EDI-Co Skin 3D-Co-culture model combined with RBM MAP analyses:
Optimized examination of human immune cell function in skin product testing

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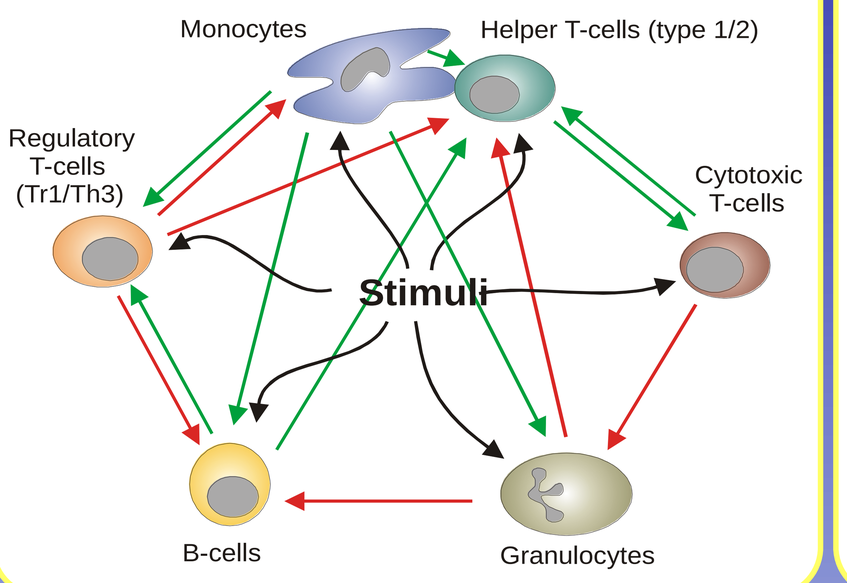
Background

Complex interaction of immunocompetent cells



Immune reaction and inflammation is highly controlled by a dialogue between cells of the immune system and cells of the various tissues. This is accomplished by the release of messenger substances (cytokines, chemokines etc.) that are part of a complex regulation network and synthesized in response to various stimuli. Application of remedies may exert a strong influence on this network in health and diseases. Organo-typical conditions to investigate drug effects on immunocompetent cells can be obtained e.g. by combining differentiated human 3D-epidermis together with human whole blood cell cultures in a two-chamber culture model. To study the effect of two standard drugs used in the treatment of atopic dermatitis we analyzed the interactions of the glucocorticosteroid *Prednicarbate* and *Pimecrolimus,* a calcineurin inhibitor on immune cells from two healthy donors in the EDI-Co skin, a co-culture model of epidermis and whole-blood. Mediators were tested by RBM (Luminex) MAP assays.

Multi-Analyte Profiles (MAPs)

Cytokines

- **Th1-related:** Interferon-γ, Interleukin-12p40, Interleukin-12p70
- **Th2-related:** Interleukin-4, Interleukin-5, Interleukin-13
- **Treg-related:** Interleukin-10
- **Monocyte/MØ-related:** G-CSF, Interleukin-1 ra, Interleukin-1 α, Interleukin-1 β, Interleukin-6, Tumor Necrosis Factor-α
- **Others:** Epidermal Growth Factor, FGF-basic, GM-CSF, IGF-1, Stem Cell Factor, Tumor Necrosis Factor-β, Interleukin-2, Interleukin-3, Interleukin-7, Interleukin-15, Interleukin-16

Chemokines: ENA-78, Eotaxin, Interleukin-8, Lymphotactin, MCP-1, MDC, MIP-1 α, MIP-1 β, RANTES

Cancer antigens: Alpha-Fetoprotein, Cancer Antigen 19-9, Cancer Antigen 125, Carcinoembryonic Antigen, Prostatic Acid Phosphatase, PSA, Free

Cardio-/vascular disease related proteins: Apolipoprotein A-1, Apolipoprotein C-III, CK-MB, Endothelin-1, Fatty Acid BP, Lipoprotein (a), Myoglobin, PAPP-A

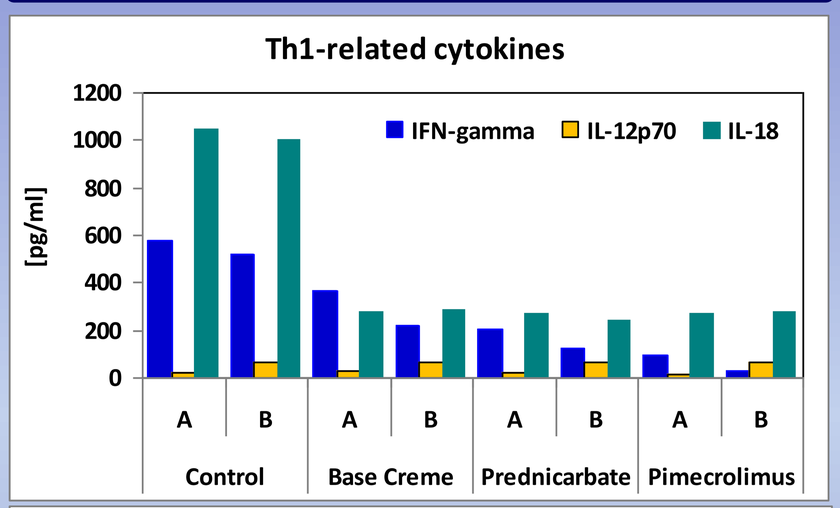
Enzymes: GST, MMP-2, MMP-3, MMP-9, Myeloperoxidase, SGOT

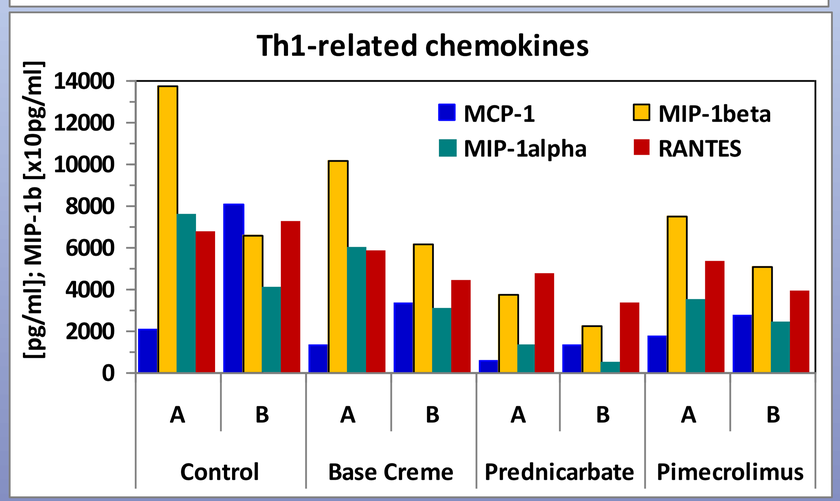
Hormones/BP: Adiponectin, Calcitonin, Erythropoietin, Growth Hormone, Insulin, Leptin, Sex Hormone Binding Globulin, Thrombopoietin, Thyroid Binding Globulin, Thyroid Stimulating Hormone

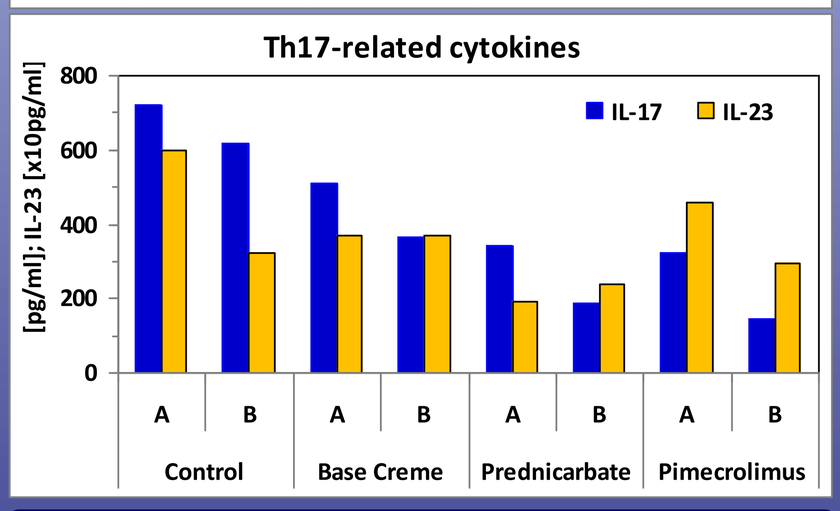
Receptors/ Ligands: CD40, CD40Ligand, ICAM-1, Tissue Factor, Tumor Necrosis Factor RII, VCAM-1

Others: Alpha-1 Antitrypsin, Alpha-2 Macroglobulin, Apolipoprotein H, BDNF, Beta-2 Microglobulin, Complement 3, C-Reactive Protein, ENRAGE, Ferritin, Haptoglobin, Serum Amyloid P, Immunoglobulin A, Immunoglobulin M, Immunoglobulin E, VEGF, Factor VII, Fibrinogen, von Willebrand Factor, PAI-1, TIMP-1

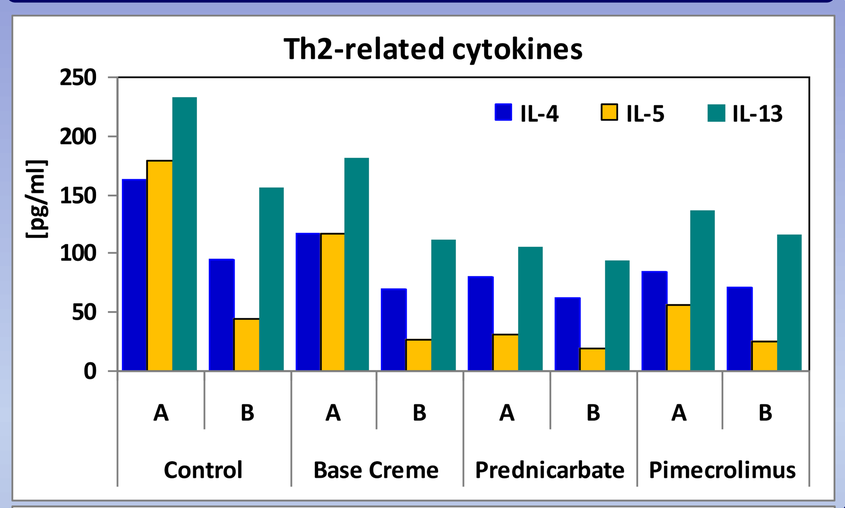
Th1 / Th17-related mediators

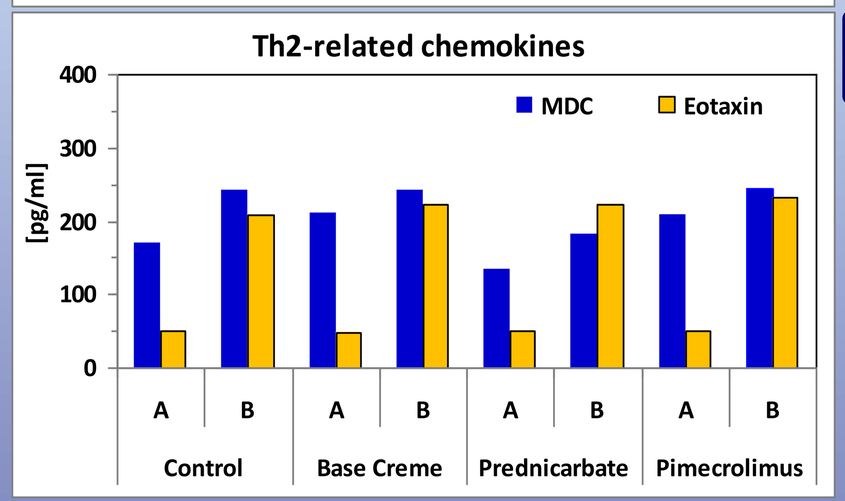




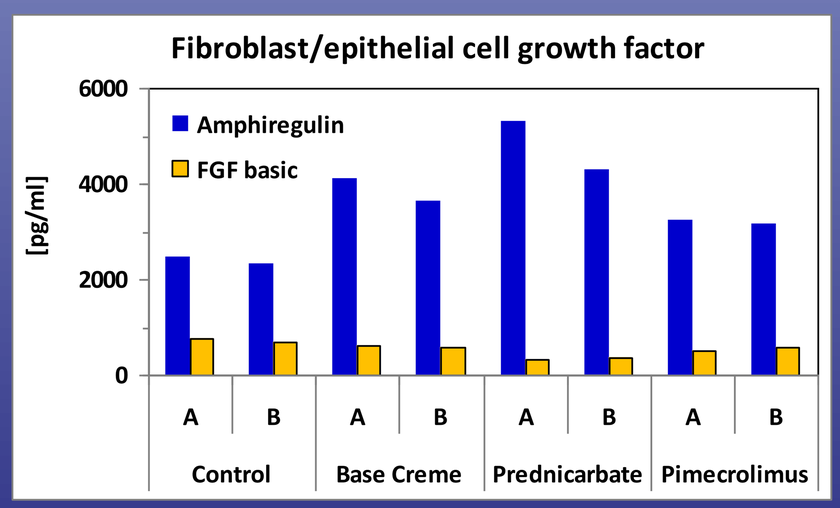


Th2-related mediators

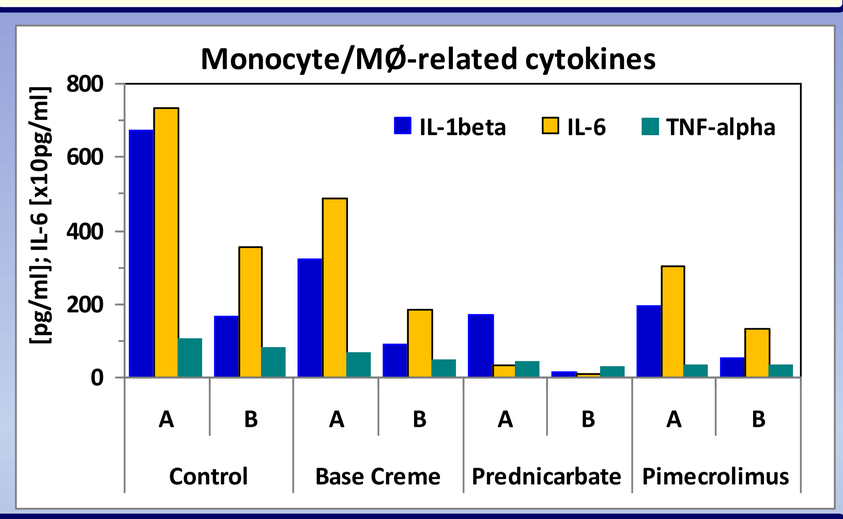




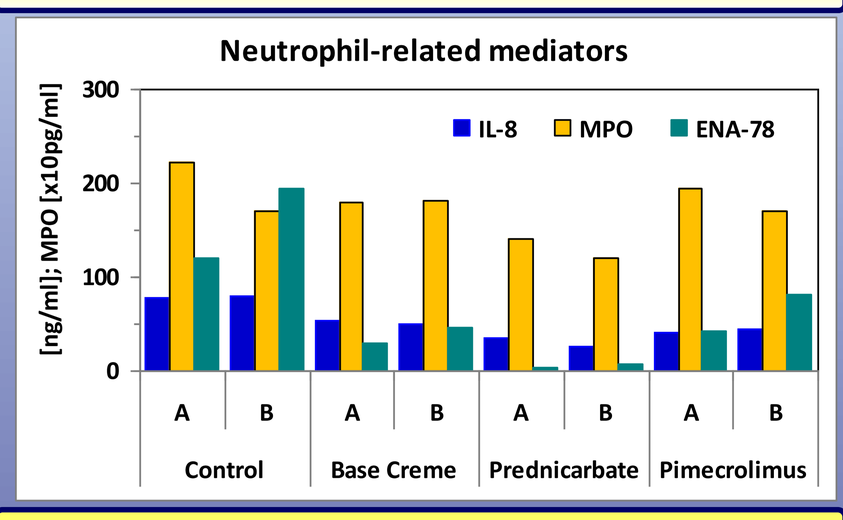
Fibroblast / epithelial cell growth factors



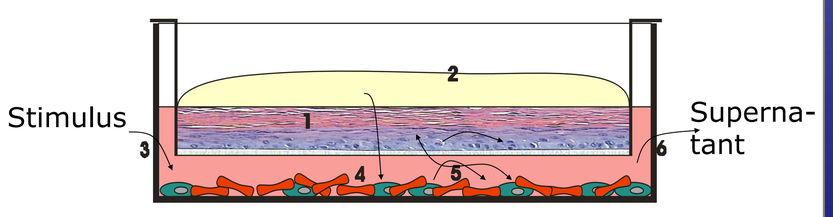
Monocyte/MØ-related mediators



Neutrophil-related mediators



EDI-Co skin
A completely human 3D Co-Culture System



1. Differentiated, multi-layered epidermis in the upper chamber
2. Incubation with drug preparation (soluble or even insoluble)
3. Immune cell activation (whole-blood in lower chamber)
4. Drug transportation/penetration
5. Mediator cross-talk within and between the compartments
6. Determination of various endpoints (mediators, enzymes, etc.)

Results and Conclusions

EDI-Co skin, a co-culture model consisting of differentiated human 3D epidermis together with whole-blood was used to get deeper insight into the complex interaction of the glucocorticosteroid *Prednicarbate* and the calcineurin inhibitor *Pimecrolimus* with the human immune system during dermal application in an inflamed skin environment. Mediators were analyzed by RBM MAP analysis. We could demonstrate that both drugs were able to inhibit a variety of inflammatory mediators being either Th1- or Th17-cell, monocyte/MØ- or granulocyte-associated. *Pimecrolimus* exerted its strongest inhibitory activity on the release of the Th1 cytokine IFN-γ, while Th2-cell mediators were less affected. *Prednicarbate* proved very potent in this model with respect to the inhibition of most inflammatory cytokines and chemokines. Interestingly, among a variety of 98 mediators amphiregulin was the only mediator which was clearly up-regulated. This molecule has been suggested to be involved into keratinocyte and epithelial cell proliferation, probably indicating that, besides an anti-inflammatory activity of the two drugs, further mechanisms may be of relevance.

From the data presented here, it is evident that the complex drug effects can be tested under in-vivo-like conditions with even final formulations of skin products when combining the EDI-Co skin co-culture model with the multiplexed Luminex mediator analysis MAP, as offered by RBM.

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