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Modelling atopic dermatitis during the morphogenetic process involved in reconstruction of a human epidermis



É. De Vuyst, A. Mound, C. Lambert de Rouvroit, Y. Poumay*

URPHYM, Namur research institute for life sciences, University of Namur, 61, rue de Bruxelles, 5000 Namur, Belgium

ARTICLE INFO

Article history:

Received 10 October 2016

Accepted 12 October 2016

Available online 17 November 2016

Keywords:

Atopic dermatitis

Reconstructed human epidermis

Cholesterol

Interleukins

Epidermal barrier

ABSTRACT

Most crucial role of epidermis is to maintain efficient barrier between the organism and its environment. This barrier is however perturbed in inflammatory skin conditions like atopic dermatitis (AD), one common chronic disease. This review depicts characteristics of a model intending to reproduce epidermal features of AD in vitro. Firstly, methyl- β -cyclodextrin (M β CD) during reconstruction of epidermis was used to deplete cholesterol from plasma membrane because this condition reproduces characteristics of AD at transcriptomic level in monolayer cultures. Major changes are confirmed after same treatment inside reconstructed human epidermis (RHE). However, since early treatment do not reveal impairment to reconstruct a functional epidermal barrier and given the importance of the Th2 dysregulated immune response in AD, cholesterol-depleted RHE at day 11 of reconstruction were then incubated with three Th2-related cytokines (IL-4, IL-13 and IL-25) previously reported as playing important roles in the development of AD, as well as altering overall function of epidermal barrier. When combining both treatments, essential epidermal features of AD are observed. Indeed, RHE then exhibit spongiosis, disappearing granular layer, alteration of barrier function, as well as dysregulated expression levels for genes involved in AD pathogenesis. Moreover, while trying to identify individual roles for each component used to create AD-like alterations, incubation with IL-4 following cholesterol depletion from plasma membrane was found inducing most of the reported alterations. This model suggests potential for better investigations of epidermal AD features and may be considered for eventual in vitro screening of cosmetics or therapeutic compounds.

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Plasma membranes of eukaryotic cells organize as phospholipid bilayers in order to define cell boundaries. Phospholipids constituting the plasma membrane are amphiphilic, allowing them to naturally create a bilayer as they orient hydrophobic tails in the centre of the bilayer where they can associate with each other and expose hydrophilic heads towards the exterior of the membrane, where they will be in contact either with the extracellular liquid in case of plasma membrane or with the cytosol. Phospholipids associate with sphingolipids and sterols to become the major lipid constituents of the plasma membrane.

It was reported that plasma membranes contain particularly high concentrations of cholesterol and sphingolipids within specialized specific regions of the membrane called membrane lipid domains. Such domains exhibit elevated affinities for multiple proteins involved in precise signalling pathways, such as the epidermal growth factor receptor (EGFR) as one example [1–3].

Cholesterol being one crucial component of the specialized membrane lipid domains in animals, a widely used experimental approach of the role of cholesterol in the function and structure of these lipid domains utilizes various cholesterol-depleting agents to extract cholesterol from the plasma membrane. One of these agents is the molecule called methyl- β -cyclodextrin (M β CD), commonly used as an externally hydrophilic molecule that is able to solubilize cholesterol in aqueous environments, like body fluids or culture media. M β CD is totally unable to cross cell membranes, but contains a central hydrophobic cavity which can, due to its steric characteristics, specifically extract cholesterol from plasma membranes. Thus, using M β CD to deplete cholesterol is supposed to effectively disrupt molecular organization of every cholesterol-containing membrane domain, as well as linked signalling pathways.

M β CD has become of interest in the context of atopic dermatitis (AD) when transcriptomic microarray analyses of cholesterol-depleted keratinocytes by M β CD have shown regulations of this cell type's phenotype that were quite similar to the abnormal

* Corresponding author. Tel.: +3281724257; fax: +3281724261.
 E-mail address: yves.poumay@unamur.be (Y. Poumay).

phenotype of keratinocytes analysed in the skin of AD patients [4]. Such similarities were occurring in keratinocytes during the first hours of recovery following M β CD treatment. Those observations of similarities were further confirmed by using RT-qPCR to measure transcript levels produced by cholesterol-regulated genes in lesional and non-lesional AD skin biopsies. Thereby, elevated levels of involucrin (IVL), transglutaminase-1 (TGM-1), heparin-binding EGF-like growth factor (HB-EGF), interleukin (IL)-8 and plasminogen activator urokinase receptor (PLAUR) expression were observed, simultaneously with decreased levels of filaggrin (FLG) and loricrin (LOR) expression in keratinocytes which had been treated to create cholesterol depletion, as well as in keratinocytes analysed directly in lesional skin biopsies of AD patients.

In keratinocytes, cholesterol deprivation by incubation with M β CD is quickly followed by repletion of cholesterol in their plasma membranes since this cell-type exhibits a strong potential to resynthesize cholesterol, as can be expected from their normal behaviour in serum-free culture conditions. This property enables keratinocytes to recover normal cholesterol content within a few hours after the M β CD treatment [5–8]. Strong similarities, at transcriptomic levels, between keratinocytes bordered by membrane depleted in cholesterol on one hand, and keratinocytes in AD skin on the other hand, precisely occurs during the first hours of recovery that follow M β CD treatment [4]. In addition, studies of keratinocyte monolayer cultures revealed that, for several hours of recovery after M β CD, activation by dimerization and tyrosine transphosphorylation of the EGFR occurred in a ligand-independent way, which in turn induced activation through phosphorylation of extracellular signal-regulated kinase (ERK) [9]. Interestingly, mitogen-activated protein kinase p38 (p38 MAPK) was simultaneously activated after cholesterol depletion, together with enhanced expression of the differentiation markers IVL, as well as of the potential EGFR-ligand HB-EGF [5,6,8]. However, activation of p38 MAPK was demonstrated as independent of the activated tyrosine kinase of EGFR, suggesting thereby that unidentified signalling pathways unrelated to EGFR are activated after cholesterol-depletion in human keratinocytes. Whereas the involved mechanisms are still to be investigated in order to understand how multiple signalling processes are simultaneously activated in such circumstances, available data suggested that transient depletion of plasma membrane cholesterol is able to modulate the keratinocyte phenotype in a way that mimics certain characteristics of AD, at least at some transcriptomic levels.

Atopic dermatitis (AD) is probably the most common disease among chronic inflammatory skin conditions and is responsible for eczema in childhood. AD condition is characterized by dryness of the skin, intense pruritus and erythematous lesions, as a result

created by scratching of itching dry skin. AD develops as a chronic, usually relapsing disease that most often starts early in childhood and may disappear during infancy in a significant proportion of children [10]. From an etiological point of view, AD is a complex multifactorial disease, still certainly not completely understood, which involves genetic predispositions and environmental triggering factors, but also multiple interplay between alterations of the epidermal barrier function and abnormal T helper 2 immune response [11–14]. So far, two different hypotheses have been proposed regarding the aetiology of the pathology. The first one is the “outside-inside” hypothesis which suggests that primary defects probably originate from a leaky epidermal barrier itself, allowing enhanced penetration of allergens and pathogens, thereby leading to inflammation and immune sensitisation. Conversely, the second hypothesis, known as “inside-outside” hypothesis, proposes that primary defects probably reside at the level of the immune system, causing inflammation of the skin and leading to subsequent barrier defects. As an evidence, precise aetiology of AD is still a puzzling question, even though it very likely involves together epidermal and immune actors, both of them being potentially responsible for induction of the other one, in a vicious circle that is prone for development of this disease, and in a rather complicated scheme where environmental as well as genetic factors are at some point involved [13,15,16] (Fig. 1).

Prevalence of AD has tripled over the last 30 years and it has been considered that between 15–30% of children and 2–10% of adults can be affected by the disease in most developed countries [17]. Recent reports highlight an increase in prevalence in developing countries also and even in low-income countries [18,19]. Moreover, AD can represent an initial step of the so-called “atopic march” which corresponds to the observation that between 30–50% of patients, particularly those suffering from severe AD, will develop asthma and/or allergic rhinitis during their life-course [20–22]. The “atopic march” can find some explanation in the potential role played by thymic stromal lymphopoietin (TSLP), a cytokine induced either by trauma, infection by microbes, toll-like receptor (TLR) activation, or by a combination of inflammatory cytokines [23–27]. Induction of TSLP in skin has been shown to be accompanied by elevated circulating TSLP levels in blood. Thus, TSLP could be considered as a systemic driver of bronchial hyper-responsiveness because elimination of TSLP signalling [28], or inducible ablated TSLP in keratinocytes [29,30] of mice prevents occurrence of the atopic march, suggesting that TSLP produced by AD keratinocytes may be involved in the link between AD and allergic asthma.

AD is thus becoming a growing public and global health problem. Existing treatments are mainly aimed at restoring epidermal barrier defects and reducing skin inflammation, as well

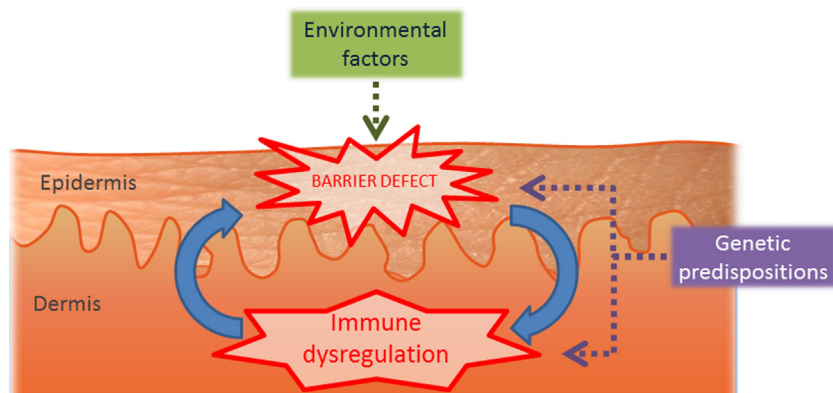


Fig. 1. Vicious circle observed in AD concerning barrier alteration and immune response dysregulation. Genetic predispositions are observed regarding both actors and this circle can further be influenced by environmental factors.

as avoidance of different environmental factors that may negatively influence severity of the disease [31]. However, since the aetiology is still understood, no specific treatment targeting causes currently exists.

In addition, in regard of developing innovative protective strategies, authorities of the European Union have a few years ago prohibited testing cosmetic finished products or their ingredients on laboratory animals. In accordance, they also have prohibited the marketing of finished cosmetic products if those products or their compounds have been tested on animals outside the EU (regulation [EC] no. 1223/2009).

Altogether, these restrictions have thus long ago stimulated the development and assessment of *in vitro* experimental models for products devoted for cosmetic use. Indeed, models developed on the basis of cultured human tissues have appeared as reliable alternative methods to animal models for studies involving human epidermal pathologies, as well as in order to screen toxicological properties of new or still untested molecules. In order to further and better investigate AD, our laboratory has recently developed and proposed a simple and easily reproducible *in vitro* model of AD, using the “open source” concept that relies on fully published procedures.

Initially, the study performed by Mathay et al. in our laboratory [4] had suggested highly significant similarities between transcriptomic profiles characterized inside human keratinocytes with cholesterol-depleted plasma membrane or profiles analysed in keratinocytes from lesional AD patients. However, since that study had been performed exclusively on keratinocytes cultured as monolayers, it was controversial to rely on this type of culture since major characteristics of AD concern alterations of the epidermal barrier. Yet, keratinocytes cultured as monolayers cannot produce any functional barrier. Thus, in order to mimic AD in an *in vitro* model, reconstructed human epidermis (RHE) were investigated after incubation of the cultured keratinocytes in the presence of MβCD in order to produce cholesterol depletion inside the plasma membrane of cells embedded in the RHE.

For this purpose in mind, we first reconstructed RHE according to the open source procedure and incubated them in the presence of MβCD during their reconstruction, at day 5, when the epidermal barrier is still under development. Initially, choosing to treat on day 5 of RHE reconstruction was based on previously published procedures [32] which had demonstrated that, at day 5, parameters like the trans-epidermal electrical resistance (TEER) was indicating by increase in its measurement that the future cornified layer was progressively developing at the morphological level, together with creating a functional barrier. Regarding potential induction of epidermal signalling pathways, as well as regulations of specific mRNA expression levels, many similarities with data obtained with monolayer cultures of keratinocytes were observed. However, despite concurrent observations, cholesterol-depletion in keratinocyte inside RHE was not able to significantly alter RHE morphology. Similarly and deceptively, no epidermal barrier alteration reminiscent of an AD-like phenotype could ever be reproduced in such conditions [33].

Therefore, in a second row of experiments, procedures have been chosen to analyse and challenge RHE on day 11 of reconstruction, when these tissues are starting to better mimic the *in vivo* epidermis. Furthermore, we reasoned that since AD is characterized by a dysregulated immune response and since RHE contain keratinocytes only and thus no immune cells, actors of the immune response had to be part of the model. Therefore, following cholesterol depletion from keratinocyte plasma membrane that induces signalisation and transcriptomic responses in RHE similar to what is observed in AD, RHE were incubated in the presence of interleukins [34]. Three interleukins, IL-4, IL-13 and IL-25, were chosen based on experiments already published showing elevated

expression levels of these cytokines in lesional AD skin [35–39]. Even more interesting, these interleukins have been demonstrated as able to induce a significant decrease in filaggrin expression by human primary keratinocytes [35,40]. Furthermore, Th2 cytokines IL-4 and IL-13, had already been demonstrated to reproduce features that mimic AD *in vitro*, and thus have already been used in several trials to produce potential *in vitro* models [41–44]. IL-25 was added for other reasons. Indeed, besides the fact that IL-25 is found overexpressed in AD lesions [37–39], this interleukin is reported as playing a dual role in AD, promoting type 2 cell immunity on one hand [39,45], but also inhibiting filaggrin (FLG) expression in keratinocytes on the other hand [38,40], thereby being potentially able to affect skin barrier in AD patients. Thus, acting both on immune and epidermal cells, IL-25 may provide, in collaboration with TSLP, some explanation for the relationship clearly observed between inflammation and barrier disruption in AD lesions [46]. Furthermore, in the skin of AD patients, expression of IL-25 and of its receptor was increased after exposure to allergens [47]. For all these reasons, we believed IL-25 could be of interest as a target for potential therapeutics and could interestingly be added in a potential model of AD in order to contribute to the development of pathological features, and to partly contribute to the vicious circle observed in AD.

Based on those arguments, RHE treated as described in order to first respond to incubation with MβCD before being exposed to IL-4, IL-13 and IL-25 exhibited morphological alterations such as tissue spongiosis and disappearance of the granular layer, together with alterations in the expression of typical AD-markers (down-regulation of FLG and LOR, upregulation of CAIL, NELL2, and HAS3), as well as in concomitance with epidermal barrier weakening. In other words, when treating RHE on day 11 of the reconstruction using MβCD for 2 hours and thereafter followed by 48 hours of incubation in the presence of IL-4, IL-13 and IL-25, we obtained an *in vitro* model which reflects several typical alterations of the epidermal tissue, as observed in AD lesions analysed in patients. Furthermore, in RHE treated with MβCD for two hours, the challenge of keratinocytes obviously induces the production of the cytokine TSLP. *In vivo*, released TSLP is able to induce immune dysregulation and further barrier alterations. While trying to reproduce the vicious circle observed in AD patients (barrier alterations ↔ immune dysregulation [Fig. 2]), and because a functional complete immune system is lacking in the RHE model, addition of exogenous interleukins has been necessary to reproduce consequences brought by Th2 cells in patients, notably activated by TSLP, to their epidermal tissue. Therefore, three interleukins related to the Th2 immune response and overexpressed in AD lesional epidermis, IL-4, IL-13, and IL-25 were added to the culture medium of RHE after the initial keratinocyte challenge obtained by cholesterol depletion from plasma membrane. Combination of the two treatments obviously appears able to significantly alter the epidermal barrier. *In vivo*, alterations of the epidermal barrier can be considered responsible for some activation of the immune system which can in turn trigger inflammation, for instance by promoting penetration of pathogens/allergens, thereby likely maintaining the vicious circle responsible for the symptoms known to characterize AD (Fig. 2).

Finally, the respective roles of each interleukin in such a model and whether their combination with preceding cholesterol depletion in keratinocyte plasma membrane was absolutely required remained an open question. Therefore, in order to better understand which components of the treatment are critical for modelling the disease, RHE were submitted to incubations either with single interleukins or with combinations of interleukins after plasma membrane cholesterol depletion, or not (Pedrosa Nascimento et al., submitted). Results demonstrate that most of the features observed when combining all treatments can be obtained

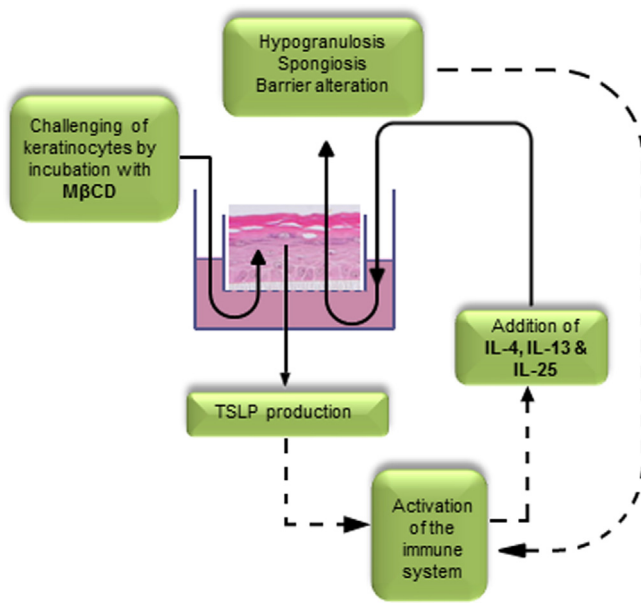


Fig. 2. Challenging keratinocytes cultured as RHE using MβCD that depletes plasma membrane cholesterol induces production of TSLP, a cytokine that could link barrier alterations and Th2 immune dysregulation. Since RHE contain keratinocytes only, exogenous addition of Th2-related interleukins in the culture medium is needed to mimic the activation of immune cells that would occur in vivo. Response of RHE to these challenges includes morphological alterations such as spongiosis and hypogranulosis as well as weakening of the epidermal barrier. In vivo, the altered epidermal barrier function would induce activation of the immune system what is thought to maintain the vicious circle observed in AD. Solid arrows represent events that can be mimicked in the in vitro model, whereas dotted arrows represent what is known from in vivo experiments. Modified from De Vuyst et al., 2016 [34].

when RHE are incubated in the presence of IL-4 after plasma membrane cholesterol depletion. IL-4 and IL-13 induce quite similar responses, however their combination does not seem to be required for the production of stronger effects on epidermal cells in RHE. However, more obvious results are observed when all treatments (MβCD and the three interleukins) are combined, especially in regard of Lucifer yellow permeation (and thus barrier alteration), as well as in regard of FLG expression. Indeed, the presence of IL-25 after MβCD incubation appears to be necessary to induce significant decrease in FLG expression.

1. Conclusions

In order to reproduce some features of the pathology *in vitro*, RHE were incubated in the presence of MβCD which is a molecule able to deplete cholesterol from plasma membranes. This molecule was used because it had been reported, in monolayer cultures of keratinocytes, to produce cellular responses that mimic major characteristics of AD at the transcriptomic level [4]. However, it is important to mention that, despite this molecule is known to induce disruption of membrane rafts, MβCD was not used because of any possible link between AD and membrane lipid domains organized by local elevated concentrations of cholesterol, but only because it can represent a certainly quite artificial, but easily reproducible method to induce in epidermal keratinocytes, in an *in vitro* model, several features usually observed in the epidermis of AD patients.

When using this molecule on RHE, since features similar to AD were reproducibly induced, mainly at the transcriptomic level, but without any barrier or morphological alterations, it was chosen to combine incubation in the presence of MβCD with Th2-related cytokines (IL-4, IL-13 and IL-25). Indeed, AD is an inflammatory skin

pathology involving both epidermal barrier alterations and Th2 immune dysregulation. While trying to mimic the pathology in an *in vitro* model, it was impossible to distinguish which phenomenon, between skin barrier alterations, or exacerbated Th2 inflammation, could be responsible for a potential initiating event of AD disease. In consequence, observations would thus argue for a likely involvement of both components, helped by potential environmental triggering conditions, to induce altogether the hypothetical vicious circle described in this dermatological disease.

The proposed protocol could certainly be improved or refined, but this simple epidermal model of AD disease is already available for useful studies of other features encountered at the epidermal level in AD. To conclude, such a model would be valuable to adapt to production at industrial scale, in order to evaluate cosmetics or therapeutic ingredients intending to relieve or prevent AD lesions, as well as to identify potentially active drugs for their potential to regulate particular keratinocyte functions.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgements

E.D.V. and A.M. performed the study and wrote the paper in collaboration with C.L.D.R. and Y.P. Financial support was provided by the government of the Région Wallonne (BAREPI-convention-1217660) and by FRFC 2.4.522.10F grant to YP.

References

- [1] Kabouridis PS, Janzen J, Magee AL, Ley SC. Cholesterol depletion disrupts lipid rafts and modulates the activity of multiple signaling pathways in T lymphocytes. *Eur J Immunol* 2000;30(3):954–63.
- [2] Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science* 2010;327(5961):46–50.
- [3] Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000;1(1):31–9.
- [4] Mathay C, Pierre M, Pittelkow MR, Depiereux E, Nikkels AF, Colige A, et al. Transcriptional profiling after lipid raft disruption in keratinocytes identifies critical mediators of atopic dermatitis pathways. *J Invest Dermatol* 2011;131(1):46–58.
- [5] Giltaire S, Lambert S, Poumay Y. HB-EGF synthesis and release induced by cholesterol depletion of human epidermal keratinocytes is controlled by extracellular ATP and involves both p38 and ERK1/2 signaling pathways. *J Cell Physiol* 2011;226(6):1651–9.
- [6] Jans R, Atanasova G, Jadot M, Poumay Y. Cholesterol depletion upregulates involucrin expression in epidermal keratinocytes through activation of p38. *J Invest Dermatol* 2004;123(3):564–73.
- [7] Lambert S, Ameels H, Gniadecki R, Herin M, Poumay Y. Internalization of EGF receptor following lipid rafts disruption in keratinocytes is delayed and dependent on p38 MAPK activation. *J Cell Physiol* 2008;217(3):834–45.
- [8] Mathay C, Giltaire S, Minner F, Bera E, Herin M, Poumay Y. Heparin-binding EGF-like growth factor is induced by disruption of lipid rafts and oxidative stress in keratinocytes and participates in the epidermal response to cutaneous wounds. *J Invest Dermatol* 2008;128(3):717–27.
- [9] Lambert S, Vind-Kezunovic D, Karvinen S, Gniadecki R. Ligand-independent activation of the EGFR by lipid raft disruption. *J Invest Dermatol* 2006;126(5):954–62.
- [10] Illi S, von Mutius E, Lau S, Nickel R, Gruber C, Niggemann B, et al. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *J Allergy Clin Immunol* 2004;113(5):925–31.
- [11] Boguniewicz M, Leung DY. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. *Immunol Rev* 2011;242(1):233–46.
- [12] Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009;129(8):1892–908.
- [13] Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, MacGowan A, et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. *J Allergy Clin Immunol* 2006;118(1):3–21.
- [14] Kabashima K. New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity. *J Dermatol Sci* 2013;70(1):3–11.
- [15] Bussmann C, Weidinger S, Novak N. Genetics of atopic dermatitis. *J Dtsch Dermatol Ges* 2011;9(9):670–6.

- [16] Lee YL, Li CW, Sung FC, Yu HS, Sheu HM, Guo YL. Environmental factors, parental atopy and atopic eczema in primary-school children: a cross-sectional study in Taiwan. *Br J Dermatol* 2007;157(6):1217–24.
- [17] Bieber T. Atopic dermatitis. *Ann Dermatol* 2010;22(2):125–37.
- [18] Odhiambo JA, Williams HC, Clayton TO, Robertson CF, Asher MI, Group IPTS. Global variations in prevalence of eczema symptoms in children from ISAAC phase III. *J Allergy Clin Immunol* 2009;124(6):1251–8 e23.
- [19] Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. *Allergy* 2014;69(1):3–16.
- [20] Spengel JM. Epidemiology of atopic dermatitis and atopic march in children. *Immunol Allergy Clin North Am* 2010;30(3):269–80.
- [21] Zheng T, Yu J, Oh MH, Zhu Z. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. *Allergy Asthma Immunol Res* 2011;3(2):67–73.
- [22] van der Hulst AE, Klip H, Brand PL. Risk of developing asthma in young children with atopic eczema: a systematic review. *J Allergy Clin Immunol* 2007;120(3):565–9.
- [23] He R, Geha RS. Thymic stromal lymphopoietin. *Ann N Y Acad Sci* 2010;1183:13–24.
- [24] Allakhverdi Z, Comeau MR, Jessup HK, Yoon BR, Brewer A, Chartier S, et al. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potentially activates mast cells. *J Exp Med* 2007;204(2):253–8.
- [25] Angelova-Fischer I, Fernandez IM, Donnadieu MH, Bulfone-Paus S, Zillikens D, Fischer TW, et al. Injury to the stratum corneum induces in vivo expression of human thymic stromal lymphopoietin in the epidermis. *J Invest Dermatol* 2010;130(10):2505–7.
- [26] Le TA, Takai T, Vu AT, Kinoshita H, Chen X, Ikeda S, et al. Flagellin induces the expression of thymic stromal lymphopoietin in human keratinocytes via toll-like receptor 5. *Int Arch Allergy Immunol* 2011;155(1):31–7.
- [27] Vu AT, Baba T, Chen X, Le TA, Kinoshita H, Xie Y, et al. *Staphylococcus aureus* membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the toll-like receptor 2-toll-like receptor 6 pathway. *J Allergy Clin Immunol* 2010;126(5):985–93, 93e1–3.
- [28] Demecri S, Morimoto M, Holtzman MJ, Kopan R. Skin-derived TSLP triggers progression from epidermal-barrier defects to asthma. *Plos Biol* 2009;7(5):e1000067.
- [29] Zhu Z, Oh MH, Yu J, Liu YJ, Zheng T. The role of TSLP in IL-13-induced atopic march. *Sci Rep* 2011;1:23.
- [30] Leyva-Castillo JM, Hener P, Jiang H, Li M. TSLP produced by keratinocytes promotes allergen sensitization through skin and thereby triggers atopic march in mice. *J Invest Dermatol* 2013;133(1):154–63.
- [31] Ring J, Alomar A, Bieber T, Deleuran M, Fink-Wagner A, Gelmetti C, et al. Guidelines for treatment of atopic eczema (atopic dermatitis) part I. *J Eur Acad Dermatol Venereol* 2012;26(8):1045–60.
- [32] Frankart A, Malaisse J, De Vuyst E, Minner F, de Rouvroit CL, Poumay Y. Epidermal morphogenesis during progressive in vitro 3D reconstruction at the air-liquid interface. *Exp Dermatol* 2012;21(11):871–5.
- [33] De Vuyst E, Giltair S, Lambert de Rouvroit C, Chretien A, Salmon M, Poumay Y. The activation of cultured keratinocytes by cholesterol depletion during reconstruction of a human epidermis is reminiscent of monolayer cultures. *Arch Dermatol Res* 2015;307(4):309–18.
- [34] De Vuyst E, Giltair S, Lambert de Rouvroit C, Malaisse J, Mound A, Bourtembourg M, et al. MBCD concurs with IL-4, IL-13 and IL-25 to induce alterations reminiscent of atopic dermatitis in reconstructed human epidermis. *Exp Dermatol* 2016. <http://dx.doi.org/10.1111/exd.13113>.
- [35] Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2009;124(3 Suppl. 2):R7–12.
- [36] Tazawa T, Sugiura H, Sugiura Y, Uehara M. Relative importance of IL-4 and IL-13 in lesional skin of atopic dermatitis. *Arch Dermatol Res* 2004;295(11):459–64.
- [37] Aktar MK, Kido-Nakahara M, Furue M, Nakahara T. Mutual upregulation of endothelin-1 and IL-25 in atopic dermatitis. *Allergy* 2015;70(7):846–54.
- [38] Hvid M, Vestergaard C, Kemp K, Christensen GB, Deleuran B, Deleuran M. IL-25 in atopic dermatitis: a possible link between inflammation and skin barrier dysfunction? *J Invest Dermatol* 2011;131(1):150–7.
- [39] Wang YH, Angkasekwinai P, Lu N, Voo KS, Arima K, Hanabuchi S, et al. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J Exp Med* 2007;204(8):1837–47.
- [40] Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Mechin MC, et al. Defects of filaggrin-like proteins in both lesional and non lesional atopic skin. *J Allergy Clin Immunol* 2013;131(4):1094–102.
- [41] Bernard FX, Morel F, Camus M, Pedretti N, Barrault C, Garnier J, et al. Keratinocytes under fire of proinflammatory cytokines: bona fide innate immune cells involved in the physiopathology of chronic atopic dermatitis and psoriasis. *J Allergy* 2012;2012:718725.
- [42] Danso MO, van Drongelen V, Mulder A, van Esch J, Scott H, van Smeden J, et al. TNF-alpha and Th2 cytokines induce atopic dermatitis-like features on epidermal differentiation proteins and stratum corneum lipids in human skin equivalents. *J Invest Dermatol* 2014;134(7):1941–50.
- [43] Kamsteeg M, Bergers M, de Boer R, Zeeuwen PL, Hato SV, Schalkwijk J, et al. Type 2 helper T-cell cytokines induce morphologic and molecular characteristics of atopic dermatitis in human skin equivalent. *Am J Pathol* 2011;178(5):2091–9.
- [44] Rouaud-Tinguely P, Boudier D, Marchand L, Barruche V, Bordes S, Coppin H, et al. From the morphological to the transcriptomic characterization of a compromised three-dimensional in vitro model mimicking atopic dermatitis. *Br J Dermatol* 2015;173(4):1006–14.
- [45] Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 2013;210(13):2939–50.
- [46] Deleuran M, Hvid M, Kemp K, Christensen GB, Deleuran B, Vestergaard C. IL-25 induces both inflammation and skin barrier dysfunction in atopic dermatitis. *Chem Immunol Allergy* 2012;96:45–9.
- [47] Corrigan CJ, Wang W, Meng Q, Fang C, Eid G, Caballero MR, et al. Allergen-induced expression of IL-25 and IL-25 receptor in atopic asthmatic airways and late-phase cutaneous responses. *J Allergy Clin Immunol* 2011;128(1):116–24.