

ImmunoTools *special* Award 2014



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EFFECTS OF ALLERGENS ONTO THE PHENOTYPE AND FUNCTION OF LANGERHANS CELLS AND KERATINOCYTES IN HUMAN SKIN MODELS

Langerhans cells (LCs) reside suprabasally within the epidermis and represent a subset of immature dendritic cells that play important roles as immune sentinels in skin. They maintain immune homeostasis and activate protective skin resident memory T cells upon infectious challenge. During human skin development, their precursors have first been identified in the epidermis by 7-9 weeks estimated gestational age (EGA), based on the expression of several markers. The characteristic features of LCs (i.e. Birbeck granules, CD207, CD1a) are demonstrable only after 11 weeks EGA. Intriguingly, the scavenger receptor CD36 is found on a subset of dendritically or round-shaped epidermal cells in the embryonic skin and decrease in numbers and are eventually – like in healthy adult skin – not detectable at the end of the first trimester. As their precise phenotype has to date not been determined in humans it is the goal of this research proposal to:

- further characterize the phenotype of LC precursors in first trimester human epidermis in situ (Specific Aim 1)
- evaluate the LC-differentiation potential of defined cord blood-derived cell subsets, phenotypically similar to those described in embryonic skin, in a human three-dimensional full-thickness skin model and the further applicability of such an established model (Specific Aim 2)

As CD36 expression on hematopoietic and non-hematopoietic cells does not indicate the affiliation to a specific cell type due to its broad expression, we plan to perform triple immunofluorescence staining on cryostat sections to localize and better characterize the phenotype of embryonic CD36⁺ skin cells and in particular CD36⁺ epidermal cells.

In subsequent experiments CD34⁺ hematopoietic precursors will be separated from cord-blood samples of healthy human donors, cultured and sorted by flow cytometry to high purity according to the expression of markers in human embryonic skin that will be characterized in "Specific Aims 1".

Human skin equivalents are a highly valuable tool to study the origin and development of LCs. These skin equivalents will be generated essentially as described. In such a model, LC precursors should ideally develop from precursors without additional exogenous cytokines since all important stimuli are supposed to be provided by the artificial epidermis and dermis. Indeed we already know that our skin equivalents are endowed with the molecular prerequisites to promote the development of LCs (manuscript under revision). Now we need to further explore the differentiation capacity of distinct progenitors using immunofluorescence and/or immunohistochemistry with the proposed monoclonal antibodies.

In additional experiments we plan to investigate the consequences of skin barrier disruption onto LCs. For this purpose above defined precursors will be integrated into an already established filaggrin-deficient human skin model. At selected time points allergens will be applied onto the top of the equivalent and LCs will be analyzed either in the equivalent and/or collected from tissue wells and tested for maturation markers (Specific Aim 2).

The results and unique tools established within this study will offer an option to further study human LC biology in situ under controlled in vitro conditions and will facilitate the development of skin models studying the effect of allergens onto the phenotype and function of LCs and keratinocytes.

ImmunoTools *special* AWARD for **Albert Botta i Orfila** includes 25 reagents

FITC - conjugated anti-human human CD1a, CD8, CD11b, CD25, CD45, CD45RA, CD45RB, HLA-DR,

PE - conjugated anti-human CD4, CD11c, CD14, CD45, CD80,

PerCP - conjugated anti-human CD45,

APC -conjugated anti-human CD25,

recombinant human cytokines rh BMP-7, rh GM-CSF, rh IFNgamma, rh IGF-I, rh IL-4, rh IL-10, rh SCF, rh TSLP,

[DETAILS](#)