasone valerate and pimecrolimus predicted mechanism of action of both drugs on human skin.	Subramanian et al[57]

(Continued)

TABLE I. *(Continued)*

Model type	Scientific merits	Clinical utility	Limitations	Key features	Key findings/ predictions	References
Population PK/PD models	Understanding of differences and variability in pharmacologic effects among a target population from clinical trial data	Prediction of optimal dose regimen Testing effects of weight, sex, etc	Requires large clinical data to have sufficient predictive power	PK/PD model for serum nemolizumab and pruritus VAS developed from 299 patients' time course data	An appropriate flat dose regimen that is independent of body weights is used.	Saito et al[58]
				Two-compartment PK model for dupilumab developed from data of 197 healthy volunteers and patients with AD from 6 studies	Production rate of IL-4Ra is similar for patients with AD and healthy volunteers and does not change over time.	Kovalenko et al[59]
Machine learning predictive models	Unbiased analyses of differences between disease and nondisease (including treated) tissue/ patients and prediction of clinical outcomes (prognostic and therapeutic)	Identification of disease and therapeutic targets Findings can feed into mechanistic models	Causative mechanisms remain largely unknown Machine learning applications to atopic eczema relatively limited at present	Piecewise linear mixed models to predict EASI scores at 3 future time points from baseline biomarkers Developed from data of 150 serum biomarkers measured in 193 patients with AD	Combination of TARC, IL-22, and sIL-2R provides a good predictor for future EASI score.	Thijs et al[60]
				Multivariate logistic regression model to identify predictors of long-term response to topical maintenance treatment in AD on 169 patients	Serum total IgE (rather than the initial severity) is the most important factor predicting a good long-term treatment outcome.	Kiiski et al[61]

EASI, Eczema Area and Severity Index; *IL-4Ra*, IL-4 receptor antagonist; *PI3Kγ*, phosphoinositide 3-kinase γ; *PK/PD*, pharmacokinetics/pharmacodynamics; *sIL-2R*, soluble IL-2 receptor; *VAS*, visual analog scale.

clinical and molecular features (including, for example, a greater heterogeneity in transcriptomic profile of lesional skin compared with psoriasis).[63]

FUTURE DEVELOPMENTS

The development of more sophisticated human and computational models of AD that integrate large-scale clinical and "omics" data offer the potential for a deeper understanding of disease endotypes, molecular mechanisms underlying key pathogenic events and clinical hallmarks of AD, as well as prediction of therapeutic outcomes, including comorbidity at the level of an individual patient. Accepting that, by definition, these models are based on a reductionist approach, they need to reflect the complexity of AD pathogenesis, including epidermal barrier dysfunction, altered penetration of chemicals and allergens, host/environment interaction, type 2 immunity, and tissue remodeling. We have illustrated in this review that the main approaches available today are *in vitro* models, identification and characterization of human inherited syndromes resembling AD, *in vivo* challenges of patients with AD, and *in silico* models. Here we speculate how the future of AD research will likely inform the development of more refined models of AD.

Refinement is likely to depend, at least in part, on methodological advances in the field and the additional information generated by novel approaches. For example, single-cell sequencing has recently identified novel rare but important immunologic subsets,[64] and intravital photon microscopy has enabled visualization of cell-cell communication during inflammation.[65,66] Application of this technology to AD is likely to inform the inclusion of distinct epithelial and immune cell types[64] and/or genetically modified primary human cells.[67] Furthermore, small-scale spheroid organoids can enhance high-throughput approaches in the field.[68] Finally, we expect that a technological breakthrough in the development of 3-dimensional skin models will be facilitated by cell printers.[69,70]

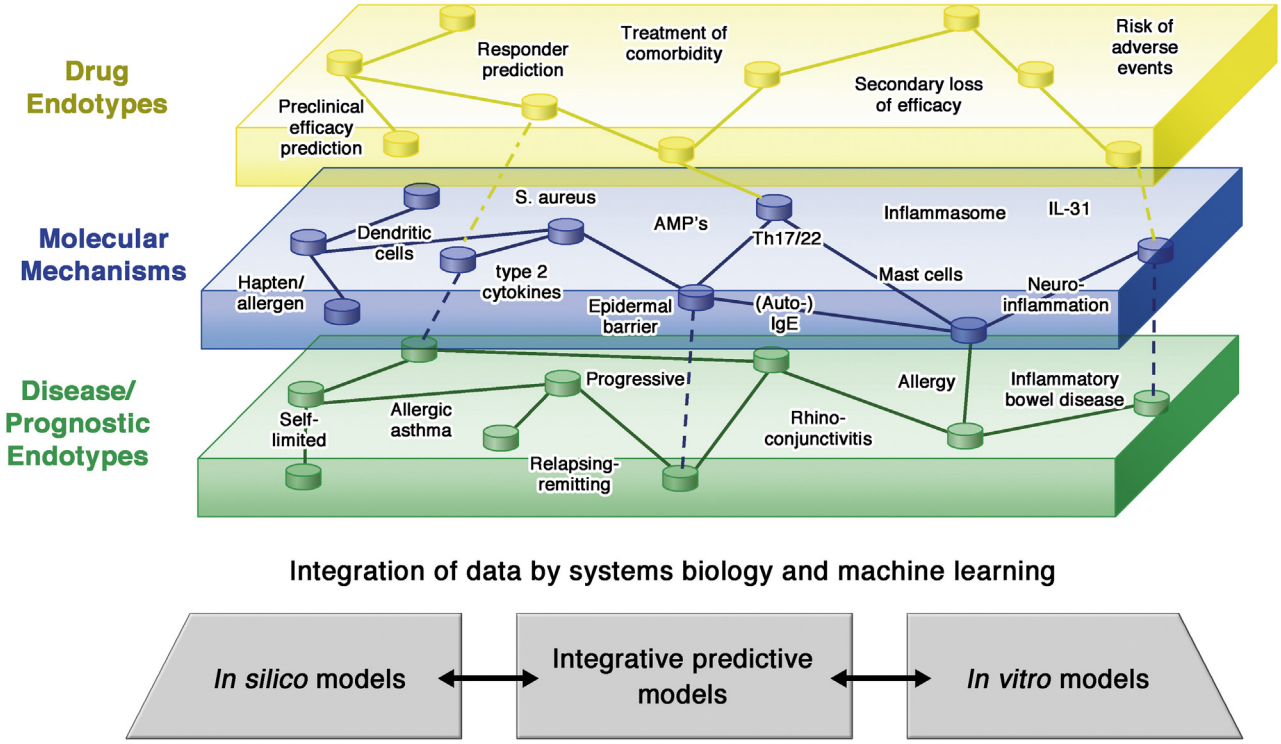

FIG 3. Interconnected multilayer networks: the future of human AD modeling. A combination of innovative *in vitro* and *in silico* models obtained by a systems biology approach and machine learning algorithms will be needed to answer clinically relevant questions, such as identification of distinct disease endotypes, elucidation of molecular pathomechanisms, or prediction of therapeutic response.

Deep neural networks are being applied as artificial intelligence tools to facilitate physician interpretation in the field of melanoma diagnostics[71] and increasingly as methods to enable large data set integration. The first examples of disease classifiers[72] and prediction of disease severity from biomarker sets[61,73,74] have recently been published, and we expect this line of development to continue while ensuring a sufficient level of validation. We anticipate that refinement of these methods, in combination with *in silico* models, can lead to computational approaches and predictive models applied to diagnostics and therapeutic stratification.

The descriptive disease ontology of inflammatory skin diseases will need to be revised by shifting to pathogenesis-oriented structure[75] and, in the future, by better definition of disease endotypes based on integration of multiomics data, clinical features, and clinical response to therapy in light of *in silico* models as assessed in large-scale and longitudinal cohorts.[76] These advances are likely to inform the development of many of the current models.

However, to achieve a substantial breakthrough, we expect that different approaches will need to be combined, integrated, standardized, and performed at larger scale (Fig 3). For example, observations made in rare human disease variants or through specific challenge models in patients with AD can be validated *in vitro* and mapped to disease signatures *in silico*. Validation of functional hypotheses will increasingly depend on cross-referencing of data derived from clinical samples with outputs from *in vitro* models. Integration of clinical, biomarker, pharmacokinetic and pharmacodynamic (topical and/or systemic), and clinical outcome data will inform therapy development and precision medicine. Notably, all of our models depend on how precisely a particular question is asked and the quality of the clinical input, including the clinical metadata and integration with "omics" data derived from clinical samples. Finally, advanced statistical and machine learning analysis combined with *in silico* predictive modeling will be required to integrate information throughout all described layers and data sets to elucidate underlying mechanisms (and endotypes), further highlighting the importance of data standardization and scientific networking.

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Table 1. Genetic disease models of AD

Genetic disease	Gene and mutation type(s)	Phenotype(s)	Mechanistic insights	Clinical utility	Limitations	Pathway relevance for drug development	Refs.
Skin barrier dysfunction							
Ichthyosis vulgaris (IV)	*FLG* Loss of function mutations semi-dominant in IV and complex trait in AD	Early onset, severe and persistent AD with & without other atopic diseases; predisposition to eczema herpeticum (EH)	Understanding that skin barrier dysfunction predates atopic inflammation	Illustrates importance of barrier repair	Molecular mechanisms and control pathways remain unclear		1, 2
Generalised peeling skin	*CDSN* Loss of function mutation autosomal recessive	Ichthyosiform erythroderma, pruritus and food allergies	Confirms the role of corneodesmosin in epidermal adhesion	Understanding that skin barrier dysfunction predates atopic inflammation			3
AD and eczema herpeticum	*IFNGR1* Loss of function mutation Complex trait	AD and eczema herpeticum (EH)	Defective systemic IFN-gamma immune response accounts for disseminated viral skin infections	Helps to explain why a subset of AD patients suffer recurrent EH	Does not explain all cases of EH		4
Netherton syndrome	*SPINK5* Loss of function mutation Autosomal recessive	Congenital ichthyosis, severe atopic disease, elevated IgE, hypereosinophilia, infections	Single nucleotide variants associated with AD. Illustrates role of epidermal protease inhibitors and kallikrein proteases in regulating epidermal barrier function	Understanding that skin barrier dysfunction predates atopic inflammation		Protease inhibitors	5
Systemic atopic inflammation							
Atopic disease	*IL4RA* Gain of function Complex trait	Elevated IgE with & without AD	Mutation found in severe cases is also a common risk allele in the population	Evidence of role for IL-4 in atopic inflammation		IL-4RA	6
Severe atopic disease	*CARD11* Heterozygous mutations Loss of function and dominant negative effect	Severe AD with & without infection	Illustrates importance of lymphocyte receptor signalling	mTORC1 and IFN-gamma production defects can be partially rescued by glutamine supplementation	Unclear whether this mechanism plays a role in prevalent AD	NFKB and MALT1	7
Skin inflammation and gastrointestinal inflammation							
SAM (Severe dermatitis, multiple Allergies and Metabolic wasting)	*DSG1* Homozygous loss of function mutations	Ichthyosiform erythroderma, atopic disease and failure to thrive	*DSG1* mutations lead to loss of cell-cell adhesion in epidermis	Structural epidermal defects lead to atopic inflammation			8
SAM	*DSP* Heterozygous mutation	Ichthyosiform erythroderma, atopic disease and failure to thrive	*DSP* mutations result in disrupted keratin filament attachment to desmosomes	Structural epidermal defects lead to atopic inflammation	Other *DSP* mutations cause different phenotypes without atopic manifestations		9
Immunodeficiency syndromes							
Hyper-IgE	*STAT3* Dominant negative mutations	AD-like skin inflammation, elevated IgE, immunodeficiency leading to infection	Illustrates role of STAT3 in signal transduction for multiple cytokines	Biologic treatments targeting IgE have limited clinical efficacy for AD	Immunodeficiency is not a prominent feature of AD	STAT6: downstream of JAKs in Th2 inflammation	10
	DOCK8 Autosomal recessive loss of function mutations	AD-like skin inflammation, elevated IgE, immunodeficiency leading to infection	Aberrations of T cell and NK cell migration to skin can cause atopic inflammation	Antiviral and antibacterial prophylaxis, immunoglobulin replacement and HSCT			11
Omenn syndrome	Hypomorphic missense mutations in a range of genes involved T and B cell development *eg. RAG1*, *RAG2*	AD-like skin inflammation, elevated IgE, immunodeficiency leading to infection	Skin inflammation can occur in the absence of adaptive immunity, also seen in mice				12
Hyper-IgE like syndrome	*PGM3* Autosomal recessive loss of function mutations	AD-like skin inflammation, atopy, immune deficiency, autoimmunity and neurocognitive impairment	Role of glycosylation in immune regulation and systemic atopy				13
Wiskott-Aldrich	*WAS* X-linked mutations	AD-like skin inflammation, severe immunodeficiency, autoimmunity and malignancy	Systemic imbalances in Treg populations can drive cutaneous AD like inflammation	Requires HSCT		OX40	14
IPEX and IPEX-like syndromes	*FOXP3, MALT1, IL2RA, CTLA-4* Autosomal recessive	Immune dysregulation, polyendocrinopathy, enteropathy and AD-like skin inflammation	Role of autoimmunity in AD-like inflammation	Immunosuppressive treatment or HSCT		*FOXP3* as possible target for gene editing	15, 16

	mutations						

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Abbreviations

EH, eczema herpeticum; HSCT, haematopoetic stem cell transplantation; IPEX, Immunodysregulation Polyendocrinopathy Enteropathy X-linked; IV, ichthyosis vulgaris; SAM, Severe dermatitis, multiple Allergies and Metabolic wasting

Table S2. Human *In vivo* Models of AD

Atopy Patch Test	System Interplay/ Application	Key Findings	Scientific Merit/ Clinical Relevance	Limitations	Reproducibility	Refs.
APT: clinical usage		Reviewed in: EAACI position paper				(2)
Epidemiology	Frequency of patch test reactions to inhalant allergens in AD patients (n=56)	D. farinae: 33.9%; D. pteronyssinus: 35.8%; American cockroach: 21.8%	Positive APT reactions occur frequently in AD patients	Small cohort		(1)
Validity/relevance	Comparison of APT and SPT in children with AD (n=253)	APT: higher specificity (69-92% depending on the allergen) than SPT (44-53%) and IgE levels (42-66%)	APT may be useful to diagnose clinically relevant sensitizations to inhalant allergens	Clinical relevance mainly evaluated by history only		(2)
	Comparison of APT and LTT in AD patients (n=96)	48% of aeroallergen sensitized patients had a positive APT; this correlated highly significant with a positive LTT	APT reactions are significantly correlated to allergen specific lymphocyte proliferation			(3)
	Comparing AD groups: with and w/o clinical symptoms (n=79)	66.7% of cases with and 10.5% of cases without a predictive history of exacerbations during pollen season	APT indicates clinically relevant positive reactions to inhalant allergens	Only investigated grass pollen, small cohort	Several studies with similar results, e.g. (1)	(4)
	Comparing APT, SPT, and sIgE with food challenge in children with AD	In a large cohort (n=1007 APT, 873 challenges), APT to food allergens added only a small predictive value to SPT and sIgE	APT to food allergens is less robust compared to APT to inhalant allergens; higher specificity and lower sensitivity than SPT and sIgE			(5)
Reproducibility	Reproducibility of APT reactions in AD patients (n=16)	15/16 (94%) patients had a reproducible APT reaction	APT results are highly reproducible	Small cohort		(6)
	Different vehicles and allergen concentrations (AD patients)	Petrolatum as a vehicle and allergen concenrations of at least 1000 protein nitrogen units/ml give best outcome	Validity of APT reactions depends on vehicles and allergen concentrations			(7)
APT: immunological relevance		Reviewed in:				(8, 9)
Th2 immunity	Tissue cell culture from APT reactions (AD patients)	APT reactions contain Der p specific Th2 cells	APT can be used as a model for acute AD	Early proof of concept study	Reproduced in several studies, also for other allergens	(15)
	Comparison of APT reactions to lesional AD in AD patients	Dust mite induces a Th2/Th9 skewing, but also Th17/Th22 activation	Reaction to dust mite does not fully reflect human AD, e.g. regarding barrier	n=15		(16)
	Gene expression in lesional APT skin (AD patients)	APT to different food allergens induces Th2, Th17 responses and IL-33	APT reflects a type 2 dominated immune response			(17)
Dynamics/kinetics of immune response	Histology/ gene expression/ TCC from dust mite APT in AD patients	Early APT reactions are mediated by Th2, while other T cell responses occur in the course of the reaction	APT reflects acute immunity as well as later stages of AD immunity		Highly reproducible also for other allergens, e.g. (10, 11)	(18)
	Interaction of allergen and microbiota in AD patients	Superantigens cause increased APT reaction Superantigens induce IL-17 and IL-22 in APT reactions	Microbial products influence AD		Reproduced in (12)	(19) (20, 21)
	Immune-histochemistry and flow cytometry of DCs in AD (n=66) compared to CD (n=12)	Inflammatory epidermal dendritic cells migrate early in APT reactions where they persist; FceRI is associated to extrinsic AD	APT is a useful model to investigate DC subtypes	No functional analysis		(22)
Specificity	APT in patients with co-existing psoriasis and AD (n=8)	Dust mite induces a Th2 mediated eczematous reaction in sensitized psoriasis patients	At least a subgroup of AD is caused by adaptive immunity	Special, small cohort of patients	Reproduced in (13)	(23)
	APT to autoantigens in AD patients	AD patients with T cell-mediated autoimmunity against manganese superoxide show APT reactivity	Identification of manganese superoxide as autoantigen in AD		Reproduced for Malassezia sympodialis thioredoxin (14)	(24)
Contact allergens	**System Interplay/ Application**	**Key Findings**	**Scientific Merit/ Clinical Relevance**	**Limitations**	**Reproducibility**	**Refs.**
Patch testing: clinical usage		Reviewed in:				(25, 26)
Epidemiology	Patch tests to haptens in AD patients	AD patients with severe disease have lower prevalence of contact allergy.	Clinically relevant ACD in AD patients needs to be ruled out by patch testing.	No definite information about severity	Reproduced in a large meta-analysis	(29)
Immunological relevance	Patch tests to experimental haptens in AD patients	AD patients have attenuated ACD reactions compared to controls and in a severity-dependent manner.	Immune bias in AD reduces the ability to amount a contact allergic response.		Highly reproducible, e.g. (27, 28)	(30)
	Gene expression following patch tests	Nickel induces Th1/Th17 responses, fragrances induce a Th2/Th22 immune response	Haptens induce distinct molecular profiles; some of			(31)

	to different haptens (n=24 ACD patients w/o AD)		them might mimic AD			
	Patch tests in AD patients (n=18) and healthy volunteers (n=10)	DNCB-specific immune responses in controls were Th1 dominated; Th1 immunity was less in AD, but here a specific and stable Th2 immunity was induced towards DNCB	AD patients show a Th2 skewed ACD reaction	Small cohort (n=16 AD patients); experimental hapten		(32)
	Repetitive application of hapten	Repetitive hapten challenge caused a switch in immune response towards Th2 immunity including barrier damage	Immune responses towards a hapten might change after repetitive challenge	Murine study		(33)
Other challenge models	**System Interplay/ Application**	**Key Findings**	**Scientific Merit/ Clinical Relevance**	**Limitations**	**Reproducibility**	**Refs.**
Aero-challenge	Pollen chamber challenge of sensitized AD patients	AD patients sensitized to grass pollen reacted with worsening of AD symptoms and biomarkers	IgE might play a role in AD	No direct causal link to IgE		(34)
Treatment Standardization	Application of vehicles and/ or topical treatments in AD patients	Standardized application of different topical treatments, assessment of TSS, TEWL, and biomarkers	Approach of standardized clinical assessment of topical treatments			(35)
	Application of petrolatum (n=13 AD patients, n=36 healthy volunteers)	Petrolatum enhances antimicrobial peptides and epidermal barrier genes	Barrier restoration might also repair immune abnormalities in AD	No evidence for a specific effect of petrolatum		(36)
Trigger challenge	Application of established AD triggers (AD patients)	Hard water increases IL-4, IL-10 and IFN-gamma	Domestic hard water exposure during infancy increase risk of AD.	Experimental design does not mimic real world exposure		(37)

Abbreviations: APT: Atopy Patch Test; SPT: Skin Prick Test; LTT: Lymphocyte Transformation Test; ACD: allergic contact dermatitis; TSS: total sign score; TEWL: transepidermal water loss; DC: dendritic cell; CD: contact dermatitis

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Supplementary Table 1. Human *In vitro* Models of AD

2D Models	System Interplay/Application	Key Findings	Scientific Merit/Clinical Relevance	Limitations	Ref
Patient-derived cells	Epidermis→Immune	AD HEK vs. NHEK: ↑GM-CSF ; conditioned media from AD keratinocytes induced PBMC proliferation	GM-CSF associated with population-specific AD pathogenesis and severity (1-3)	No epidermal characteristics assessed; small patient cohort (*n* = 8)	(4)
FLG knockdown (KD)	Barrier→Immune/Epidermal Differentiation	NHEK: *lentiviral KD*; ↑Th2 cytokines: IL-2/4/5/13; ↓IFNγ ; ↓KRTs, ↓IVL, ↓TGM1, ↑Lor	FLG KD induces keratinocyte cytokine release (5); FLG changes are associated with AD	No assessment of lipids or barrier function; no rescue experiment	(6)
IL-4/IL-13 treatment	Immune→Barrier	NHEK: ↓FLG mRNA and protein	Cytokines known to drive AD	No epidermal characteristics assessed	(7)
AD drug discovery model	Barrier	Compound library screened by FLG reporter assay in HaCaT cells; NHEK: ↑ FLG mRNA and promoter activity by compound JTC801; ↑FLG in 3D and explant cultures	JTC801 ↑FLG and suppressed AD-like phenotype in NC/Nga mice	Only FLG taken into consideration as a target	(8)
Immune cells only	Immune	TSLP receptor is increased in AD-derived skin-associated Th2 cells; TSLP increases IL-4 producing T-cells	TSLP highly expressed in AD keratinocytes and known to trigger dendritic cells (9)	No epidermal component	(10)
3D Models	**System Interplay/Application**	**Key Findings**	**Scientific Merit/Clinical Relevance**	**Limitations**	**Ref**
FLG KD Models			***FLG* is relevant in pathogenesis of AD (11)**	**FLG loss associated with 20-50% of AD (12); *FLG* KD does not always cause AD-like phenotype *in vitro* (13, 14)**	
Human epidermal equivalent (HEE)[a]	Barrier→Immune/Epidermal Differentiation	NHEK: *lentiviral shRNA KD*; epidermal thickening; FLG loss associated with changes in proteases, inflammatory, and stress-related pathways based on proteomic profiling	Findings validated in AD patient samples; data can enhance systems biology modeling of AD	No evidence changes in protein expression underlie AD phenotype	(15)
	Barrier→Epidermal Differentiation	NHEK: *lentiviral shRNA KD*; hypogranulosis; ↓corneodesmosomes; ↓NMF; ↑barrier permeability; ↑UV sensitivity; altered differentiatic	FLG loss is clinically associated with barrier dysfunction; similar results with *FLG2* KD (16)	Epidermal thinning; immune component not assessed; no rescue experiment	(17)
Human skin equivalent (HSE)[b]	Barrier→Epidermal Differentiation	NHEK: *siRNA KD*; hypogranulosis; ↑barrier permeability; ↑UV sensitivity	FLG loss is clinically associated with barrier dysfunction; siRNA produced similar phenotype in other studies (18-20)	↔differentiation or lipid synthesis; immune component not assessed; no rescue experiment	(21)
***FLG* KD + IL-4/IL-13 HSE**	Barrier→Immune/Epidermal Differentiation	NHEK: *siRNA KD*; spongiosis, ↑proliferation; ↑epidermal thickness; ↓IVL; ↓LOR; ↓OCLN; ↑TSLP; ↑DEFB4A	Combination of FLG loss and immune activation	Barrier function not assessed; no rescue experiment	(22)
Co-culture Models			**Multiple systems contribute to AD**		
CD45RO+ T-cell HSE	Epidermis→Immune	HaCaT: spongiosis; ↑apoptosis; ↓TEER; ↑cytokine release; ↑ICAM-1; ↑NT-4	Activated T-cells drive AD; dexamethasone or tacrolimus reversed 3D model phenotype	Primary keratinocytes not used	(23)
***FLG* KD + CD4+ T-cell**	Immune→Epidermis→ Immune	NHEK: *siRNA KD*; ↑IL-8 and IL-6 secretion; ↑skin surface pH; ↓ IVL; ↑barrier permeability; ↑TSLP; ↑T-cell migration; CD4+ T-cells shift to Th2/Th22	TSLP-dependent T-cell migration indicates direct T-cell/keratinocyte cross-talk	No histological changes vs. *FLG* KD without T-cells	(24)
AD cell-derived HSE	Dermis→Epidermis→Dermis	Healthy NHEK + AD Fibroblasts: ↓FLG/*FLG* mRNA; ↓KRT10; epidermal thickening AD HEK + Healthy Fibroblasts: rescues FLG, KRT10, KRT5	Fibroblasts may mediate immune cell infiltration in skin (25)	Immune component and barrier not assessed; AD patient samples with variable FLG status;	(26)
Nerve HSE	Neurons→Epidermis	NHEK: Innervated cultures alone or with substance P+CGRP neuropeptides ↑epidermal thickness and ↑ Ki67; AD HEK vs. NHEK: ↑innervation; ↑ epidermal thicknes	Increased nerve fibers in AD (27); used for drug discovery of neuron-modulating agents (28)	Immune component and barrier not assessed; porcine dorsal root ganglia used for neurons	(29)
Cytokine Models			**Immune modulators are relevant to AD**		
IL-4-treated HSE	Immune→Epidermal Differentiation	N/TERT: ↑proliferation; ↓KRT10; ↓IVL; suprabasal integrin -β1	Assesses the effects of a single cytokine; similar effects on proliferation in NHEK (30)	Primary keratinocytes not used; IL-4 alone shown not reduce FLG in NHEK (30)	(31)
IL-4/IL-13-treated HSE	Immune→Barrier	NHEK: spongiosis; ↑apoptosis; ↑phosphorylated STAT6; ↑*CA2* mRNA; ↑*NELL2* mRNA	mRNA levels matched AD biopsies No change in psoriasis-associated genes	Barrier not assessed; dexamethasone or tacrolimus did not reverse phenotype	(32)
IL-17-treated HSE	Immune →Barrier/ Epidermal Differentiation	NHEK: ↓TEER; ↑barrier permeability; ↓TJ proteins; SC thickening; Δ in FLG and LOR localization	Loss of TJ proteins confirmed in small cohorts of normal and AD patients	Changes in keratinocyte immune signaling not assessed	(33)
IL-31RA expression + IL-31-treated HSE	Immune→Epidermal Differentiation	HaCaT: ↓FLG[†]; ↓desmosomal transcripts*; ↓*CASP14* mRNA*; ↓ *CDSN* mRNA; ↔ TJ proteins; ↑barrier permeability; ↑IL-1α release* NHEK: ↓FLG; ↑antimicrobial peptid	IL-31 expression associated with AD (34); similar effects seen in HaCaT cells (35)	Most experiments performed with HaCaT cells	(36)
Cytokine cocktail-treated HEEs	Immune→Epidermal Differentiation	NHEK: *Cocktail: poly(I)C, TNFα, IL-4, IL-13* ↓FLG/*FLG* mRNA; altered differentiation and inflammation; ↑TSLP and ↑IL-8 secretion	Transcriptomic profiling after cocktail correlates with AD datasets; ↓FLG with TNFα/IL-4, IL-13, IL-22 cocktail (37)	Barrier function not assessed	(38)
		NHEK: *Cocktail: IL-4, IL-13, IL-25 with or without methyl-β-cyclodextrin (disrupts lipid rafts)* hypogranulosis; spongiosis; ↓TEER; ↓*FLG* mRNA; ↓LOR/*LOR* mRNA; ↑ CA2/*CA2* mRNA; ↑*NELL2* mRNA	Effect on protein expression by cocktail treatment correlated with AD patient samples;	Role of membrane lipid domains not clear in AD; no change in keratinocyte TSLP	(39, 40)
		NHEK: *Cocktail: TNFα, IL-4, IL-13, IL-31* spongiosis; ↑proliferatio altered differentiation; ↑TSLP; ↓fatty acids; ↓ceramid	Tested cytokines alone and in combination	Barrier function not assessed	(30)
ILs and HMGB HSE and HEE	Immune→Immune/Epidermal Differentiation	NHEK*: ↑epidermal alarmins (IL-33 and HMGB1) with IL-25+IFNγ; IL-25, IL-33, IL-4, or HMGB1 treatment ↓FLG/*FLG* mRNA; ↓IVL; ↓LOR; ↑proliferatio	Effects seen in 3 epidermal culture models	epidermal thinning; barrier function not assessed	(41)
Allergy Models					
Histamine treatment HEE	Immune →Barrier/Epidermal Differentiation	NHEK: ↓ FLG/*FLG* mRNA*; ↓LOR/*LOR* mRNA*; ↓KRT10/*KRT10* mRNA*; ↓DSG1; CDSN; ↓TJ proteins; ↑barrier permeability	Histamine mediates mast cells which are correlated to inflamed skin (42)	No change in histamine-treated explant cultures	(43)
Explant Models					
Patient samples	AD vs. normal tissue	In AD explants: ↓LOR; ↓IVL; ↓desquamation enzyme	Explants maintain AD biopsy phenotypes	Barrier function not assessed; no system perturbations	(44)
Cytokine cocktail	Immune→Skin→Immune	*Cocktail: IL-4, IL-5, IL-13, TNFα*; ↑TSLP release;↑IL-8; induction of dendritic cell maturation	Use of skin explants and epidermal explants; TSLP release relevant to AD (9)	AD skin samples not used; barrier function or differentiation status not tested	(45)

a, epidermal equivalents are 3D cultures with only keratinocytes; b, skin equivalents are 3D cultures with components of the dermis, e.g., collagen lattice and/or fibroblasts; ↑: increase, ↓: decrease, ↔: no change; *, effects observed in 2D cultures of the same cell type; †, effect observed in explant culture

Abbreviations: AP-1: activator protein 1; CGRP: calcitonin gene-related peptide, CAII: carbonic anhydrase II, CASP14: caspase 14, CDSN: cormeodesmin, DSG1: desmoglein 1 FLG: filaggrin, GM-CSF, granulocyte-macrophage colony-stimulating factor, HMGB1: high-mobility group box 1, ICAM-1: intracellular adhesion molecule 1, IFN: interferon, IL: interleukin, IVL: involucrin, KRT: keratin, LOR: loricrin, NELL2: neural epidermal growth factor–like 2, NHEK/HEK: normal human epidermal keratinocytes (primary), NMF: natural moisturizing factor, NT-4: neurotrophin 4 PBMC: peripheral blood mononuclear cell, STAT6: signal transducer and activator of transcription 6, TJ: tight junction, TEER: transepithelial electrical resistance, TGM1: transglutaminase 1, TSLP: thymic stromal lymphopoietin

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