ATOPIC DERMATITIS MODEL ON RHE IN PRESENCE OF S. AUREUS AND INFILTRATING IMMUNO-COMPETENT CELLS

Marisa MELONI, Elisa CAVIOLA, Silvia BALZARETTI
Francesco CARRIERO and Jennifer BOURLAND
Contact: jennifer.bourland@vitroscreen.com

INTRODUCTION & AIM

Atopic dermatitis (AD) is a common skin disease linked to a dysregulation of the immune system and an impaired epidermal barrier. Barrier impairment and immune modulation are thought to create a vicious circle leading to AD. Resulting lesions show an increased susceptibility to infection by *S. aureus*, which triggers the production of thymic stromal lymphopoietin (TSLP) by keratinocytes. TSLP has a pivotal role and is involved in immune modulation and allergic reaction. Based on Reconstructed Human Epidermis with a double porosity polycarbonate filter (RHE-CMM) allowing THP-1 monocyte (test system of OECD 442E) infiltration, a unique *in vitro* model has been developed to better investigate the cutaneous immune response activated by *S. aureus* in AD.

HYPOTHESIS

The colonization of reconstructed human epidermis by *Staphyloccocus aureus* and the basal infiltration of immune cells induces a phenotype of atopic dermatitis by recapitulating biological cross-talk in a 3D environment.

MATERIAL & METHODS

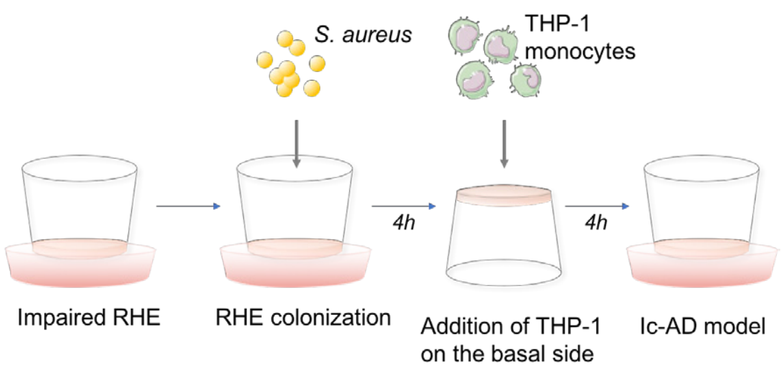

Figure 1: Production of a 3D human immunocompetent and colonized atopic dermatitis model (Ic-AD model). RHE-CMM (Episkin, France) were mechanically impaired and subsequently colonized with *S. aureus* (1.10^6 CFU/RHE) for 4 hours. Immune cell infiltration was performed during 4 hours through a double porosity polycarbonate membrane (0.4 µm and 3 µm). Tissues were analyzed after 4h in presence of THP-1 or after 16h of recovery.

ADVANTAGES

- **Based on human cell co-culture with spontaneous recruitment of immune cells**
- **Combine the "inside-out" effect of immune system with the "outside-in" concept of barrier impairment and environmental factor with *S. aureus* colonization**

RESULTS

Filaggrin & Barrier function

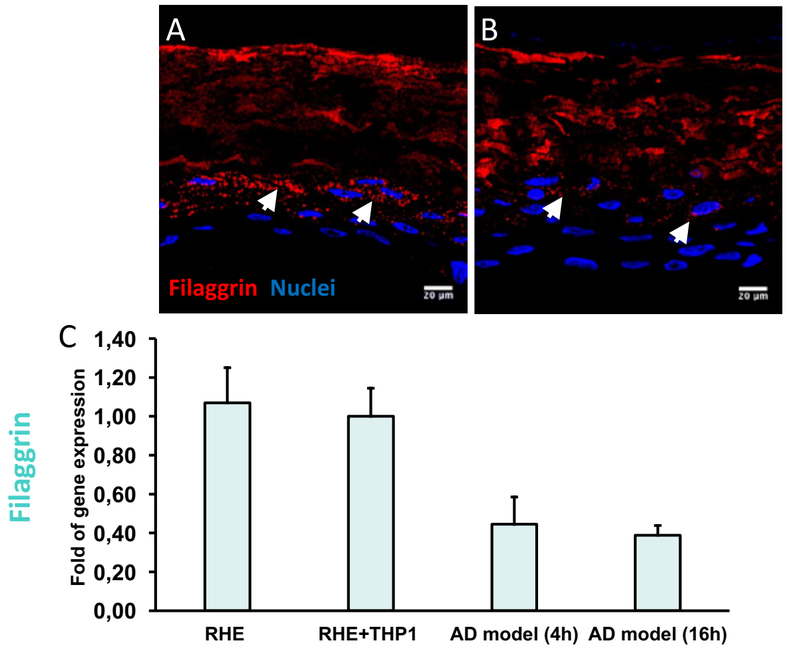

Figure 2: Alteration of epidermal barrier function in the AD model. (A-B) Immunostaining of filaggrin (red signal) on cryosections of (A) normal RHE and of (B) Ic-AD model at 16h. Nuclei are labeled with DAPI (blue signal). (C) Modulation of filaggrin gene expression as quantified by qRT-PCR. Data in fold of the non-colonized RHE with THP-1.

- **AD barrier impairment and filaggrin reduction are recapitulated in Ic-AD model**

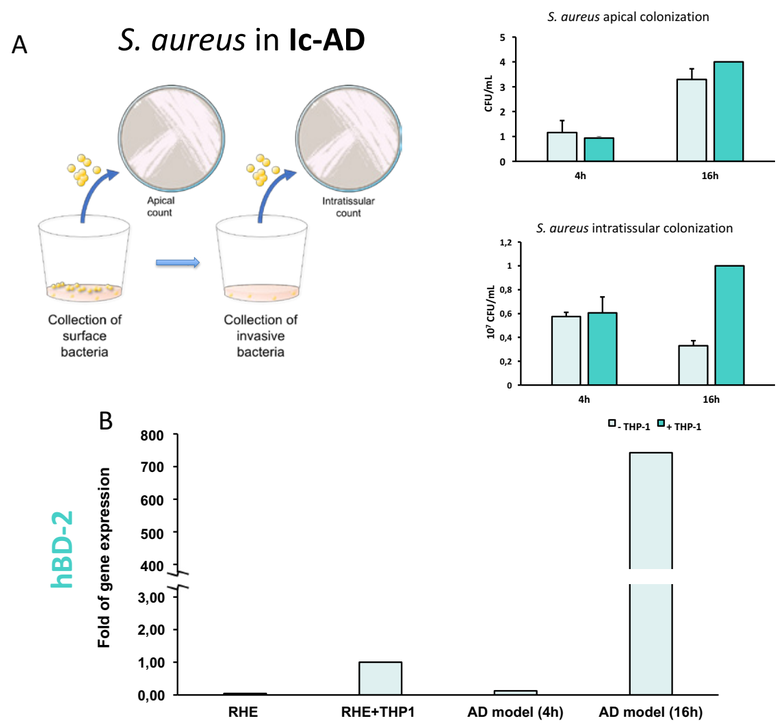

Figure 3: *S. aureus* colonization in presence of THP-1 and influence on skin innate response. (A) Apical and intratissular viable bacterial count at 4h and 16h of colonization in presence or absence of THP-1. (B) Modulation of anti-microbial peptide β-defensin-2 gene expression (DEFB4) as quantified by qRT-PCR. Data in fold of the non-colonized RHE with THP-1.

- **Synergic effect of THP-1 and *S. aureus* in AD phenotype**
- **Colonization by *S. aureus* increases defensin-β2 expression**

IcAD immune response

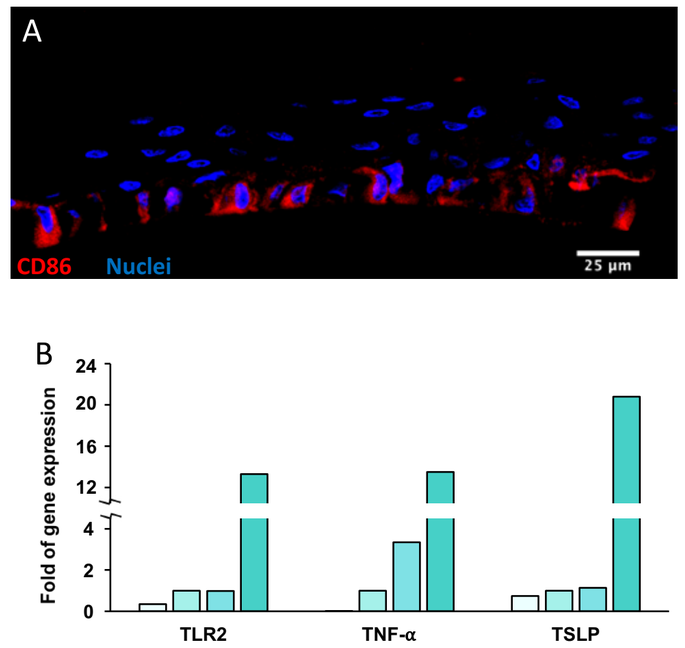

Figure 4: Inflammatory profile of the AD model. (A) Infiltration of immune cells in the RHE as indicated by the immunostaining against CD86 (red signal). Inflammation in presence of THP-1 and *S. aureus* colonization is visible in gene expression of (B) TLR2 (innate immunity), TNF-α (inflammation), TSLP (immune modulation) and (C) IL-8. Gene expression represented in fold of RHE with THP-1 as quantified by qRT-PCR (Taqman probes).

- **In our Ic-AD model THP-1 infiltrate and differentiate in macrophages**
- **The combination of THP-1 and *S. aureus* induces pro-inflammatory gene over-expression**
- **As in vivo, TSLP is up-regulated in Ic-AD model**

CONCLUSION

- **Unique AD model with both microbiota and immune cell contributing to the AD phenotype**
- **Based on our expertise in 3D models and currently under further characterization**
- **Can be used to test products as prevention or as treatment based on different mechanisms of actions (anti-inflammatory or anti-bacterial anti-biofilm products)**
- **Allows topical or systemic-like application of compounds.**