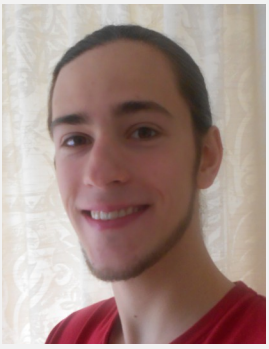
ImmunoTools *special* Award 2015



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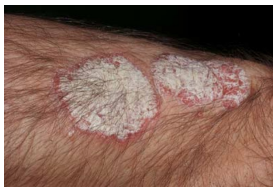
Influence of Inositol-C2-PAF on Th cell proliferation, differentiation and activity

The prevalence of psoriasis in Europe is 3%. But what does that actually mean? The answer is: Over 22 million europeans and even more people worldwide suffer from an incurable disease. A disease that does not only lead to stigmatizing skin lesions but is also associated with an increased risk for cardiovascular events.



psoriatic skin lesion [1]

Affected parts of the skin are characterized by elevated, red areas covered with scales. The pathology is based on a genetic predisposition in combination with different environmental trigger factors that lead to an uncontrolled inflammation in the skin [2].

Within a psoriatic inflammation there is a cytokine cross talk with positive feedback mechanisms between immune cells. Since they coordinate immune responses, T helper cells (Th) are of special interest in this context.

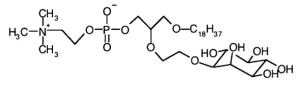
Th cells can be subdivided into pro-inflammatory effector T cells (Teff) and anti-inflammatory regulatory T cells (Treg). Treg cells can inhibit Teff cells through the emission of cytokines such as Interleukin 10 (IL-10) [3]. In psoriatic lesions, however, IL-6 produced by activated immune cells is known to induce Teff cell resistance against the suppression by Treg cells [4]. Consequently, the fine tuned balance between Treg and Teff cells is disturbed.

Teff cells can be further subdivided into Th1, Th2 and Th17 cells according to their specific cytokine profiles.

Triggered by IL-12 naïve Th cells differentiate into Interferonγ (IFNγ) producing Th1 cells. IFNγ provokes the secretion of IL-12 that reinforces Th1 differentiation. It also increases the IL-6 level, which promotes Treg deficiency. Moreover, IFNγ triggers its own expression as well as the expression of Tumor Necrosis Factor α (TNFα).

Both IFNγ and TNFα are able to provoke the release of IL-23. IL-23 is necessary for Th17 differentiation of naïve Th cells. In addition, IL-1β and IL-6 are required. If naïve Th cells receive a combination of these signals they will differentiate into Th17 cells. IL-17 secreted by Th17 cells is a booster for their own development and for the inflammation through inducing IL-6, IL-1β and TNFα [5,6].

In contrast to increased Th1 and Th17 cells, Th2 cells and their major cytokine IL-4 are underrepresented in psoriatic skin [7]. A reason for that is the suppression of Th2 development by IFNγ from Th1 cells [8].



Molecular structure of Inositol-C2-PAF [9]

A promising agent in facing psoriasis is called Inositol-C2-PAF (Ino). Due to its amphiphilic properties it easily accumulates in cellular membranes where it influences intracellular signaling pathways [10]. In vitro studies with Ino treated HaCaT cells confirmed a down regulation of pro-inflammatory genes as well as an antiproliferative effect [11]. In vivo, Ino significantly improves skin lesions in psoriatic mouse models and decreases Th cell numbers within them [12].

In this context we want to address the question weather Ino interferes with Th cell proliferation, differentiation and activity. In the planned experiments T cells from PBMCs will be activated with anti-CD3 and anti-CD28 in the presence of different Ino concentrations.

Reflecting Th cell differentiation and activity, cytokine levels in the supernatant will be measured with ELISA after 24 and 48 hours. For proliferation analyzes, T cells will be additionally stimulated with IL-2 over 7 and 9 days and counted in the Neubauer counting chamber.

In order to investigate the influence of Ino on the Th17 subset in detail, Th17 cells will be generated by isolating naïve Th cells from PBMCs and then stimulating them with anti-CD3, anti-CD28, IL-1β, IL-6 and IL-23. The subsequent treatment and analyses will be identical to the one described above.

Through the treatment with Ino we hope to positively influence the imbalances within the Th subsets and their associated cytokines. Since the experiments require a considerable amount of reagents, winning the ImmunoTools Award would be an enormous step forward in the realization of my project.

References

[1] Fritsch, P. *Dermatologie und Venerologie für das Studium.* (Springer Medizin, 2009).

[2] Coors, E. & Weiß, J. *Duale Reihe Dermatologie.* 7th edn (Thieme, 2010).

[3] Schütt, C. & Bröker, B. *Grundwissen Immunologie.* 3rd edn (Spektrum Akademischer Verlag, 2011).

[4] Buckner, J. H. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nat. Rev. Immunol.* **10,** 849–859 (2010).
[5] Michalak-Stoma, A. *et al.* Cytokine network in psoriasis revisited. *Eur. Cytokine Netw.* **22,** 160–168 (2011).
[6] Lowes, M. A., Suárez-Fariñas, M. & Krueger, J. G. Immunology of psoriasis. *Annu. Rev. Immunol.* **32,** 227–255 (2014).
[7] Ghoreschi, K., Weigert, C. & Röcken, M. Immunopathogenesis and role of T cells in psoriasis. *Clin. Dermatol.* **25,** 574–580 (2007).
[8] Schütt, C. & Bröker, B. *Grundwissen Immunologie.* 3rd edn (Spektrum Akademischer Verlag, 2011).
[9] Danker, K., Reutter, W. & Semini, G. Glycosidated phospholipids: uncoupling of signalling pathways at the plasma membrane. *Br J Pharmacol* **160,** 36–47 (2010).
[10] Hildmann, A. & Danker, K. Modified phospholipids: From detergents towards small molecular response modifiers. *Eur. J. Lipid Sci. Technol.* **116,** 1108–1113 (2014).
[11] Semini, G., Klein, A. & Danker, K. Impact of alkylphospholipids on the gene expression profile of HaCaT cells. *Pharmacogenet. Genomics* **21,** 375–387 (2011).
[12] Forkel, S. *et al.* Inositoylated platelet-activating factor (Ino-C2-PAF) modulates dynamic lymphocyte-endothelial cell interactions and alleviates psoriasis-like skin inflammation in two complementary mouse models. *J. Invest. Dermatol.* **134,** 2510– 2520 (2014).