Modelling Atopic Dermatitis *in vitro*: Involvement of the Microbiota, Immunocells and Keratinocytes in a 3D tissue

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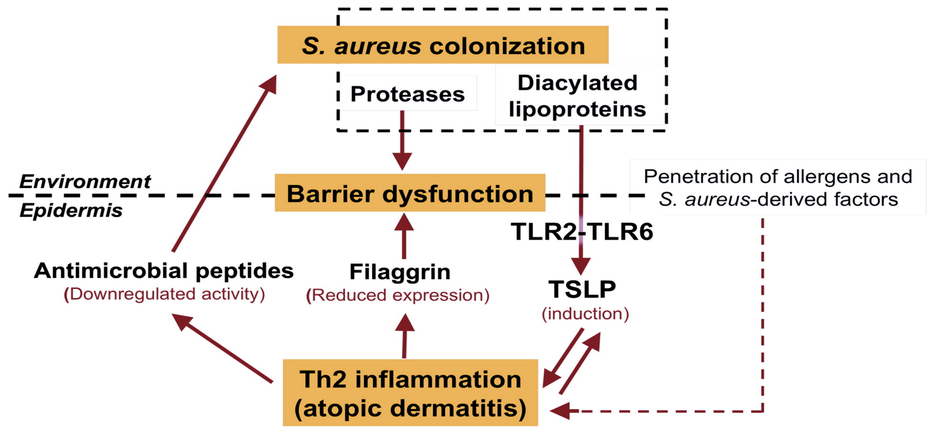
Introduction:
Atopic dermatitis (AD) is the most common form of eczema. It affects mainly young children and is characterized by lesions and pruritus. It involves a dysregulation of the immune system and the microbiota as well as an impairment of barrier function. However, its development is a complex process not entirely understood.

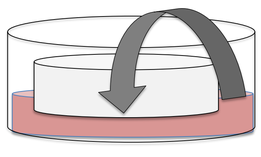
Objective:
To better understand this pathology as well as assess the efficacy of new products, we have developed an *in vitro* model of AD based on reconstructed human epidermis (RHE) infiltrated by immune cells further colonized by *Staphylococcus aureus*.



Vicious cycle between colonization by *S. aureus* and AD.
S. aureus–derived TLR2-TLR6 ligands and extracellular proteases promote AD. The inflammatory TH2 (TH2/TNF) cytokine milieu in patients with AD further promotes the *S aureus* colonization. *J Allergy Clin Immunol 2010;126:985-93*

PRODUCTION OF A 3D HUMAN IMMUNOCOMPETENT AND COLONIZED ATOPIC DERMATITIS (Ic-AD) MODEL
RHE-CMM (Episkin, France) were mechanically impaired and subsequently colonized with *S. aureus* (1.10^6 CFU/RHE) for 4h on the apical side. Immune cell infiltration was performed during 4h through a double porosity polycarbonate membrane (0.4 µm and 3 µm) on the basal site. Tissues were analyzed after 4h or 16h of recovery from infiltration to assess tissue and immunocells response to bacteria colonization.

STEP 1: MODEL INDUCTION

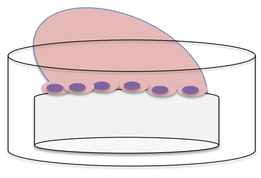


Impaired barrier+ *S.aureus* colonization

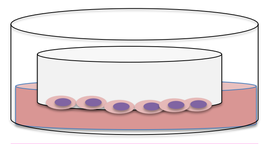
STEP 2: THP-1 INFILTRATION

4h

THP-1 on a turned upside down insert



STEP 3: POST INCUBATION



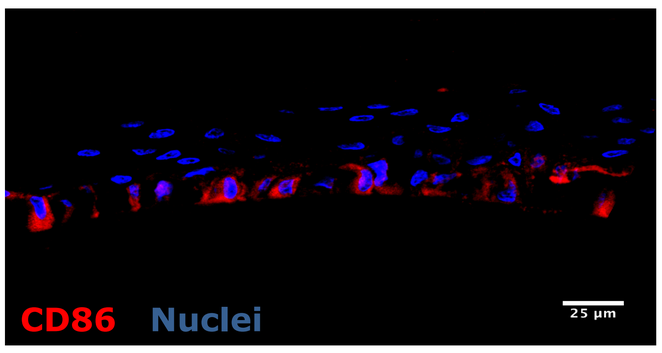
Co-culture

ENDPOINTS :

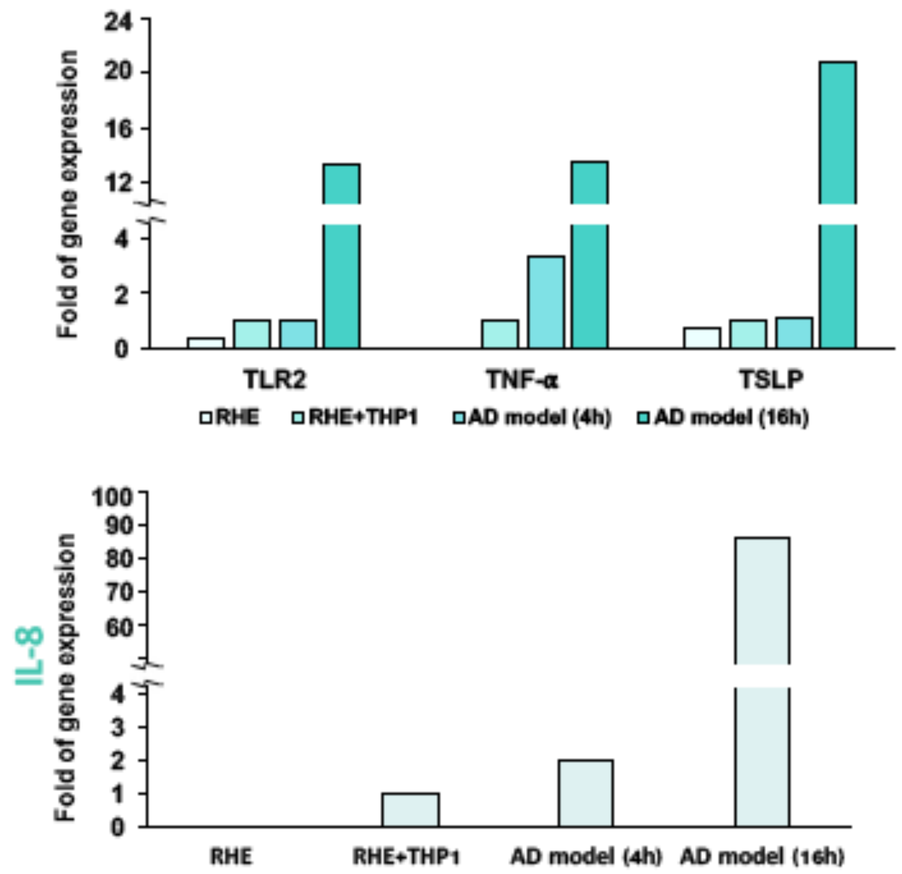
1) **Tissue response within Ic-AD model**:
- Gene expression by qRT-PCR of inflammatory markers (TNF-α, TSLP, TLR-2), barrier proteins (filaggrin, CLDN1, ZO-1) and skin antimicrobial peptides (DEFB4)

2) **Cells infiltration evaluation**:
- Immunostaining of barrier proteins (CD86)
- IL-8 expression

Ic-AD IMMUNO RESPONSE
The immunostaining against CD86 (red signal) suggests that THP-1 infiltrate into the RHE tissue and differentiate into macrophages.



The combination of THP-1 and *S. aureus* induces pro-inflammatory genes (TNF-a, IL-8) over-expression as quantified by qRT-PCR (Taqman probes) indicating an inflammation in the THP-1-RHE model in presence of *S. aureus*. As *in vivo*, TSLP is up-regulated in the Ic-AD model.



AD gene signature Functional class	Gene Name	Function in Atopic Dermatitis
Barrier Function	Filaggrin	Its down regulation as a sign of loss of epidermal integrity (increased paracellular passage and higher susceptibility to bacterial colonization).
Anti-microbial Response	Defensin beta 2	DEFB4 is secreted and overexpressed by keratinocytes as defense against *S. aureus* infection.
Immune mediated Response	Toll-like receptor 2	TLR2 receptors are responsible for increased barrier stability, integrity and functionality.
	Tumor Necrosis Factor alpha	TNF-α acts to inhibit key skin barrier proteins. Its overexpression is characteristic of Th2 response and inflammation in AD.
	Thymic stromal lymphopoietin	TSLP is an epithelial cell-derived cytokine involved in immuno modulation and allergic reactions. It is overexpressed in AD.
Inflammatory Response	Interleukin 8	IL-8 is an inflammatory marker (Th2 cytokine genes) produced and secreted by atopic phenotype.

Conclusions
- The THP-1-RHE cell migration model seems to better recapitulate the features of AD *in vitro* compared to other models by taking into account the keratinocyte innate and inflammatory response and the immuno-mediated response in presence of a *S. aureus* colonization.
- The Ic-AD model allows the assessment of products by topical or systemic-like exposure.
- It can be used to test products as prevention or as treatment based on different mechanisms of actions (anti-inflammatory, anti-bacterial or anti-biofilm products).