ImmunoTools *special* Award 2014

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FACTORS CONTROLLING LANGERHANS CELLS ACTIVATION AND PROLIFERATION IN THE EPIDERMAL MICROENVIRONMENT

Human epidermis contains tissue resident subset of Dendritic Cells (DCs) known as Langerhans Cells (LCs). What distinguishes them from the other members of DCs family members is a steady state, *in situ* proliferation capacity that takes place independently from the bone marrow [1]. Studies of prenatal skin revealed that precursors of LCs populate human epidermis very early during prenatal life (between 7-9 weeks of estimated gestational age) [2]. Several years of research identified anti-proliferative cytokine TGF-β_1 as a critical factor in LCs biology. This cytokine was considered to be essential for differentiation of LCs [3, 4]. However, recent findings have challenged this view. It was shown that at the time when LCs network is firstly established, TGF-β_1 is not expressed in the epidermis. The immunohistology also revealed that in the later time points its expression is not detectable in basal keratinocytes layers which are known to be proliferation sites of LCs in the adult skin. It has been recently observed that Bone Morphogenic Protein 7 (BMP-7) is highly expressed together in the prenatal skin and in LCs germinal layer in adult epidermis having an inverse expression pattern to TGF-β_1. In *in vitro* model of LCs differentiation replacement of TGF-β_1 with BMP-7 induces very high numbers of LCs without any effect on proliferation capacity of investigated cells. On the other hand, BMP-7 generated LCs produce higher amounts of pro-inflammatory cytokines in response to microbial signals than TGF-β_1 generated LCs (late addition of TGF-β_1 abrogates this effect) [5].

In my project I would like to test hypothetical model where in the steady state human epidermis there are two different subsets of LCs.

SUBSET 1 – in the outer epidermal layers → TGF-β_1 dependent LCs
TGF-β_1 responsible for the maintenance of the network prevents hyper-activation of LCs; terminally differentiated cells have no proliferative capacity

SUBSET 2 – in the basal keratinocyte layer → BMP-7 dependent LCs
BMP-7 induces differentiation and proliferation of LCs

In my work I will use *in vitro* system of LCs generation from human $CD34^+$ hematopoietic progenitors isolated from cord blood. I want to answer a question how different/similar are TGF-β_1 vs. BMP-7 generated LCs and fully characterize them. The advantage of this model is use of serum free media with specific cytokines mixes that drive precursors into LCs committed pathway. These defined conditions will allow me to select factors that play a critical role in differentiation and investigate their influence on function of LCs generated with different methods (TGF-β_1 vs. BMP-7). In parallel to *in vitro* work I will also isolate and characterize LCs from human epidermis. I want to investigate how much *in vitro* generated LCs correspond to the cells isolated *ex vivo*. Study design where *in vitro* experiments will be combined with isolations of cells *ex vivo* will allow me to fully investigate my hypothetical model. I will perform functional assays, measure cytokine production, and analyse expression of marker molecules with use of specific antibodies in multicolour flow cytometry.

The results of this study can contribute to identification of a new subset of human LCs and potentially lead to a development of a novel LCs *in vitro* differentiation model. Establishment of the system that allows generation of very high number of LCs will provide a new, valuable tool for further investigation of biology of these cells and can be important for cancer vaccinations research.

References

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