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Reconstructed human epidermis for *in vitro* studies on atopic dermatitis: A review

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disease causing a strong impact on quality of life. Its pathophysiology is the result of complex interactions involving immunological, genetic and environmental factors. Although there are several published *in vitro* three-dimensional models mimicking AD, none of them have taken all these pathophysiological features into account; thus, finding the right model may be complicated. This paper reviews the literature on the different reconstructed epidermis models of AD as well as their relevance. We focused our attention on both the defect of the epidermal barrier and the inflammation linked to the immune system.

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1. Introduction

Atopic dermatitis (AD) (AD; OMIM # 603165) is a common chronic relapsing inflammatory disease that typically begins in early childhood. Its prevalence has been evaluated as 10.7% in the pediatric population and as 7.2% in the adult population of the United States [1,2]. AD is a multifactorial disease involving environmental, immunological and genetic factors. Clinically, AD is characterized by erythematous and itchy skin lesions over the entire body that cause a strong impact on the quality of life of patients [3,4].

AD treatment is currently in full revolution with the advent of new treatments. Consequently, the need for a realistic *in vitro* model is becoming essential for testing new molecules. Moreover, studies testing some medications and even more cosmetic products using animal models are limited by their cost and ethical.

Reconstructed human epidermis (RHE) is defined as an *in vitro* three-dimensional reconstruction of human epidermis from

primary keratinocytes extracted from human skin (usually abdominal skin or foreskin) of healthy subjects. Cells are isolated, amplified on either an inert polycarbonate filter or collagen matrix and placed at the air-liquid interface to allow their differentiation into a stratified human epidermis [5]. These models are histologically (cell proliferation and expression of differentiation markers) and functionally similar to *in vivo* human epidermis. However, it is not possible to reconstruct the real immunological response in RHE models. The induction of a RHE mimicking AD can be carried out due to the modification of culture medium for different RHE models, which is summarized in Table 1.

The etiology of AD is complex and not fully understood to date. Both the impairment of the epidermal barrier and systemic inflammation, which result in an abnormal immune response, lead to a vicious cycle. Indeed, defects of the epidermal barrier allow for an increase of cutaneous permeability and consequently trigger inflammation and immune system activation (the “outside-inside” hypothesis) [6]. Conversely, skin inflammation leads to defects of the epidermal barrier with perturbation of keratinocyte differentiation (the “inside-outside” hypothesis) [7].

The acute inflammation phase begins with a predominant response of T helper 2 (Th2) lymphocytes and then evolves toward a chronic T helper 1 (Th1) lymphocyte response [8]. In addition, AD

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Table 1
Summary table of the different RHE models.

Model	Conditions of culture	Inflammation	Proteins	Lipids
Castex-Rizzi et al. [33]	IL-4, IL-13, TLR ligand poly (I:C) and Pam3CKS4	↑ TSLP	– ↓ of filaggrin – Alteration of claudin-1	ns
De Vuyst et al. [34]	methyl-β-cyclodextrin	↑ IL-8	ns	Cholesterol depletion
De Vuyst et al. [36]	methyl-β-cyclodextrin, IL-4, IL-13, IL-25	↑ TSLP	↓ of loricrin	Cholesterol depletion
Danso et al. [39]	IL-4, IL-13, IL-31, +/- TNF-α	↑ TSLP	– Spongiosis – Alteration of keratin 10, loricrin and filaggrin	Modification of ceramide, cholesterol and saturated fatty acids
Rouaud-Tinguely P et al. [40]	Poly I: C, TNF-α, IL-4, IL-13	↑ TSLP and IL-8	– Spongiosis – Alteration of loricrin, involucrin and filaggrin	ns
Pendaries et al. [41]	shRNA FLG	ns	– ↓ of filaggrin-2, hornein, caspase-14, keratin-10, loricrin and bleomycin hydrolase – ↑ of corneodesmosine and claudin-1	ns
Pendaries et al. [42]	shRNA FLG	ns	– ↓ of loricrin, procaspase-14, bleomycin hydrolase – ↑ of claudin-1	ns
Son et al. [45]	Extracts of <i>S. aureus</i> , cytokines IL-6 or IL-8	ns	↓ of keratin 1 and 10	ns

The table lists the culture conditions of each model of reconstructed human epidermis that we studied in this review. The main factors studied in each model are shown in the last column. FLG: filaggrin; IL: interleukin; Poly I: C: polyinosinic-polycytidylic acid; shRNA: shot hairpin; TNF: tumor necrosis factor; TSLP: thymic stromal lymphopoietin protein; ns: not studied.

skin lesions are characterized by the overexpression of pro-inflammatory type 2 molecules such as interleukin (IL) -4, -13 and thymic stromal lymphopoietin (TSLP) secreted by keratinocytes [9]. The inflammation is also maintained, enhanced or triggered by neurogenic inflammation [10].

Concerning the epidermis, both the organization and composition of proteins and lipids play an important role in epidermal barrier function.

Genetically, mutations in *FLG*, the gene encoding for filaggrin, are one of the major factors predisposing AD [11]. A defect in filaggrin leads to disorders of the skin barrier function, which involves the entry of allergens, and disorders of epidermal differentiation [12].

The aim of this review is to describe the different models of RHE that mimic AD. We will focus more precisely on two hypotheses (the “inside-outside” and “outside-inside” hypotheses, as previously

defined) to describe these models, and evaluate their relevance (Fig. 1).

2. AD Pathophysiology

Recently, studies on AD are in full effervescence, particularly with the marketing of new treatments such as biotherapies but also because of the dynamic research on itching. In this context, *in vitro* models are important for understanding mechanisms involved in AD (i.e., the “outside-inside” and “inside-outside” hypotheses), especially for discovering new therapeutic approaches.

Models using RHE allow for the study of these two hypotheses, either by generating inflammation *via* interleukins (ILs) or by producing a defect in the epidermal barrier.

Skin is the first barrier of the body to the outside environment. It requires a stratified and differentiated epidermis, with a basal

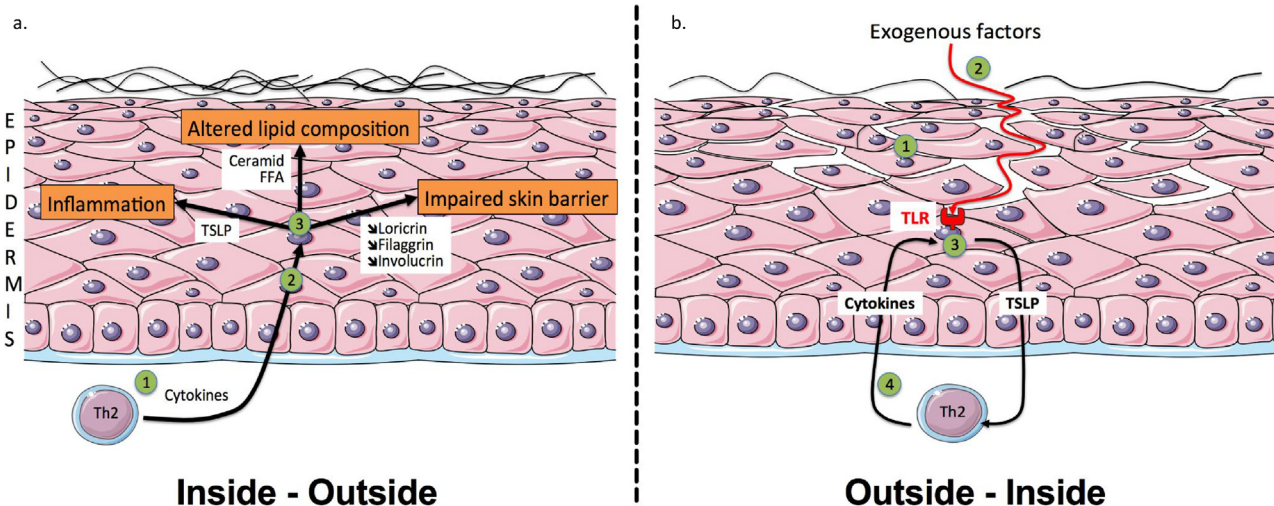


Fig. 1. The “inside-outside” and “outside-inside” hypotheses with a reconstructed human epidermis.

(a) (1) The pro-inflammatory environment is linked to a production of Th2 cytokines by immune cells. (2) The impact of Th2 cytokines on the epidermis and especially on keratinocytes. (3) The induction of TSLP secretion by keratinocytes, defect of the epidermis with an alteration of the organization and composition of lipids, and alteration of protein differentiation. (b) (1) Defect of the epidermis with defects of filaggrin, loricrin, involucrin and keratin 10 and alteration of lipid composition. (2) An increase of penetration of the exogenous factor. (3) Keratinocyte stimulation (especially by TLR) leading to TSLP secretion and activation of dendritic cells. (4) Inflammation induction with stimulation of Th2 cytokines. FFA: Free fat acids; Th2: helper T lymphocytes; TLR: toll-like receptor; TSLP: thymic stromal lymphopoietin.

layer (BL), a spinous layer (SL), a granular layer (SG) and a stratum corneum (SC), to protect and regulate the organism. Differentiated keratinocytes synthesize proteins such as keratin (K) 10, loricrin, involucrin and filaggrin [13,14]. These last three proteins participate in the formation of the SC and their decreased expression is often associated with a breakdown of the epidermal barrier in AD, as confirmed by immunohistochemistry and histology showing spongiosis and exocystosis of the epidermis [15,16].

Among these proteins, filaggrin is the most studied because genetic variations and loss of function variants within the *FLG* gene have been evidenced as a major risk factor in AD [17,18]. Filaggrin precursor (profilaggrin) is produced by granular keratinocytes which is known as main component of the keratohyalin granules. During cell transition from GL to SC, profilaggrin is rapidly dephosphorylated and cleaved by serine proteases (e.g., kallikrein 5) to generate filaggrin monomers [12]. These monomers are associated with keratin filaments suspected to induce the formation of the fibrous matrix of the SC, which is involved in the construction of the epidermal barrier. Hence, a strong correlation between the presence of AD lesions and mutations of the *FLG*, which is located on the chromosomal region 1q21.3, has been demonstrated [19]. Indeed, a mutation in this gene was observed in more than 20% of AD patients, suggesting a key role in the disease by its protective function of the skin barrier [20].

Proteins are not the only constituent of the impermeable barrier. Lipids, mainly ceramides (with ester linked ω -hydroxyl ceramide (EO ceramide)), free fatty acids (FFA) and cholesterol, constitute the lipid matrix that is organized in lamellar bodies and located between corneocytes [21]. In AD, this lipid matrix is disorganized, and an alteration of the epidermal barrier is induced. AD patients show a reduction of long chains, an increase of unsaturated FFAs and a decrease of very long chains of ceramides in the skin [22].

With regard to inflammation, AD is characterized by an overexpression of pro-inflammatory molecules such as Th2 cytokines (IL-4, IL-5 and IL-13), IL-22 or thymic stromal lymphopoietin (TSLP) [9]. The non-lesional skin of AD patients already shows signs of low-level inflammation with presence of Th2 and Th22 cell infiltration [23]. IL-31 and IL-33 are also related to pathogenesis of AD [24–26]. In the acute phase of AD, skin barrier defects allow the penetration of epicutaneous antigens and lead to the production of TSLP by keratinocytes. In the chronic phase, continuous activation of Th2 and Th22 increase in the affected epidermal barrier accelerating also local inflammation leading to a vicious cycle [27].

TSLP secretion by keratinocytes [28] is induced by sensitizing antigens, Th2 cytokines or the activation of toll-like receptor (TLR) pathways [29,30]. It plays a role in immune processes at the epithelial interface with the outside by acting on dendritic cells, mast cells and Th-lymphocytes. Activation of dendritic cells by TSLP promotes the transformation of Th-naïve lymphocytes to the Th2 phenotype and the secretion of Th2 cytokines [31].

TSLP directly activates cutaneous sensory nerve endings *via* its specific receptor and then promotes itching [28]. Due to the narrow associations between skin, immune system, and nervous system, nerve endings are very important in the pathophysiology of inflammatory dermatoses, especially in AD. Many neurotransmitters and nerve growth factors released from nerve endings in the blood or skin are involved in neurogenic inflammation, dramatically enhancing and maintaining the inflammation induced by immune cells. During times of stress, their release is highly enhanced [10,32]. Due to the multimodality of AD, none of the actual RHE are mimicking all the components of the disease and focus only on specific components of the pathophysiology of AD instead.

3. Reconstructed human epidermis and atopic dermatitis: inflammation (Fig. 1a)

Inflammation plays a major role in AD and is the target of new therapies. The majority of authors are logically interested in inflammation and TSLP secretion by keratinocytes that is the best pathway for studying inflammation in RHE since immune cells are absent (Table S1).

Danso et al. studied the effects of Th2 cytokines (IL-4, IL-13 and IL-31) with and without tumor necrosis factor (TNF)- α on the secretion of TSLP, and noticed an increase in its release by ELISA in all cases.

Rouaud et al. have supplemented their culture medium with Th2 cytokines (IL-4 and IL-13), TNF- α and polyinosinic-polycytidylic acid (poly I:C). They obtained a significant increase of TSLP as well as IL-8 levels measured by ELISA. These authors claim to have higher levels of TSLP than models without poly I:C, suggesting that poly I:C can mimic an immune response *via* its TLR-3 receptor expressed on keratinocytes.

In their model, Castex-Rizzi et al. supplemented the use of poly I:C by Pam3CKS4, an activator of TLR-4 and showed also an increase of TSLP in their model by ELISA [33].

In a first model, De Vuyst et al. did not add ILs in the culture but depleted their RHE in cholesterol only, and IL-8 mRNA level was transiently increased (between 1 and 8 h after depletion) [34]. In a second time, they added IL-4, -13 and -25 and then observed an absence of TSLP mRNA expression increase. Surprisingly, IL-8 was not studied [35,36]. The authors explain this phenomenon by the absence of TNF- α or IL-1 in their culture medium in association with the ability of ILs to stimulate TSLP production.

In all of these models of inflammation, Th2 cytokines as well as activators of TLRs are always used to enhance inflammation revealed by a TSLP increased. Furthermore, the addition of TNF- α seems to be an important feature, and its absence in the De Vuyst model could explain the absence of TSLP [37].

To study the genes involved in AD, many candidates have been identified and are divided into two groups: genes involved in the barrier function of filaggrin and genes involved in the immune response. The second group one of the identified gene clusters is located on chromosome 5q31–33 and gathers includes Th2 type cytokine genes, such as IL-3, IL-4, IL-5, IL-13, and factors of granulocyte and monocytes stimulation (GM-CSF). IL-18 improves the production of IL-4, IL-13 and IL-10, and IL-31 play various roles in immune regulation and the anti-inflammatory response. Farley et al. used an RHE to study mechanisms of inflammatory responses induced by Fas ligand using neonatal keratinocytes and their immortal derivatives from the knockdown of ERK 1/2 [38]. The authors described the role of Fas ligand in the induction of keratinocytes apoptosis in dermatitis and in AD. Nevertheless, this model focused on inflammation in general but not in AD indicating that their model is not appropriate for studying AD.

4. Reconstructed human epidermis and atopic dermatitis: defect of epidermal barrier (Fig. 1b)

4.1. Morphology and protein alteration

The study of the morphological characteristics of the epidermis may be helpful for evaluating the skin barrier (Table S2). To reproduce the features of AD in the epidermis, different strategies are used. The first and most common is the induction of an inflammatory environment.

Danso et al. studied the influence of different cytokines on epidermal organization and differentiation [39]. The addition of Th2 cytokines with TNF- α , leads to spongiosis and epidermal thickening similar to condition with Th2 cytokines but without

TNF- α . Then, Danso et al. analyzed the expression of the advanced terminal products of differentiation by immunohistochemical staining. In their model supplemented with single Th2 cytokines (IL-4, IL-13 or IL-31) or TNF- α , K10 is not expressed in BL or in the deeper layers of the SL. When the mix of Th2 cytokines (IL-4, IL-13 and IL-31) was added in the presence or absence of TNF- α , the expression of K10 and filaggrin were also delayed in the upper layers (as evidenced by immunohistochemical staining, qPCR and ELISA tests). The expression of loricrin was present in GL but discontinuous and fragmented in immunohistochemical staining. Nevertheless, only the addition of IL-13 alone or in association with other Th2 cytokines clearly reduced the expression of loricrin, as shown by ELISA.

Rouaud-Tinguely et al. exhibited a stratified epidermis where the AD RHE was less compact and thicker than the RHE control with spongiosis in the deepest layers after incubation with IL-4, IL-13, TNF- α and Poly I: C [40]. The expression of filaggrin and loricrin in this model was reduced. Involucrin expression was slightly marked only in the superficial layers of the AD RHE compared to the untreated RHE. These authors have characterized the differences of gene expression between the control RHE and those cultured with an inflammatory cocktail. Transcriptomic analysis revealed a clear difference in the expression of 26 genes between control and AD RHE. By performing RT-qPCR, they evidenced a decreased expression of differentiation-associated genes, such as *FLG*, *LOR* and *K10*, while an increase in inflammation-associated genes was observed. Castex-Rizzi et al. described a model treated specifically with the activators of TLR-3 and TLR-4. By histological analysis, a reduction of filaggrin and an alteration of Claudin-1 expression were observed [33].

First, De Vuyst et al. depleted a single cholesterol using methyl- β -cyclodextrin (M β CD), and treated their RHE with a mixture of IL-4, IL-13 and IL-25 [34]. This model mimics an AD-like epidermal phenotype with spongiosis and hypogranulosis, which are associated with barrier alteration (as performed by transepithelial electrical resistance measurement). In addition, the same team has studied the impact of the different ILs used in association with M β CD [36] and showed a decreased expression of genes associated with keratinocyte differentiation, such as *FLG* and *LOR* (performed by RT-qPCR). In particular, *FLG-1* and *FLG-2* expression levels were decreased when IL-25 was associated with M β CD. IL-4 led to the most epidermal alteration, with an attenuation of loricrin expression, and in contrast, an increase of hyaluronan synthase expression and inflammation-related gene expression, such as *Carbonic anhydrase II (CAII)* and *NELL2*.

4.2. Study of filaggrin

Filaggrin was shown to be an advanced terminal product of epidermal differentiation, and mutations on its gene seem to induce AD.

The team of Pendaries et al. was interested in the deficiency of the *FLG* in the RHE. From shFLG-RNA, they demonstrated that the RHE model obtained mimicked the hallmarks of human AD on a protein level. In a first study, they observed a decrease in epidermal thickness and SC as well as a loss of keratohyalin granules, which was confirmed by electron microscopy analysis [41]. Skin barrier function was impaired (Lucifer Yellow test) and measurements of urocanic acid and pyrrolidone carboxylic acid were significantly decreased in the shFLG-RNA RHE. These elements suggested an increase in UV sensitivity (increased caspase-3 activity after irradiation) (Table S3). Moreover, in this model filaggrin-2, loricrin, caspase-14 and bleomycin hydrolase expression were decreased as shown by western blotting and RT-qPCR tests. This study demonstrated the important role of filaggrin mutations in AD patients. Thus, it suggests that the shFLG-RNA model is a good

model for *in vitro* experimentations. The same team investigated the role of filaggrin-2 using shRFLG2-RNA RHEs and found differences between FLG and FLG2 shRNA [41]. These differences are summarized in the Supplementary data (Table S4) and are particularly related to SC, cutaneous pH and the expression of epidermal differentiation genes and proteins. These different models using shRNA bring together many characteristics present in AD and are therefore relevant for studying the inside-outside hypothesis of AD at morphological level.

Moreover, there are RHE models that use culture medium containing pro-inflammatory Th2 cytokines +/- TNF- α in order to study the impact of inflammation on the expression of filaggrin, as previously described.

4.3. Lipids alterations

Epidermal lamellar bodies are embedded in corneocytes. In AD patients, the organization and composition of these lipid complexes are altered and participate in a vicious cycle.

Danso et al. used a culture medium with pro-inflammatory cytokines (IL-4, IL-13 and IL-31) with or without TNF- α [39]. The authors demonstrated, by liquid chromatography and mass spectrometry, that IL-31 and TNF- α alone or associated with Th2 cytokines influence the composition of lipids in RHE. Thus, TNF- α leads to a significant reduction of FFAs and EO ceramides (as after incubation with IL-31) in RHE (Table S5). According to Danso et al., there is a clear relationship between the effects of the cytokines added in the culture medium and the effect of keratinocyte proliferation and differentiation in their model. However, these changes did not reflect the changes in the lipid composition of SC. Therefore, Danso et al. suggested that the changes induced by cytokines during epidermal differentiation and lipid synthesis in the SC were influenced by different pathways.

De Vuyst et al. analyzed the effects of cholesterol depletion on signaling pathways and gene expression (Table S7) [34,35]. They used M β CD on day 5 of the air-liquid interface step which induced a transient depletion of cholesterol 1 h after treatment and a return to basal line after 24 h. They found in their RHE an activation of cell signaling pathways and an alteration of gene expression. This model did not allow for a mimic of AD because the epidermal barrier and morphology were not modified. Thus, in their second model, they included ILs in the RHE culture medium.

5. Relevance and limits of the reconstructed human epidermis model

RHE models are valuable tools for studying specific aspects of the epidermal pathophysiology, especially for AD. Nevertheless, the absence of fibroblasts, immune cells and nerve endings remains one of the major issues of the RHE model. Indeed, inflammation of the skin is mediated by immune cells, especially Th2 cells, and maintained by neurogenic inflammation in AD [32]. The majority of models try to mimic inflammation by adding cytokines to the culture medium. Expression of TSLP by keratinocytes confirms their activation by an inflammatory environment. In both the models of Rouaud-Tinguely and Castex-Rizzi, it is claimed that the activators of TLR-3 and -4 are a good complement to Th2 cytokines [33,40]. Conversely, the absence of TNF- α associated with Th2 cytokines, similar to the De Vuyst's model, does not allow for the production of TSLP. Nevertheless, this model is very interesting because it tries to reproduce the vicious cycle of AD with both Th2 cytokines, to mimic skin inflammation, and a depletion of cholesterol, to mimic a disruption of the epidermal barrier.

Pendaries et al. tend to approach the inside-outside hypothesis by disrupting the epidermal barrier. However, the inflammatory aspect (TSLP secretion) was not studied but would be interesting to consider, even if immune cells are absent [41,42].

Keratinocytes are in permanent contact with their environment and interact with the cells that make up its environment. These cells, especially fibroblasts, play a major role in their differentiation and stratification. Skin explant models allow this communication and are obviously closer to realistic AD [43]. Adding sensory innervation would make the model even more realistic because none of the current models include nerve endings.

Moreover, murine models are studied more frequently. These animal models are very useful for understanding the molecular mechanisms and pathogenesis of AD, although the pathogenesis of inflammation is not truly identical to that of humans [44]. Another major default is the ethical limitations for the use of animals in human research.

In summary, RHE offers several advantages, such as the ease of obtaining these models and the simplified study of AD. While no model using RHE will be able to mimic every aspect of AD, they allow high throughput screening of pharmacological drugs. Moreover, the presence of single keratinocytes is an advantage for studying and understanding their role in the pathophysiology of AD.

6. Conclusion

RHE is a frequent research technique that results in numerous axes of studies on pathologies with epidermal involvement. Several teams have created their own AD models by mimicking its features as best as possible. Each team used methods to modify their culture medium, which are summarized in Table I, to induce AD. Although these *in vitro* models of RHE that mimic AD are numerous, none of them could reproduce an epidermis with all the characteristics of AD, in particular the hyperinnervation.

Each model focuses on one or more aspects of the pathophysiology of AD. Thus, in the case of a study of AD through RHE, the choice of its model is paramount according to the study objectives and characteristics of the model.

One of the advantages of RHE models of AD is the possibility to test the effects of local treatments developed by pharmaceutical companies *in vitro* with high throughput while limiting the use of animals.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jdermsci.2017.11.015>.

References

- [1] J.I. Silverberg, N.K. Garg, A.S. Paller, A.B. Fishbein, P.C. Zee, Sleep disturbances in adults with eczema are associated with impaired overall health: a US population-based study, *J. Invest. Dermatol.* 135 (2015) 56–66.
- [2] T.E. Shaw, G.P. Currie, C.W. Koudelka, E.L. Simpson, Eczema prevalence in the United States: data from the 2003 national survey of children's health, *J. Invest. Dermatol.* 131 (2011) 67–73.
- [3] F.J. Dalgard, U. Gierler, L. Tomas-Aragones, L. Lien, F. Poot, G.B.E. Jemec, L. Misery, C. Szabo, D. Linder, F. Sampogna, A.W.M. Evers, J.A. Halvorsen, F. Balieva, J. Szepletowski, D. Romanov, S.E. Marron, I.K. Altunay, A.Y. Finlay, S.S. Salek, J. Kupfer, he psychological burden of skin diseases: a cross-sectional multicenter study among dermatological out-patients in 13 European countries, *J. Invest. Dermatol.* 135 (2015) 984–991.
- [4] A.M. Drucker, A.R. Wang, W.-Q. Li, E. Severson, J.K. Block, A.A. Qureshi, The burden of atopic dermatitis: summary of a report for the national eczema association, *J. Invest. Dermatol.* 137 (2017) 26–30.
- [5] A. Frankart, J. Malaisse, E. De Vuyst, F. Minner, C.L. de Rouvroit, Y. Poumay, Epidermal morphogenesis during progressive *in vitro* 3D reconstruction at the air-liquid interface, *Exp. Dermatol.* 21 (2012) 871–875.
- [6] H. Hammad, B.N. Lambrecht, Barrier epithelial cells and the control of type 2 immunity, *Immunity* 43 (2015) 29–40.
- [7] P.M. Elias, M. Schmuth, Abnormal skin barrier in the etiopathogenesis of atopic dermatitis, *Curr. Opin. Allergy Clin. Immunol.* 9 (2009) 437–446.
- [8] W. Peng, N. Novak, Pathogenesis of atopic dermatitis, *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* 45 (2015) 566–574.
- [9] A. Heratizadeh, T. Werfel, Anti-inflammatory therapies in atopic dermatitis, *Allergy* 71 (2016) 1666–1675.
- [10] L. Misery, Atopic dermatitis and the nervous system, *Clin. Rev. Allergy Immunol.* 41 (2011) 259–266.
- [11] E. Rodríguez, H. Baurecht, E. Herberich, S. Wagenpfeil, S.J. Brown, H.J. Cordell, A.D. Irvine, S. Weidinger, Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease, *J. Allergy Clin. Immunol.* 123 (2009) 1361–1370 e7.
- [12] A. Sandilands, C. Sutherland, A.D. Irvine, W.H.I. McLean, Filaggrin in the frontline: role in skin barrier function and disease, *J. Cell Sci.* 122 (2009) 1285–1294.
- [13] E. Candi, R. Schmidt, G. Melino, The cornified envelope: a model of cell death in the skin, *Nat. Rev. Mol. Cell Biol.* 6 (2005) 328–340.
- [14] M. Le Lamer, L. Pellerin, M. Reynier, L. Cau, V. Pendaries, C. Leprince, M.-C. Mèchin, G. Serre, C. Paul, M. Simon, Defects of corneocyte structural proteins and epidermal barrier in atopic dermatitis, *Biol. Chem.* 396 (2015) 1163–1179.
- [15] N.A. Soter, Morphology of atopic eczema, *Allergy* 44 (Suppl. 9) (1989) 16–19.
- [16] B.E. Kim, D.Y.M. Leung, M. Boguniewicz, M.D. Howell, Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6, *Clin. Immunol. Orlando Fla* 126 (2008) 332–337.
- [17] S.J. Brown, K. Kroboth, A. Sandilands, L.E. Campbell, E. Pohler, S. Kezic, H.J. Cordell, W.H.I. McLean, A.D. Irvine, Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect, *J. Invest. Dermatol.* 132 (2012) 98–104.
- [18] S.J. Brown, W.H.I. McLean, One remarkable molecule: filaggrin, *J. Invest. Dermatol.* 132 (2012) 751–762.
- [19] S. Bonness, T. Bieber, Molecular basis of atopic dermatitis, *Curr. Opin. Allergy Clin. Immunol.* 7 (2007) 382–386.
- [20] S. Weidinger, E. Rodríguez, C. Stahl, S. Wagenpfeil, N. Klopp, T. Illig, N. Novak, Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis, *J. Invest. Dermatol.* 127 (2007) 724–726.
- [21] P.M. Elias, Structure and function of the stratum corneum extracellular matrix, *J. Invest. Dermatol.* 132 (2012) 2131–2133.
- [22] M. Janssens, J. van Smeden, G.S. Gooris, W. Bras, G. Portale, P.J. Caspers, R.J. Vreeken, T. Hankemeier, S. Kezic, R. Wolterbeek, A.P. Lavrijsen, J.A. Bouwstra, Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients, *J. Lipid Res.* 53 (2012) 2755–2766.
- [23] S. Weidinger, N. Novak, Atopic dermatitis, *Lancet Lond. Engl.* 387 (2016) 1109–1122.
- [24] A.S. Paller, K. Kabashima, T. Bieber, Therapeutic pipeline for atopic dermatitis: end of the drought? *J. Allergy Clin. Immunol.* 140 (September (3)) (2017) 633–643.
- [25] C. Cornelissen, Y. Marquardt, K. Czaja, J. Wenzel, J. Frank, J. Lüscher-Firzlaff, B. Lüscher, J.M. Baron, IL-31 regulates differentiation and filaggrin expression in human organotypic skin models, *J. Allergy Clin. Immunol.* 129 (February (2)) (2012) 426–433 433. e1–8.
- [26] J. Seltmann, L.M. Roesner, F.W. von Hesler, M. Wittmann, T. Werfel, IL-33 impacts on the skin barrier by downregulating the expression of filaggrin, *J. Allergy Clin. Immunol.* 135 (June (6)) (2015) 1659–1661 e4.
- [27] S. Noda, J.G. Krueger, E. Guttman-Yassky, The translational revolution and use of biologics in patients with inflammatory skin diseases, *J. Allergy Clin. Immunol.* 135 (2015) 324–336.
- [28] S.R. Wilson, L. Thé, L.M. Batia, K. Beattie, G.E. Katibah, S.P. McClain, M. Pellegrino, D.M. Estandian, D.M. Bautista, The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch, *Cell* 155 (2013) 285–295.
- [29] T. Takai, X. Chen, Y. Xie, A.T. Vu, T.A. Le, H. Kinoshita, J. Kawasaki, S. Kamijo, M. Hara, H. Ushio, T. Baba, K. Hiramatsu, S. Ikeda, H. Ogawa, K. Okumura, TSLP expression induced via toll-like receptor pathways in human keratinocytes, *Methods Enzymol.* 535 (2014) 371–387.
- [30] K. Tatsuno, T. Fujiyama, H. Yamaguchi, M. Waki, Y. Tokura, TSLP directly interacts with skin-homing Th2 cells highly expressing its receptor to enhance IL-4 production in atopic dermatitis, *J. Invest. Dermatol.* 135 (2015) 3017–3024.
- [31] V. Soumelis, P.A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, K. Smith, D. Gorman, S. Zurawski, J. Abrams, S. Menon, T. McClanahan, R. de Waal-Malefyt, R. Bazan, R.A. Kastelein, Y.-J. Liu, Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP, *Nat. Immunol.* 3 (2002) 673–680.
- [32] O. Gouin, N. Lebonvallet, K. L'Herondelle, C. Le Gall-Ianotto, V. Buhé, E. Plée-Gautier, J.-L. Carré, L. Lefeuvre, L. Misery, Self-maintenance of neurogenic

- inflammation contributes to a vicious cycle in skin, *Exp. Dermatol.* 24 (2015) 723–726.
- [33] N. Castex-Rizzi, M. f. Galliano, M. f. Aries, H. Hernandez-Pigeon, C. Vaissiere, H. Delga, A. Caruana, C. Carrasco, M. L  v  que, H. Duplan, S. Bessou-Touya, In vitro approaches to pharmacological screening in the field of atopic dermatitis, *Br. J. Dermatol.* 170 (2014) 12–18.
- [34]   . De Vuyst, S. Gilt  re, C. Lambert de Rouvroit, A. Chr  tien, M. Salmon, Y. Poumay, The activation of cultured keratinocytes by cholesterol depletion during reconstruction of a human epidermis is reminiscent of monolayer cultures, *Arch. Dermatol. Res.* 307 (2015) 309–318.
- [35] E. De Vuyst, S. Gilt  re, C. Lambert de Rouvroit, J. Malaisse, A. Mound, M. Bourtembourg, A.F. Nikkels, A. Chr  tien, M. Salmon, Y. Poumay, M  CD concurs with IL-4, IL-13 and IL-25 to induce alterations reminiscent of atopic dermatitis in reconstructed human epidermis, *Exp. Dermatol.* (2016), doi: <http://dx.doi.org/10.1111/exd.13113>.
- [36] T. do Nascimento Pedrosa, E. De Vuyst, A. Mound, C. Lambert de Rouvroit, S.S. Maria-Engler, Y. Poumay, Methyl-  -cyclodextrin treatment combined to incubation with interleukin-4 reproduces major features of atopic dermatitis in a 3D-culture model, *Arch. Dermatol. Res.* 309 (2017) 63–69.
- [37] S.I. Bogiatzi, I. Fernandez, J.-C. Bichet, M.-A. Marloie-Provost, E. Volpe, X. Sastre, V. Soumelis, Cutting edge: proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes, *J. Immunol. Baltim. Md* 178 (2007) (1950) 3373–3377.
- [38] S.M. Farley, D.E. Purdy, O.P. Ryabinina, P. Schneider, B.E. Magun, M.S. Iordanov, Fas ligand-induced proinflammatory transcriptional responses in reconstructed human epidermis. Recruitment of the epidermal growth factor receptor and activation of MAP kinases, *J. Biol. Chem.* 283 (2008) 919–928.
- [39] M.O. Danso, V. van Drongelen, A. Mulder, J. van Esch, H. Scott, J. van Smeden, A. El Ghalbzouri, J.A. Bouwstra, TNF-   and Th2 cytokines induce atopic dermatitis-like features on epidermal differentiation proteins and stratum corneum lipids in human skin equivalents, *J. Invest. Dermatol.* 134 (2014) 1941–1950.
- [40] P. Rouaud-Tinguely, D. Boudier, L. Marchand, V. Barruche, S. Bordes, H. Coppin, M.P. Roth, B. Closs, From the morphological to the transcriptomic characterization of a compromised three-dimensional in vitro model mimicking atopic dermatitis, *Br. J. Dermatol.* 173 (2015) 1006–1014.
- [41] V. Pendaries, J. Malaisse, L. Pellerin, M. Le Lamer, R. Nachat, S. Kezic, A.M. Schmitt, C. Paul, Y. Poumay, G. Serre, M. Simon, Knockdown of filaggrin in a three-dimensional reconstructed human epidermis impairs keratinocyte differentiation, *J. Invest. Dermatol.* 134 (December (12)) (2014) 2938–29294.
- [42] V. Pendaries, M. Le Lamer, L. Cau, B. Hansmann, J. Malaisse, S. Kezic, G. Serre, M. Simon, In a three-dimensional reconstructed human epidermis filaggrin-2 is essential for proper cornification, *Cell. Death Dis.* 6 (2015) e1656.
- [43] N. Lebonvallet, C. Jeanmaire, L. Danoux, P. Sibille, G. Pauly, L. Misery, The evolution and use of skin explants: potential and limitations for dermatological research, *Eur. J. Dermatol.* 20 (2010) 671–684.
- [44] A. Tanaka, Y. Amagai, K. Oida, H. Matsuda, Recent findings in mouse models for human atopic dermatitis, *Exp. Anim.* 61 (2012) 77–84.
- [45] E.D. Son, H.J. Kim, T. Park, K. Shin, I.H. Bae, K.M. Lim, E.G. Cho, T.R. Lee, *Staphylococcus aureus* inhibits terminal differentiation of normal human keratinocytes by stimulating interleukin-6 secretion, *J. Dermatol. Sci.* 74 (April (1)) (2014) 64–71.

Further reading

T. Werfel , J.P. Allam, T. Biedermann, K. Eyerich, S. Gilles, E. Guttman-Yassky, W. Hoetzenecker, E. Knol, H.U. Simon, A. Wollenberg, T. Bieber, R. Lauener, P. Schmid-Grendelmeier, C. Traidl-Hoffmann, C.A. Akdis, Cellular and molecular immunologic mechanisms in patients with atopic dermatitis, *J. Allergy Clin. Immunol.* 138 (August (2)) (2016) 336–349.

Supplementary data

Table S1: Studies of skin inflammation using RHE models.

Model	Culture medium	Inflammatory response
Rouaud-Tinguely P <i>et al.</i> 2015 [40]	poly I :C, TNF- α , IL-4 et -13	↑TSLP and IL-8
Danso <i>et al.</i> 2014 [39]	IL-4	↑TSLP
	IL-13	↑TSLP
	IL-31	↑TSLP
	IL-4, IL-13, IL-31	↑TSLP
	TNF- α	↑TSLP
	IL-4, IL-13, IL-31, TNF α	↑TSLP
De Vuyst <i>et al.</i> 2015 [34]	methyl- β -cyclodextrin	↑IL-8 (between H1 and H8)
De Vuyst <i>et al.</i> 2016 [35]	methyl- β -cyclodextrin and IL-4, -13 and -25	↑TSLP in the 6 H
Castex-Rizzi <i>et al.</i> 2014 [33]	IL-4 and IL-13, TLR ligand Poly (I :C)	↑TSLP
	and Pam3CKS4	

This table lists the RHE models that study inflammation. For each culture medium used, the corresponding inflammatory response that was evaluated is described. IL= interleukin; H= hours; poly I: C= polyinosinic-polycytidylic acid; TLR= toll-like receptor; TNF= tumor necrosis factor; TSLP= thymic stomal lymphopoietin protein.

Table S2: Morphological aspects of the RHE models that were studied.

Model	Spongiosis	K10	LOR	INV	FLG	Others
Castex-Rizzi <i>et al.</i> 2014 [33]	ns	ns	ns	ns	Decreased	Alteration of claudin 1 staining
De Vuyst <i>et al.</i> 2016 [35]	-	ns	Decreased	ns	ns	ns
Danso <i>et al.</i> 2014 [39]	Deep layer of epidermis	Delayed	Fragmented	ns	Decreased	Increase of the proliferation of basal cells/no augmentation of epidermal thickness
Rouaud-Tinguely P <i>et al.</i> 2015 [40]	Deep layer of epidermis	ns	Reduction and expression only in deep layer	Reduction and expression in all suprabasal layers	Decreased	Epidermis less compact and thinner
Son <i>et al.</i> 2013 [45]	ns	Decreased	ns	ns	ns	Decreased expression of K1

This table summarizes the expression levels of keratin (K), loricrin (LOR), involucrin (INV) and filaggrin (FLG) proteins in the different models that study the morphology of RHE. The level of spongiosis is also described. ns = not studied.

Table S3: Comparison of the RHE characteristics of the model by Pendaries *et al.*, 2014 with AD [41].

	RHE shRNA <i>FLG</i>	Atopic dermatitis
Reduction of SC thickness	+	+/-
Increase of SC permeability	+	+
Increase of pH	-	+
Decrease of pro-caspase 14 activation	+	+
Decrease of loricrin expression	+	+
Decrease of bleomycin hydrolase expression	+	+
Decrease of urocanic acid and pyrrolidone carboxylic acid	+	+

The model of Pendaries *et al.*, 2014 used a culture medium with shRNA FLG and analyzed several parameters, as indicated in the table. Each characteristic of the Pendaries *et al.*, 2014 model is compared with those found in the atopic dermatitis (Werfel *et al.*, 2016).

+ = present; - = absent.

Table S4: Comparison of the two main models studying filaggrin and AD.

	shRNA FLG (Pendaries <i>et al.</i> , 2014) [41]	shRNA FLG 2 (Pendaries <i>et al.</i> , 2015) [42]	Atopic dermatitis (Werfel <i>et al.</i> , 2016) [46]
Morphology (standard histology +/- electronic microscopy)	- ↓ epidermis and SC thickness, keratohyalin granules. - Dilatation of extracellular space	- ↓ epidermis thickness - Parakeratosis, no modification of stratum corneum and keratohyalin granules - No modification of extracellular space	- ↓ epidermis, +/- parakeratosis, - Dilatation of extracellular space
SC properties	- Alteration of epidermal barrier (Lucifer Yellow) - ↓ of urocanic acid and pyrrolidone carboxylic acid	- No alteration of epidermal barrier (Lucifer Yellow) - ↓ of urocanic acid but no effect on pyrrolidone carboxylic acid	- Alteration of epidermal barrier - ↓ of urocanic acid and pyrrolidone carboxylic acid
Cutaneous pH	No modification	↑	↑
Epidermal differentiation			
- protein by WB	- ↓ of Flg-2, hornein, caspase-14, K10, Lor et bleomycin hydrolase - ↑ of corneodesmosine and claudin- 1	- ↓ Lor, procaspase-14, bleomycin hydrolase - ↑ of claudin- 1	- ↓ Flg, hornein, caspase-14, Lor and bleomycin hydrolase - persistence of corneodesmosomes
- RNA by qRT-PCR	- ↓ of Flg-2, caspase-14, Lor and bleomycin hydrolase - ↑ corneodesmosine	- ↓ Inv, Lor E-cadherin, procaspase-14 and bleomycin hydrolase	- ↓ Flg, hornein, caspase-14, Lor and bleomycin hydrolase

The table compares the 2 main RHE models that studied filaggrin. Each feature studied is compared with what was found in the AD (Werfel *et al.*, 2016). Sh = shot hairpin; SC = Stratum corneum; Flg= Filaggrin; Lor = Loricrin; K = keratin; Inv = involucrin; ↑ = increase; ↓ = decrease.

Table S5: Study of the effects of culture media on the organization of lipids of the stratum corneum in the model of Danso *et al.* [39]

	TNF- α	TNF- α + cytokines	Cytokines	IL-31
Total ceramides	↑	↑	ND	ND
EO total surface ceramides	↓	ND	ND	↓
Cholesterol	↓	↓	ND	ND
Mono-saturated free fatty acids	no modification	no modification	no modification	no modification
Saturated fatty acids	↓	↓	no modification	↑

The results were obtained by liquid chromatography and mass spectrometry. The culture media contained TNF- α , TNF- α with cytokines, cytokines, or IL-31 alone. The study of lipids included the study of total ceramides, EO (esterified omega-hydroxylated) total surface ceramides, cholesterol, mono-saturated free fatty acids, and saturated fatty acids. The results were compared to the RHE control. ND = not determined; ↑ = increase; ↓ = decrease.

Table S6: Effect of the culture medium of the De Vuyst *et al.* model [35, 36] on the cell signaling pathways and expression of genes in the RHEs relative to the RHE control.

Activation of signaling pathways (phosphorylation) (WB)
↑ EGF receptor (1 hour post treatment)
↑ p38 MAPK (1 hour post treatment)
↑ ERK (4 and 24 hour post treatment)
Gene expression (qRT-PCR)
<i>HB-EGF</i> : ↑ 1 and 4 hour post treatment
<i>INV</i> : ↑ 4 and 8 hour post treatment
<i>IL-8</i> : ↑ 1, 4 and 8 hour post treatment
<i>TGM-1</i> : ↑ 4, 8 and 24 hour post treatment
<i>PLAUR</i> : ↑ 1 and 4 hour post treatment
<i>KRT10</i> : no modification
<i>FLG</i> : no modification
<i>LOR</i> : no modification

The activation of the signaling pathways was visualized by the phosphorylation of the proteins by western blot. The expression of the genes was quantified by qRT-PCR. RHEs were compared to the RHE control.

WB = western blot; ↑ = increase.