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MβCD concurs with IL-4, IL-13 and IL-25 to induce alterations reminiscent of atopic dermatitis in reconstructed human epidermis

Evelyne De Vuyst[1], Séverine Giltaire[1], Catherine Lambert de Rouvroit[1], Jérémy Malaisse[1], Abdallah Mound[1], Maureen Bourtembourg[1], Arjen F. Nikkels[2], Aline Chrétien[3], Michel Salmon[3] and Yves Poumay[1]

1 URPhyM-NARILIS, University of Namur, Namur, Belgium.

2 Department of Dermatology, University Medical Center of Liège, Liège, Belgium.

3 StratiCell, Les Isnes, Belgium.

Correspondence: Professor Yves Poumay, Cell and Tissue Laboratory, URPhyM-NARILIS, University of Namur, 61, rue de Bruxelles, B-5000 Namur, Belgium.

E-mail: yves.poumay@unamur.be

Phone: +32 81 72 42 57

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Abbreviations: AD, atopic dermatitis; TSLP, thymic stromal lymphopoïetin; RHE, reconstructed human epidermis; MβCD, methyl-β-cyclodextrin; IL, interleukin; TEER, trans-epidermal electrical resistance; FLG, filaggrin; LOR, loricrin; CA2, carbonic anhydrase II; NELL2, neural epidermal growth factor-like 2 ; HAS, hyaluronic acid synthase; HA, hyaluronic acid.

BACKGROUND

Reconstructed human epidermis (RHE) mimic normal human *in vivo* epidermis in terms of histology, distribution of differentiation markers, and barrier functionality (1).

A typical transcriptional profile and the activation of signalling pathways reminiscent of atopic dermatitis (AD) lesional skin can be obtained in RHE upon incubation with methyl-β-cyclodextrin (MβCD) (2, 3), a molecule that extracts cholesterol from plasma membranes, thereby disrupting lipid microdomains. However, barrier function and morphology remain unaltered in those conditions, requiring further refinement of the model.

QUESTION ADDRESSED

Because of the crucial role played by Th2 immune response in AD, a mixture of interleukins linked to this Th2 response (IL-4, IL-13 and IL-25) was used in addition to MβCD, in an attempt to induce most of the epidermal AD features in RHE. Ultimately, a valid RHE model of acute AD would allow studying the epidermal component of pathogenesis.

EXPERIMENTAL DESIGN

RHE were incubated for two hours with MβCD in order to induce cholesterol depletion (Figure S1), then for 48 hours with the interleukin mix and compared to control tissues, and to tissues incubated with either MβCD or interleukins.

RESULTS

When allowed to recover in fresh culture medium after incubation with MβCD for 2 hours, RHE revealed no obvious histological alteration when compared with untreated RHE (Figure 1a). Conversely, RHE treated with IL-4, IL-13 and IL-25 for 48 hours displayed intercellular space widening similar to spongiosis (already reported for IL-4 and IL-13 (4-6)) and hypogranulosis, two histological hallmarks of lesional AD skin (Figure S2). These morphological alterations were enhanced when keratinocytes were challenged by MβCD before being incubated with the three interleukins (Figure 1a).

Barrier function, weakened in AD, was studied using two assays: measurement of trans-epithelial electrical resistance (TEER) and assessment of permeability to the fluorescent dye lucifer yellow (LY) through the RHE. A significant decrease in TEER was observed in RHE incubated with the interleukins, and worsened upon membrane cholesterol depletion (Figure 1b). Accordingly, permeability of RHE towards LY was significantly increased after combined treatments, whereas cholesterol depletion alone or incubation with interleukins only were insufficient to elicit an effect (Figure 1c).

Then, expression of atopic dermatitis markers was investigated. Gene expression of filaggrin (FLG) and loricrin (LOR), epidermal differentiation genes usually downregulated in lesional AD, was analyzed through RT-qPCR (Figure 2a). FLG and LOR exhibited reduced mRNA levels in RHE incubated with IL-4, -13 and -25, as already reported in the literature regardless of the different concentrations and timings used (5). This decrease was exacerbated and became significant when keratinocytes were challenged by cholesterol depletion before incubation with the interleukins. Simultaneously, relative expression levels of carbonic anhydrase II (CA2) and neural epidermal growth factor-like 2 (NELL2), two genes upregulated in AD (7), were significantly enhanced in RHE in response to incubation with interleukins (Figure 2a).

Immunohistological analysis confirmed decreased expression of LOR and increased expression of CA2 in AD lesions (Figure S3a). Similar changes were observed in RHE treated with interleukins and became even more evident in tissues previously treated with MβCD (Figure S3b).

Expression levels for hyaluronan synthase 3 (HAS3) were found elevated in RHE exposed to interleukins and further increased after combined treatments (Figure 2a), in agreement with the upregulation observed in AD (4, 8). Though, no significant changes were found with respect to HAS1 expression levels (data not shown), unlike data collected from AD lesions (8).
Fluorescent detection of hyaluronic acid (HA) revealed increased staining of intercellular spaces between keratinocytes in AD lesions (Figure S4a). HA was also more strongly detected in RHE incubated with interleukins, particularly after previous challenging by MβCD (Figure S4b). Accordingly, increased HA concentrations were measured in culture medium of treated RHE (Figure S4c).

TSLP is a cytokine, upregulated in AD, which contributes to Th2 immune response activation, promotes itch in skin and the 'atopic march' in general (9). However, despite significant induction in response to two hours of MβCD (Figure S1c), mRNA expression levels for TSLP were no longer above baseline 48 hours later (Figure 2a). However, in the meantime, over the 6 hours period following cholesterol depletion (Figure 2b), data illustrate that interleukins and MβCD concur to significantly enhance TSLP expression in challenged RHE.

CONCLUSIONS

This study illustrates that challenging RHE through cholesterol depletion, or by incubation with IL-4, IL-13 and IL-25, results in multiple epidermal alterations. But interestingly, a combination of those two treatments has additive effects, allowing mimicking an AD-like epidermal phenotype in vitro in the absence of immune cells. Indeed, morphological alterations such as tissue spongiosis and hypogranulosis, alterations in mRNA expression levels and histological localizations of typical AD and differentiation markers, modulations of epidermal HA synthesis, and epidermal barrier weakening represent hallmarks of AD epidermis.
In vivo, such alterations become responsible for activation of the immune system because they promote penetration of pathogens or allergens, thereby creating some vicious circle likely responsible for AD lesions development (Figure 2c).
Most probably, challenging of keratinocytes by MβCD models alterations in cell signalling through disorganization of specific lipid microdomains in this cell type (3). Interestingly, gene expression levels for IL-13Rα2, IL-13Rα1, IL-4Rα and IL-17RA (subunit of IL-25 receptor) were upregulated in keratinocytes incubated with MβCD (3). This indicates that cholesterol-containing lipid microdomains could regulate signalling through these receptors and could therefore explain the additive effects observed in this study.
In conclusion, this study confirms that membrane cholesterol depletion in keratinocytes concurs with Th2-related cytokines to elicit an AD-like phenotype. The present model could be used in order to study other features encountered in AD epidermis, but also evaluate compounds intending to relieve, prevent AD lesions or restore keratinocyte functions.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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FIGURE LEGENDS

Figure 1. Morphology and barrier efficiency in treated RHE.

(a) Histological sections of RHE treated for 2 hours with MβCD followed by 48 hours of recovery (MβCD 2h + recovery 48h) or treated for 48 hours with IL-4, IL-13 and IL-25 (IL 48h), or a combination of both treatments (MβCD 2h + IL 48h), compared to non-treated RHE (Ctrl 48h). Images are representative of three independent cultures. Scale bar=50µm.
Analysis of barrier function, by (**b**) trans-epidermal electrical resistance (TEER) or (**c**) fluorescence quantification of lucifer yellow permeation in the culture medium, of RHE treated for 2 hours with MβCD (MβCD2h), followed or not by 48h incubation with interleukins 4, 13 and 25 (IL 48h). Data represent measurements performed on three independent cultures (error bars = SEM, one-way RM ANOVA and *P<0.05, **P<0.01).

Figure 2. Transcriptional regulation of atopic dermatitis markers in treated RHE and hypothetical simplified model for AD-like pathogenesis.

(a) RT-qPCR analysis of the expression of filaggrin (FLG), loricrin (LOR), thymic stromal lymphopoietin (TSLP), carbonic anhydrase II (CA2), neural epidermal growth factor-like 2 (NELL2) and hyaluronan synthase 3 (HAS3) were performed on total RNA extracted from RHE treated with MβCD (MβCD2h)

and/or interleukins 4, 13 and 25 (IL48h). Levels of mRNA expression in control conditions were arbitrarily fixed at 1. Error bars represent 95% confidence intervals (n=3 independent cultures, one-way RM ANOVA, *P<0.05, **P<0.01, ***P <0.001).

(**b**) TSLP mRNA expression was quantified by RT-qPCR in RHE incubated for 2 hours with MβCD, followed or not by incubation with IL-4, IL-13 and IL-25 for 2h, 4h or 6h. Values are expressed relative to control condition (no MβCD + no IL). Error bars represent 95% confidence intervals (n=3 independent cultures, two-way RM ANOVA, *P<0.05, **P<0.01, ***P <0.001)

(**d**) Plasma membrane cholesterol depletion with MβCD in RHE challenges keratinocytes which in turn produce TSLP, a Th2 response-activating cytokine. Addition of Th2-related interleukins in the culture medium intends to mimic consequences of the activation of immune cells *in vivo*. Response of challenged RHE includes morphological alterations as well as weakening of the epidermal barrier. *In vivo,* activation of the immune system resulting from altered barrier function, would maintain the vicious circle observed in AD.