ImmunoTools *special* Award 2014

Kaat Dierckx, PhD student

Supervisor: Professor Dr. Julien Lambert

University of Antwerp, Department of Translational Pathophysiological Research, Laboratory of Dermatology, Wilrijk (Antwerp), Belgium

Skin-derived dendritic cells: role in the development, disease course and treatment of alopecia areata

Background

Alopecia areata (AA) is an autoimmune-mediated disease, characterized by bald patches on hairy skin. The prevalence of AA is 0.1% to 0.2% worldwide with a lifetime risk of about 1.7% in the general population. There is a great variability in time of onset, duration and extent of disease[1]. It is now generally accepted that AA is a T cell-mediated disease of the hair follicle, where a collapse of the immune privilege leads to destruction of the hair. Although the underlying mechanisms in the development of AA have been the subject of extensive research, the mechanisms responsible for the breakdown of the immune privilege are not fully understood[2]. There is evidence that dendritic cells (DCs) may play a role in the development of AA. DCs are specialized antigen-presenting cells (APCs) with the unique capacity to regulate the balance between immunity and tolerance and form a complex network of phenotypically and functionally distinct subsets[3].

DCs differentiate continuously from $CD34^+$ hematopoietic progenitor cells in bone marrow to both conventional DCs (cDCs) and plasmacytoid DCs (pDCs). In humans, all DCs express high levels of HLA-DR and lack lineage markers CD3, CD16, CD19, CD20 and CD56. In the skin and blood there are two types of cDCs, namely $CD1c^+$ $CD11c^+$ DCs and $CD141^+$ $CD11c^+$ DC. A third type of cDC, the Langerhans cells (LCs), can be found in the epidermis. LCs were the first described DCs and are characterized by the expression of CD207 and CD1a[4].

Currently, many different therapies exist for AA but none of these leads to permanent cure of the disease. Topical immunotherapy with contact allergens is considered to be the most effective therapeutical alternative in AA. Diphencyprone is the contact allergen mostly used for this purpose. Different hypotheses for the exact pathophysiological mechanism of this treatment are proposed, the exact underlying mechanism and its effect on dendritic cells is not fully understood[5].

Aims

1. The characterization of the different DC populations in skin biopsies and blood of AA patients in order to identify an inflammatory tissue-specific DC subset that can ultimately be targeted for an alternative treatment for AA.

2. Studying the effect of diphencyprone on different DC subsets to obtain more insight in a possible DC-specific mechanism of the therapy.

Methods implementing ImmunoTools reagents

In order to characterize different skin-derived DC populations, we will study the phenotype of DCs in skin biopsies using general and subset-specific monoclonal antibodies (**CD3, CD14, CD16, CD19, CD20, CD56, HLA-ABC, CD1a, CD11c**, CD207, CD141, CD303) as well as co-stimulatory (**CD80**,**CD86**), activation (CD83) and migratory markers (**CD62L**,CLA, CCR5, CCR7). Simultaneously, blood-derived DCs will be characterized.

Next, in order to investigate the *in vivo* effect of diphencyprone *in vivo*, we will characterize different DC subsets in patients following treatment with diphencyprone. Besides, the effect of diphencyprone on skin DCs will be studied *in vitro*. For this, we will isolate (i) $CD14^+$ monocytes and (ii) $CD34^+$ progenitor cells from peripheral blood mononuclear cells (PBMCs). The differentiation into LCs occurs in the presence of: (i) **GM-CSF, IL-4** and TGF-β1 or (ii) **GM-CSF**, **SCF, TNF-α, IL-4,** respectively. Recently, the Laboratory of Experimental Hematology (University of Antwerp) identified a LC-like DC subset with a high expression of CD207 and a pro-inflammatory profile. To obtain this phenotype, $CD14^+$ monocytes were cultured in the presence of **GM-CSF** and **IL-15**. We will investigate the effect of diphencyprone treatment on the phenotype and cytokine secretion of the differentiated DC populations by flow cytometry (**CD14, CD45, CD34, CD1a, HLA-ABC, CD86**, **CD80**, **HLA-DR**) and **ELISA (IL-6, IL-12p40, TNF-α)**, respectively. This *in vitro* platform offers perspectives for research about the effects of alternative and/or new therapies for AA.

References

1. Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part I. Clinical picture, histopathology, and pathogenesis. J Am Acad Dermatol 2010;62:p 177-88.
2. Gilhar A. Collapse of immune privilege in alopecia areata: coincidental or substantial? J Invest Dermatol 2010;130:p 2535-2537.
3. Cools N, Ponsaerts P, Van Tendeloo VF, Berneman ZN. Balancing between immunity and tolerance: an interplay between dendritic cells, regulatory T cells, and effector T cells. J Leukoc Biol 2007;82:p 1365-1374.
4. Collin M., McGovern N., Haniffa M. Human dendritic cell subsets. Immunology 2013; 140:p 22-30
5. Singh G, Lavanya M. Topical immunotherapy in alopecia areata. Int J Trichology 2010;2(1):p 36 -9.

ImmunoTools *special* AWARD for **Kaat Dierckx** includes 25 reagents

FITC - conjugated anti-human CD1a, CD3, CD14, CD16, CD19, CD20, CD56, CD86, HLA-ABC, Control-IgG1, Control-IgG2a,

PE - conjugated anti-human CD34, Control-IgG1,

PerCP - conjugated anti-human CD45,

APC -conjugated anti-human CD11c, Control-IgG1, Control-IgG2a, Control-IgG2b,

recombinant human cytokines rh GM-CSF, rh IL-4, rh IL-15, rh SCF, rh TNFα,

human IL-6 ELISA-set, human IL-12p40 ELISA-set, human TNF-alpha ELISA-set,

DETAILS