

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

The role of sebaceous lipids and proteins in the differentiation,  
polarization and function of dermal macrophages and T cells

by Marianna Lovászi

Supervisor: Dániel Törőcsik MD, PhD



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**The role of sebaceous lipids and proteins in the differentiation, polarization and function of dermal macrophages and T cells**

By Marianna Lovászi, Biologist

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Head of the **Examination Committee:** Margit Balázs, PhD, DSc

Members of the Examination Committee: István Balázs Németh, MD, PhD  
Lajos Széles, PhD

The Examination takes place at the Department of Preventive Medicine, Faculty of Public Health, University of Debrecen, 25th of April, 2018 at 11:00 a.m

Head of the **Defense Committee:** Margit Balázs, PhD, DSc

Reviewers:

Szilvia Benkő, PhD

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Members of the Defense Committee: István Balázs Németh, PhD

Lajos Széles, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 25th of April, 2018 at 13:00 p.m

# **1. INTRODUCTION**

For a long time, it was a widely-accepted concept in dermatology, that sebaceous glands (SGs) are atavistic remnants of the human skin and only play important roles in mammals, which resulted in the overlook of human sebaceous gland biology. However, in the last 25 years, cell lines (such as SZ95, SEB-1 and Seb-E6E7) and new molecular techniques were introduced also into SG research, leading to the identification of numerous signalling pathways, transcription factors and enzymatic molecules in sebocytes. As a result, although the primary function of the SGs is still considered to be the production of sebum, which contributes to the lipid barrier of the skin, more and more data has revealed that SGs might also have a role in various (patho)physiological functions from skin aging to UV protection and that sebocytes could also have immune properties as well.

The aim our work was therefore, to focus on their involvement in inflammatory processes and identify possible mechanisms behind it, especially addressing their possible interaction with various cells types of the immune system.

## **1.1 Sebaceous gland function**

Sebaceous glands (SGs) are found in the dermal layer of the skin all over the body except for the palms and soles. Localizing mostly to hair-covered areas, they can also be found in non-hairy regions of the skin, such as the eyelids, nipples, labia minora and the glans penis. Attached to the hair follicles, SGs form the pilosebaceous unit together with the arrector pili muscles. The primary function of human sebaceous glands is considered to be the production and secretion of sebum to cover the hair and contribute to the lipid barrier of the skin. In mammals, sebum provides waterproofing and lubrication for the skin and hair, furthermore, together with apocrine glands, SGs also play an important role in thermoregulation. Additionally, sebum offers protection from several natural hazards such as ultra violet (UV) radiation and various microbes with its antimicrobial activity.

## **1.2 The endocrine regulation of sebaceous glands**

The proliferation and differentiation as well as sebum production of sebocytes is controlled by complex endocrine processes, of which the most prominent changes take place at puberty. However human sebocytes are not only receptive of hormonal changes, but rather are capable of producing androgens, estrogens, corticotrophin-releasing hormone (CRH), all trans retinoic acid (ATRA), eicosanoids, cortisol and cholecalciferol/vitamin D3 themselves. While

androgens are known to facilitate sebaceous gland proliferation and differentiation, furthermore increase lipid production, estrogens, cortisol and ATRA have an opposite effect on the proliferation and differentiation of sebocytes, moreover the accumulation of lipids is also decreased in their presence. Notably CRH activates sebocyte proliferation and directly induces lipid synthesis. Furthermore  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) a ligand of melanocortin receptors (MC-Rs) expressed by sebocytes, have been shown to directly regulate the production of proinflammatory cytokines in sebocytes.

Neuropeptides are also present in sebaceous glands and may provide a link between stress and acne pathogenesis. Among neuropeptides substance P (SP) has a significant role in modulating sebaceous cell size and the number of sebum vacuoles, suggesting that SP promotes both sebocyte proliferation and differentiation.

In understanding the role of diet in sebaceous lipogenesis, a widely accepted mechanism is that a diet rich in carbohydrates increases the activity of the insulin-like growth factor 1 (IGF-1) pathways. The presence of the IGF-1 receptor (IGF-1R) in sebocytes has been described and was shown to activate the phosphatidylinositol 3-kinase/Akt/Forkhead box O1 (PI3K/Akt/FoxO1) pathway thus providing evidence that FoxO1 may be a key factor in the regulation of growth-factor stimulatory effects on sebaceous lipogenesis and inflammation in response to elevated IGF-1 and insulin levels.

Notably, in a previous study we have demonstrated that leptin “the satiety hormone”, besides inducing the expression of inflammatory cytokines such as interleukin (IL)-6 and CXCL-8 in sebocytes also affects lipogenesis by increasing triglyceride (TG) levels, thus increasing the ratio of monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) and by decreasing the levels of vitamin E.

Peroxisome proliferator-activated receptors (PPARs) belong to the family of nuclear hormone receptors (NHRs), which are transcriptional factors either residently or transiently located in the nucleus. All PPAR isotypes are present in sebocytes and particularly PPAR $\gamma$  came to the focus of research with its sebostatic effect via inhibiting apoptosis and thus obstructing holocrine secretion altogether leading to decreased sebum production.

Retinoids are vitamin A derivatives with well-known beneficial effects in acne patients. Target receptors for retinoids are retinoid X (RXR) and retinoic acid (RAR) receptors both of which are expressed in sebocytes. While RXRs have a role in influencing cell differentiation, RARs modulate cell proliferation.

Also belonging to the nuclear receptor family, liver X receptors (LXRs,  $\alpha$  and  $\beta$  isoforms) play a decisive role in cellular cholesterol (CH) homeostasis and lipid metabolism. LXRs regulate

various ATP-binding cassette transporters (ABC transporters), fat metabolizing enzymes and other transcription factors such as sterol regulatory element-binding protein-1c (SREBP-1c).

### 1.3 Sebum composition and production

Sebocytes are able to uptake lipids from the circulation and *de novo* synthesize polar lipids and TGs elongating the carbon chains by adding acetate groups to it. The maturation of sebocytes is followed by a cell type specific cell death. This procedure results in the holocrine secretion of sebum.

Human sebum is a lipid mixture composed of cell debris and nonpolar (neutral) lipids, namely TGs and fatty acids (FAs) (57.5%), wax esters (26%), squalenes [SQ (12%)], cholesterol esters (CHEs) (3%) and CH (1,5%). The composition of human sebum varies greatly from what is secreted by mammalian sebaceous glands, SQ, sapienic acid and sebaleic acid are only synthesized by human sebocytes. Due to its prominent lubricant and penetration efficiency SQ is capable of neutralizing the formation of UV irradiation-induced reactive oxygen species in the skin. On the other hand, peroxidated SQ has been reported to be comedogenic and therefore responsible for pathological skin conditions.

As opposed to SQ, wax esters are uniquely produced by sebocytes. FAs are direct precursors of wax esters, moreover fatty acid transport protein 4 (FATP4) is strongly expressed in sebocytes and is responsible for the uptake of long chain fatty acids (LCFAs) into sebocytes. The enhanced uptake of LCFAs results in an enhanced wax ester biosynthesis in human sebaceous gland cells.

While diglyceride-acyltransferase (DGAT) is a major enzyme partaking in sebaceous TG synthesis, stearoyl-CoA desaturase (SCD) plays a major role in the synthesis of unsaturated FAs. Importantly SCD creates a double bond between the 9. and 10. carbon atom thus creating oleic acid [OA (18:1 $\Delta$ 9)] from stearic acid [STA (18:0)]. OA (18:1 $\Delta$ 9), a major omega-9 MUFA of sebum TGs, exerts the strongest antibacterial and anti-inflammatory properties among sebaceous FAs and strongly enhances the innate antibacterial defence of the skin.

Linoleic acid [LA (18:2,  $\Delta$ 9,12)], an omega-6 polyunsaturated compound, and  $\alpha$ -linolenic acid (18:3,  $\Delta$ 9,12,15), an omega-3 polyunsaturated compound, are essential FAs.

Fatty acid desaturase 2 (FADS2) converts palmitic acid [PA (C16:0)] into sapienic acid (C16:1 $\Delta$ 6), a unique sebaceous MUFA exerting strong antibacterial and antifungal activities. The elongation and further desaturation of sapienic acid yields another unique sebaceous gland FA, sebaleic acid (18:2 $\Delta$ 5, 8).

## 1.4 Sebum alterations in various skin diseases

Variations in the composition and amount of sebum in different skin diseases have been detected, which are suggested not to be simply disease markers but may also play significant roles in disease pathogenesis.

### 1.4.1 Acne

Acne predominantly affects 60-80% of adolescents. Both genetic and environmental factors contribute to its onset, however the exact mechanisms are yet to be identified. In the recent years numerous studies have showed, that in addition to an elevated sebum production, acne patients have a markedly altered sebum composition. Due to endogenous desaturases, peroxidases as well as bacterial lipases and phosphatases, an altered ratio between saturated and unsaturated FAs has been identified, particularly, an increase in the C16:1 $\Delta$ 6/ C16:0 lipid ratio has been shown in acne, accompanied by diminishing levels of LA content. These data imply that desaturation of FAs may drive towards acne development.

#### *The role of P. acnes in the pathogenesis of acne*

Several hypotheses attribute a key role for *P. acnes* in the development of acne, although it is still not fully understood how *P. acnes*, a part of the healthy skin microbiome, can become pathogenic. According to the most accepted theory, *P. acnes* might induce the hyperproliferation of the keratinocytes in the hair follicle duct, which blocks the opening of the pilosebaceous unit forming the so called “follicular plug” thus preventing the flow of sebum. The accumulating sebum then forms a microenvironment in which *P. acnes*, could reach significant levels to induce and augment inflammation. Based on in vitro experiments, *P. acnes* not only promotes the activity of NLRP3 and thus enhances the production of IL-1 $\beta$ , but also increases the production of CXCL-8, IL-12 and tumour necrosis factor (TNF)- $\alpha$  in keratinocytes macrophages and sebocytes. Although, it is still a question whether *P. acnes* can come into direct contact with sebocytes, its products such as lipases can undoubtedly have an effect on sebaceous glands.

### 1.4.2 Seborrheic dermatitis

Seborrheic dermatitis, also known as seborrhea, is a chronic inflammation of the skin, with symptoms such as redness, itching and scaling localizing mainly to the scalp and face. In relation to seborrheic dermatitis, studies suggest that the lipases and phosphatases produced by

*Malassezia* hydrolyse sebaceous lipids, resulting in decreased TGs and a corresponding increase in free fatty acid (FFA), which may cause the characteristic irritation.

#### 1.4.3 *Rosacea*

Rosacea is a chronic inflammatory cutaneous condition that typically affects the cheeks, forehead chin and nose resulting in redness, swelling and dilated blood vessels. Despite the occurrence of rosacea in the sebaceous gland-rich areas of the body, the amount of sebum excretion does interestingly not show alterations in rosacea patients compared to healthy controls. However, there are changes in the overall relative composition of sebum. Affected individuals exhibit elevated levels of myristic acid (C14:0) and reduced levels of saturated LCFAs. These lipids have a role in maintaining the skin barrier integrity, therefore it is reasonable to assume that the altered sebaceous FA profile is an important contributor to skin barrier dysfunction in rosacea.

#### 1.4.4 *Psoriasis*

Psoriasis is a chronic inflammatory skin disease, characterized by patches of hyperproliferating skin, which severely affects the quality of life. Psoriatic lesions are usually characterized by various degrees of sebaceous gland atrophy, however there has been no correlation found between sebaceous gland size and either total Psoriasis Area Severity Index (PASI) or the degree of erythema and inflammatory infiltration. Despite having sebaceous gland atrophy, the total amount of sebum production in psoriatic skin is not significantly changed, although an increase in the levels of phospholipids, triacylglycerols, and CH were found in the epidermis of psoriatic patients, which correlated with the severity of psoriasis.

#### 1.4.5 *Atopic Dermatitis (AD)*

AD is a chronic inflammatory skin condition that often starts in early childhood and results in dry, red and itchy skin. Although AD is mainly characterized by the dysfunctional synthesis of ceramides in keratinocytes, AD patients seem to exert a severely reduced sebum production, which contribute to diminished skin hydration. Since the proportion of sebaceous lipids in the casual lipids is decreased and epidermal lipids (e.g. CH) are increased, therefore a link between an altered sebaceous gland function and skin barrier dysfunction seem to contribute to the development of the disease, although the exact mechanisms have not been elucidated.



## 1.5 Therapeutic perspectives in modulating sebum composition

The observations of altered sebum composition made so far in various skin diseases puts forward possible therapeutical solutions through the modulation of sebum amount and composition.

New compounds might be utilized as topical treatments in acne, such as long-chain PUFAs, which could target infections, e.g. with *P. acnes*, *Staphylococcus aureus* (*S. aureus*), due to their antimicrobial and anti-inflammatory properties, thus providing an alternative for antibiotics in acne treatment. PA, OA, and lauric acid have been already proposed as alternatives to antibiotic therapy in acne vulgaris. Additionally, clinical studies are currently ongoing to create sebocyte specific PPAR $\alpha$  agonists and PPAR $\gamma$  antagonist, which may beneficially decrease sebum production and inflammation in acne patients.

Controlling lipid production not only can be beneficial for acne patients, where the overproduction of sebum and its compositional imbalance represents the main problem. Severe skin dryness as observed in AD patients might be remedied by manipulation of sebum production towards a normal composition via the stimulation of androgen or PPAR receptors, which could potentially repair the defective skin barrier. Furthermore, the activation of LXRs by synthetic ligands may have therapeutic relevance, which could also augment lipid production along with exerting anti-inflammatory effects.

Novel compounds such as endocannabinoids may also provide solutions to various sebaceous gland-related diseases. Recently Dobrosi et al. provided evidence that sebocytes express cannabinoid receptor-2, more interestingly arachidonoyl ethanolamide/anandamide, 2-arachidonoyl glycerol are also present in sebocytes and can induce lipid production and apoptosis. Endocannabinoids generally up-regulate the expression of genes involved in lipid synthesis, such as PPAR transcription factors, thus cannabinoid receptor-2 antagonists or agonists may be utilized in the management of sebaceous gland disorders.

## 1.6 Inflammatory processes exerted by sebocytes

Although, sebocytes are able to alter their sebum production in response to different inflammatory stimuli, to position them in the inflammatory network, research has mostly focused on identifying the role of the co-produced proteins. Recent findings have shown that sebocytes, similarly to immune competent cells, are able to produce a vast array of cytokines and chemokines both *in vivo* and *in vitro*, such as: IL-6, CXCL-8, IL-1 $\alpha$ , IL-1 $\beta$  and tumour necrosis factor (TNF)- $\alpha$ .

Moreover, sebocytes have been shown to exert inflammatory properties with the production of antimicrobial peptides, cytokines and chemokines, in which the activation of TLR2 and -4, shown to also be expressed in sebocytes, play a crucial role. Furthermore, sebocytes have also been identified as sources of various adipokines, such as adiponectin, leptin, serpin E1, resistin and visfatin, within the skin, highlighting that sebaceous glands could represent a link between inflammation and lipid metabolism similarly to adipocytes.

Besides protein production, inflammation is also characterized by lipid mediators, such as prostaglandins (PGs) and LTs, which PGs and LTs are derivatives of AA metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) respectively. LTs and PGs both have major regulatory roles over sebocyte differentiation and lipogenesis, under physiological and pathological conditions as well.

### **1.7 Immune functions of sebum lipids**

Sebaceous lipids make an important contribution in maintaining the integrity of the skin barrier along with lipids of epidermal origin thus contributing to the body's first line of defence. The healthy human skin is populated with a characteristic microbiome. Interestingly, its diversity was found to be associated with the differences in the quantitative levels of sebum and hydration even between various parts of the face. One of the most studied colonizing member is *P. acnes*, an anaerobic bacterium, which is capable of metabolizing sebaceous TGs into FFA on the surface of the skin via its lipases and peroxidases. This bacterial lipase activity not only leads to an increased presence of FFA on the surface of the skin, but also to an altered sebum composition. These changes lead to hyperkeratinisation and chronic inflammation, which further increases the pathological microbial colonization of the skin, thus sustaining acne formation. Sebum may also act as a delivery system for antioxidants and antimicrobial peptides. Such molecules with antimicrobial properties are cathelicidin, psoriasin, dermcidin and human hBD-2. Other studies have showed that lauric and sapienic acid have broad-acting antimicrobial properties.

### **1.8 Skin resident immune cells**

Resident macrophages and dendritic cells (DCs) of prenatally seeded yolk sac- or fetal liver-derived precursors can be found in the epidermis (Langerhans cells, LCs) and the dermis. These cells are distinct in their phenotype and function from CD14<sup>+</sup> monocytes and precursor dendritic cells (pre-DCs), which are derived from bone marrow cells and circulate in the blood stream, from where upon attracting signals and stimulation they passage through the

endothelium into the surrounding tissues differentiating into macrophages and DCs. Interestingly McGovern et al. found that these CD14<sup>+</sup> cells have a rapid turnover in the dermis in contrast to resident macrophages, spending approximately 6 days in the dermal layer of the skin, followed by cell death and phagocytosis, only to be replaced by freshly arriving CD14<sup>+</sup> monocytes. With the identification of novel monocyte/macrophage and dendritic cell markers, new subsets of myeloid cells are discovered continuously, making the myeloid cell population of the skin highly heterogeneous, however the available tools to characterize their functions is very limited.

### *1.8.1 Macrophages and macrophage heterogeneity*

Being the key phagocytes of the immune system, macrophages sense and transform signals and stimuli towards the initiation of inflammation and activation of the adaptive immune system. In order to fulfil these complex roles, macrophages exert a prominent morphological and functional heterogeneity in both the healthy and the inflamed skin, outlining the importance of the different stimuli behind their activation. Based on these stimuli, a widely accepted classification separates macrophages into classically (M1) and alternatively (M2) activated subsets. While alternative macrophages are activated by IL-4/IL-13 and by various lipids that are associated with tissue maintenance and remodelling processes, and are increased in high numbers in pathological conditions, such as wound healing or tumour matrix formation, the classically activated macrophages typically develop in response to interferon gamma (IFN- $\gamma$ ) and/or TNF- $\alpha$ . Classic examples of M2 macrophages can be found in skin diseases of granuloma annulare and necrobiosis lipoidica, while a hallmark of M1 macrophages are present in tuberculoid granulomas. Human dermal macrophages in a healthy conditions typically express MHC-II, CD14, and Factor XIII subunit A (FXIII-A) and are superior at phagocytosis, but inferior at T cell activation compared to DCs.

#### *Macrophage markers used in the study*

CD163 is a high affinity scavenger receptor for the haemoglobin-haptoglobin complex. Notably it has been identified as a marker of cells originating from the monocyte/macrophage lineage. CD206, also known as mannose receptor, is a C-type lectin mainly present on the surface of macrophages and immature dendritic cells. The receptor recognises terminal mannose and N-acetylglucosamine residues found on the surface of certain microorganisms, thus playing an important role both in the innate and adaptive immune systems.

CD209, also known as DC-SIGN, is a C-type lectin receptor present on the surface of both macrophages and dendritic cells. In case of macrophages CD209 recognises mannose type carbohydrates commonly found on various pathogens, therefore CD209 has an important role in activating phagocytosis.

Factor XIII (FXIII) is a transglutaminase and an important member of the blood coagulation system that crosslinks fibrin. While circulating FXIII consists of two catalytic subunits (FXIII-A) and two carrier subunits (FXIII-B), in monocytes/macrophages FXIII-A (but not FXIII-B) can also be found in an intracellular localization where it could contribute to gene expression regulation as well as alter cellular functions such as phagocytosis. Recently FXIII-A has been identified as a marker of alternative activation and was successfully applied to detect these macrophages in granuloma annulare and necrobiosis lipoidica.

#### *Role of lipid metabolism in macrophage activation*

The importance of lipid metabolism in macrophage activation has been recently reviewed by Ménégaut et al. Both external (dietary) and internal FA sources are able to influence and drive macrophage polarization: generally, M2 polarization is associated with FA oxidation, whereas M1 polarization is linked with lipogenic pathways. Several studies highlighted the importance of PPAR $\gamma$ , which activated by oxidized FAs or IL-4, is capable of driving M2 polarization. Recent papers suggest that FA synthesis directly contributes to the inflammatory response in M1 macrophages, however it is not clear yet, whether the induction of FA synthesis is necessary for M1 polarization. Additionally, saturated FFAs, such as PA has been suggested to trigger the metabolic activation of macrophages in adipose tissue. In another study Shi et al. showed that in vivo FFA released by adipocytes potentially influenced macrophage polarization.

Based on these observations it is reasonable to assume, that sebum component lipids may also take part in the polarization of macrophages.

#### *1.8.2 DCs*

Besides macrophages, the skin also contains distinct populations of DCs, characterised by unique morphology and the surface expression of CD11c. Cutaneous DCs serve as sentinels, which along with producing inflammatory cytokines also process and present antigens to antigen-specific T lymphocytes. Depending on their location cutaneous DCs can be divided into subsets: epidermal LCs and bone marrow-derived dermal cDCs, which can be further classified into type 1 (cDC1) and type 2 (cDC2) subsets. Similarly to macrophages, DCs also

respond to changes in FA metabolism. Interestingly, the oxidation of FAs creates a state in which DCs become tolerogenic.

### *1.8.3 Helper T cell subsets*

Cell-mediated immune responses are mainly controlled by T cells. T cells mature in the thymus, and the ones that express CD4 glycoprotein on their surface are classified as T helper (Th) cells. Th cells obtain highly specialized effector functions during activation and can be categorized into several subsets. These distinct CD4<sup>+</sup> subsets differentiate from naive CD4<sup>+</sup> T cells as a result of specific cytokine exposure. Th cells were first classified into Th1 and Th2 subsets based on their distinct cytokine production of IFN- $\gamma$  and IL-4, respectively, however recently the Th family has notably expanded to include Th9, Th17 and Th22 cells, which also have their specific cytokine expression profile.

As with the case of macrophages besides specific cytokines that define the different Th differentiation pathways, lipids can also contribute to Th polarization, such as Trans fatty acids (TFAs) that promote Th17 polarization.

## 2. OBJECTIVES

In recent years sebaceous gland research has come a long way; with the establishment of sebocyte cell culture models and the development of new molecular techniques more and more information became available regarding sebocyte proliferation, differentiation and function. However, their possible role as mediators or even initiators in inflammatory skin diseases has not been defined well yet. Furthermore, their main profile namely sebum production and the potential role of sebum component lipids in influencing various inflammatory pathways and immunological processes has been barely investigated. Therefore, we aimed to:

1. determine whether sebum component lipids could contribute to the dermal lipid content.
2. evaluate whether sebocytes are able to recruit immune cells to sites of skin inflammation.
3. investigate if sebocytes could contribute to the differentiation, polarization and function of macrophages via their secreted lipids and proteins.
4. address a possible interaction between human sebocytes and *P. acnes* in driving inflammatory responses, which may be active in skin homeostasis (symbiosis) and/or in immune reactions such as acne vulgaris.
5. analyse the functional communication between human sebocytes and T cells.

### **3. MATERIALS AND METHODS**

#### **3.1 Oil-Red-O staining**

Anonymised frozen skin biopsies were sectioned and fixed in 4% Paraformaldehyde (Sigma-Aldrich, Dorset, UK) for 10 min. Slides were placed in 100% Propylene Glycol (Amresco, Solon, OH, USA) for 1 min, followed by a rinse with distilled water. The sections were stained with 0,7% Oil-Red-O (Sigma-Aldrich) solution for 7 minutes than rinsed with 85% Propylene Glycol solution. A counter staining for nuclei was done with methylene green. Finally, the slides were covered using Mount Quick Aqueous mounting medium (Bio Optica Milano, Italy).

#### **3.2 Raman spectroscopy**

Raman spectroscopy measurements were executed and evaluated by our collaborators Dr. Attila Gácsi and Dr. Erzsébet Csányi in the Institute of Pharmaceutical Technology and Regulatory Affairs, Szeged. Skin biopsies were obtained from abdominal reconstruction surgery. Approximately 1 cm<sup>2</sup> skin samples were treated with either SQ, LA, OA, PA or STA on 4 mL volume Franz diffusion cells using phosphate buffer saline solution (PBS) (Biochrom, Berlin, Germany) to avoid skin drying. In all cases the treatment duration was 24 h and the treated area was 66.5 mm<sup>2</sup>. Tissue samples of the treated areas were frozen and sectioned onto aluminium coated slides. Raman spectra of each section were obtained with a DXR Raman microscope (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a 532nm diode laser. Instrument operation and the evaluation of measurements were done by the OMNIC Dispersive Raman 8.2 software (Thermo Fisher Scientific). In all mapping measurements 24 spectra were collected, the spectral resolution was approximately 2 cm<sup>-1</sup>, and the spectral window ranged from 200 to 3200 cm<sup>-1</sup>. The individual spectra of each FA were used as a reference when comparing the treated vs untreated samples.

#### **3.3 Immunohistochemistry**

##### *3.3.1 CD163*

Anonymised FFPE sections of human skin from the tissue archive of the Department of Dermatology, University of Debrecen, were used after approval by the Regional and Institutional Ethics Committee, University of Debrecen. Paraffin sections were deparaffinised, rehydrated, and incubated in 3% H<sub>2</sub>O<sub>2</sub> in distilled water for 15 min. For antigen retrieval, slides were treated with Tris-EDTA buffer (10mM Tris Base, 1mM EDTA solution, 0.05% Tween 20, pH 9) in a boiling pressure cooker (120°C) for 20 min. To reduce non-specific binding,

slides were incubated in 5% BSA-PBS for 30 min at room temperature. Tissue sections were incubated with CD163 antibody (LifeSpan BioSciences, Seattle, WA, USA) overnight at 4°C in a humidity chamber. As negative control, samples were incubated in 2.5% BSA-PBS without primary antibody. SuperSensitive One-step Polymer-HRP-conjugated secondary antibody (BioGenex, CA, USA) was used in accordance with the manufacture's instruction. Immunoreaction was visualized by Vector VIP Kit (Vector Laboratories Ltd, Cambridgeshire, UK). Sections were counterstained with methylene green. Images were analysed with ImageJ software.

### 3.3.2 *CD206, CD209 and Factor XIII subunit A (FXIII-A)*

Frozen sections were fixed in acetone for 10 min and blocked with 5% normal goat serum (Dako, Glostrup, Denmark). FXIII-A was detected by rabbit affinity purified anti-human FXIII-A antibody (Affinity Biologicals, Ancaster, Ontario, Canada) for 2 h at room temperature. Visualization was achieved by DyLight 488 horse anti-sheep secondary antibody (Vector Laboratories Ltd). For co-expression, the detection of FXIII-A was sequentially combined with reference markers using monoclonal anti-human antibodies against CD antigens [CD206, CD209 (Abcam, Cambridge, UK), CD163 (Enzo Life Sciences, Farmingdale, NY, USA)]. Following a 10 min blocking with normal horse serum, the second primaries' specific binding was visualized by DyLight 594 goat anti-rabbit/mouse antibody (Vector Laboratories Ltd). Slides were washed in PBS and mounted with Vectashield Mounting Medium with DAPI (Vector Laboratories Ltd) to counterstain nuclei. Images were acquired with an Axioplan microscope (Carl Zeiss, Oberkochen, Germany) equipped with selective filters and connected to a CCD IMAC camera (Sony, Tokyo, Japan) and ISIS fluorescent imaging system (MetaSystems, Altlusheim, Germany).

### 3.3.3 *IL-17 and CD4*

Stainings were performed by Dr. Natalie Garzorz-Stark in collaboration with the ZAUM institute in Munich. FFPE skin biopsies of acne patients were incubated at 65°C for 25min and rehydrated by incubation with xylol (2x10 min) followed by 100% isopropanol (2x5 min), 96% ethanol, 70% ethanol and dH<sub>2</sub>O (1x5min each). Antigen retrieval was achieved by boiling in a pressure cooker with citrate buffer (pH 6) for 7min and blocking with 10% normal goat and 10% normal horse serum (both Life Technologies, Carlsbad, CA, USA) for 1h at room temperature. Slides were incubated with primary antibody mix or isotype controls (polyclonal goat anti-IL-17, R&D Systems and monoclonal rabbit anit-CD4, Zytomed Systems) for 1h at



room temperature and then overnight at 4°C. Visualization was achieved with a secondary antibody mix (Alexa Fluor 488 goat anti-rabbit IgG, Life Technologies and NorthernLights 557 donkey anti-goat IgG, R&D Systems). To quench tissue autofluorescence, sections were incubated in 0.1% Sudan Black B (Sigma-Aldrich). DAPI (Sigma-Aldrich) was used to counterstain nuclei, finally slides were mounted with VectaShield (Vector Laboratories Ltd). Fluorescence images were obtained using an Olympus IX73 inverted fluorescence microscope equipped with cell Sens Software (Olympus, Tokyo, Japan) and processed with ImageJ software.

### **3.4 Migration assay**

Migration assays were performed by our collaborators in Munich. Monocytes, neutrophils and T cells were isolated from PBMCs of healthy donors using CD14 and CD3 microbeads respectively (Miltenyi Biotech, Vienna, Austria) following the manufacturer's protocol. Cells were resuspended in complete RPMI 1640 (Sigma-Aldrich) supplemented with 0.5% BSA (Amresco) and their numbers were adjusted to achieve a final concentration of  $2 \times 10^6$  cells ml<sup>-1</sup>. The migration assays were performed by using 5 µm pore polycarbonate membranes (ChemoTx Disposable Chemotaxis System, NeuroProbe, Gaithersburg, MD, USA). according to the manufacturer's protocol. Different concentrations of recombinant CXCL-8 (10 ng/ml, 50 ng/ml and 100 ng/ml, respectively, PromoKine, Heidelberg, Germany) were used as positive controls. Cells were migrating for 2 h at 37°C in a humidified atmosphere containing 5% (v/v) CO<sub>2</sub>, transmigrated cells were collected and counted with LSRFortessa (BD Biosciences New Jersey, NJ, USA) flow cytometer. Migrated T cells were additionally stained for CD4, CD8, CD56, CD45RO and CD45RA. For CXCL-8 neutralization, the supernatant was incubated with the anti-CXCL-8 antibody (R&D Systems, Minneapolis, MN, USA) for 1 h prior to the migration assay.

### **3.5 SZ95 sebocyte cell culture and treatment**

The immortalized human sebaceous gland cell line SZ95 was cultured at 37°C in a humidified atmosphere containing 5% (v/v) CO<sub>2</sub>, in Sebomed medium (Biochrom) supplemented with 10% fetal bovine serum (FBS) (Biowest, Rue de la Caille, France), 1 mM CaCl<sub>2</sub> solution, 1% penicillin/streptomycin (Sigma-Aldrich) and 5 µg/ml epidermal growth factor (EGF) (Sigma-Aldrich). Cells were kept in culture until reaching approximately 80% confluence. Prior to supernatant collection the used medium was replaced with Sebomed medium containing 0.5% FBS, 1 mM CaCl<sub>2</sub> solution, with or without 1% penicillin/streptomycin, lacking EGF. 24 h

supernatant was collected and filtered using 0.2 µm syringe filters (Sarstedt, Nümbrecht, Germany) and used for experiments. In each *in vitro* experiment Sebomed medium containing 0.5% FBS, 1 mM CaCl<sub>2</sub> solution, with or without 1% penicillin/streptomycin, lacking EGF was used as a control treatment of monocytes or macrophages.

For lipid depletion of SZ95 sebocyte supernatant Cleanascite lipid clarification reagent (Biotech Support Group, Monmouth Junction, NJ, USA) was used according to the manufacturer's instructions. Lipids; SQ, LA, OA, PA and STA (Sigma-Aldrich) dissolved in ethanol:DMSO (1:1); were replaced individually subsequent to lipid depletion in a concentration of 150µM. ethanol:DMSO (1:1) was used as a vehicle control.

For T cell and DC culturing experiments SZ95 sebocytes were stimulated with recombinant cytokines (50ng/ml each) or lipopolysaccharide/lipoteichoic acid (LPS/LTA) (1µg/ml) or the *P. acnes* strain 889 (50:1 ratio) for 6h, extensively washed and cultured for additional 24h in Sebomed medium. SZ95 sebocyte supernatants were collected and filtered using 0.2µm syringe filters (Sarstedt) and frozen until use in experiments.

### **3.6 *P. acnes* strains**

Strain *P. acnes* 889 was cultured at the Department of Microbiology, University of Debrecen, Hungary. *P. acnes* bacteria were cultured on pre-reduced Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 5% cattle blood, vitamin K1, and hemin, incubated at 37°C for 72 h under anaerobic conditions (Ruskinn Concept 400 Anaerobic Workstation, Pencoed, UK). The 72 h culture of the bacteria was collected at a density of 10<sup>5</sup>-10<sup>6</sup> colony forming units (CFU)/ml. Cells were washed in PBS (Lonza, Verviers, Belgium) and harvested in 1 ml RPMI1640 medium (Sigma-Aldrich) lacking antibiotic/antimicotic solution and used for experiments.

### **3.7 Monocyte isolation and differentiation**

#### *3.7.1 Macrophage differentiation and culturing*

Monocytes were isolated from PBMCs of healthy donors using the CD14 microbeads (Miltenyi Biotech) following the manufacturer's protocol. 1x10<sup>6</sup> monocytes were plated in a 24-well plate in RPMI 1640 (Sigma-Aldrich) and were treated with 40% or 80% of SZ95 sebocyte supernatant. An appropriate concentration of Sebomed medium was used as control. Monocytes were differentiated in the presence of SZ95 sebocyte supernatant or Sebomed medium for 5 days at 37°C in a humidified atmosphere containing 5% (v/v) CO<sub>2</sub> and were used for experiments. For further differentiation and activation with IL-4 (20ng/ml), IFN-γ (20ng/ml) or

*P. acnes* was used (1:50). Results presented are obtained with 80% SZ95 sebocyte supernatant supplementation after confirming dose dependence.

### 3.7.2 DCs generation and mixed leukocyte reaction

Experiments were a joined effort with Dr. Martina Mattii in collaboration with the ZAUM institute in Munich.  $1 \times 10^6$  CD14<sup>+</sup> monocytes were seeded in a 24-well plate containing 500 $\mu$ l RPMI 1640 (Invitrogen) and 500 $\mu$ l of SZ95 sebocyte supernatant as well as IL-4 and GM-CSF (100U/ml of each) (PromoKine) and incubated for 5 days at 37°C, 5% CO<sub>2</sub>. At day 5, DCs were stimulated with LPS (1  $\mu$ g/ml, Invitrogen) for 24 hours, washed twice with PBS and plated in a 96-well plate in a 1:10 ratio with CD4<sup>+</sup>CD45RA<sup>+</sup> or CD4<sup>+</sup>CD45RO<sup>+</sup> T cells for the mixed leukocyte reaction. Supernatant of CD4<sup>+</sup>CD45RO<sup>+</sup> memory T cells was collected at day 3, CD4<sup>+</sup>CD45RA<sup>+</sup> naïve T cells were kept in culture for 6 days and re-stimulated for 72 hours with plate-bound human  $\alpha$ CD3 and soluble  $\alpha$ CD28 (each 0.75 $\mu$ g/ml) (BD Biosciences) before supernatant collection. Samples were assayed in duplicates.

## 3.8 Flow cytometry

For flow cytometric analysis macrophages were collected and washed with PBS (5 min, 1500 rpm) and were resuspended in staining buffer [PBS containing 1% BSA (Amresco)]. Cells were stained for surface markers CD206, CD209 using phycoerythrin (PE) conjugated mAbs (BD Biosciences) for 30 min at 4°C; PE conjugated IgG1 $\kappa$  (BD Biosciences) was used as isotype control. Data was collected by flow cytometric analysis using FACSCalibur (BD Biosciences) and was analysed with Flowing Software (Cell Imaging Core, Turku, Finland).

## 3.9 Phagocytosis assay

Macrophages were incubated with FITC-labelled *P. acnes* for 2 h at 37°C, with or without lipid depletion and replacement, to allow bacteria uptake. Phagocytosis was stopped with ice cold PBS and cells were fixed with 4% PFA. Data was collected by flow cytometric analysis using FACSCalibur (BD Biosciences) and was analysed with Flowing Software (Cell Imaging Core).

## 3.10 Western blotting

Cells were collected and processed for Western blot analysis at 12 h following *P. acnes* treatment, protein concentration was determined using BCA Protein Assay Kit (Thermo Fisher Scientific). Proteins were probed with anti-IL-1 $\beta$  (R&D Systems), anti-FXIII-A (Acris Antibodies, Herford, Germany) and anti- $\beta$ -actin (Cell Signalling, Danvers, MA, USA). The Ag-Ab complexes were labelled with appropriate HRP-conjugated secondary antibodies (Bio-

Rad Laboratories) and visualized by Immobilon Western HRP Substrate kit (Millipore, Bedford, MA, USA).

### **3.11 Chemokine and cytokine detection**

#### *3.11.1 Enzyme-linked immunosorbent assay (ELISA)*

Supernatants from macrophages cultured in the presence of SZ95 sebocyte supernatant, *P. acnes* 889 or appropriate controls were collected at 12h, 24 h or 36h after treatment and were stored at  $-20^{\circ}\text{C}$  until they were analysed for CXCL-8, IL-6, TNF- $\alpha$ , IL-1B $\beta$ , IL-17, IL-22, IFN- $\gamma$  (R&D Systems) and IL-4 (BD Biosciences) using the appropriate ELISA kits according to the manufacturer's instructions.

#### *3.11.2 Bio-Plex assay*

Experiments were performed on our samples by our collaborators in the ZAUM institute in Munich. Bio-Plex analysis was performed using the Bio-Plex Pro Human Cytokine 27-Plex Assay (Bio-Rad Laboratories) according to the manufacturer's instructions. Quantification of protein content was determined with the Bio-Plex 200 System (Bio-Rad Laboratories).

### **3.12 Purification and stimulation of naïve and memory CD4<sup>+</sup> cells**

Experiments were a joined effort with Dr. Martina Mattii in collaboration with the ZAUM institute in Munich. CD4<sup>+</sup> cells were magnetically sorted using the CD4 T cell isolation kit II followed by a positive selection with CD45RO or CD45RA beads (Miltenyi Biotech).  $2 \times 10^5$  cells were seeded in a 96-well plate and stimulated with plate-bound human  $\alpha\text{CD3}$  and soluble  $\alpha\text{CD28}$  (each  $0.75\mu\text{g/ml}$ ) (BD Biosciences) in presence of  $100\mu\text{l}$  SZ95 sebocyte supernatant and  $100\mu\text{l}$  RPMI 1640 (Invitrogen) at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . Supernatant of CD4<sup>+</sup>CD45RO<sup>+</sup> memory T cells was collected at day 3. CD4<sup>+</sup>CD45RA<sup>+</sup> naïve T cells were kept in culture for 6 days, and re-stimulated for 72 hours with plate-bound human  $\alpha\text{CD3}$  and soluble  $\alpha\text{CD28}$  (each  $0.75\mu\text{g/ml}$ ) (BD Biosciences) before supernatant collection.

### **3.13 Neutralization experiments and protein removal**

Experiments were performed on our samples by our collaborators in the ZAUM institute in Munich. SZ95 sebocyte supernatants were incubated for 1h with the following antibodies: anti-IL-1 $\beta$  ( $1\text{ng/ml}$ ), anti-TGF $\beta$  ( $1\text{ng/ml}$ ), anti-IL-6 ( $0.8\text{ng/ml}$ ) or antiCXCL-8 ( $0.8\mu\text{g/ml}$ ) (all R&D Systems). For total protein digestion, SZ95 sebocyte supernatants were treated with Proteinase K ( $0.5\text{mg/ml}$ ) (Qiagen, Hilden, Germany) and incubated for at  $56^{\circ}\text{C}$  for 30 min and  $80^{\circ}\text{C}$  for 10 min for enzyme inactivation.

### **3.14 Statistical analysis**

Statistical analysis for our joint project with the ZAUM institute was carried out by Dr. Alexander Konstantinow. Further analysis was verified by Eszter Anna Janka. Each experiment was performed in technical duplicates and at least in biological triplicates. Statistical analysis was performed using the Graph Pad Prism and Excel softwares. Statistical significance was either determined using the Wilcoxon matched-pairs signed rank test or ANOVA for multiple comparisons. Additionally, Tukey post-hoc test was used in the analysis of ELISA data.

## **4. RESULTS**

### **4.1 Sebaceous gland rich skin is enriched in its dermal lipid content**

As a starting point for our studies, using Oil-Red-O staining, we clearly demonstrated that in samples from sebaceous gland rich areas the lipid staining had an increased intensity in the dermis compared to samples from regions that were poor in sebaceous glands. Interestingly, this increase was most prominent in the subepidermal part and around the sebaceous glands. These results suggest that sebocytes might have a primary role in supplying the dermis with lipids.

### **4.2 Sebum component lipids are able to penetrate through the epidermis and accumulate in the dermis**

Next, applying SQ, OA, PA, LA and STA individually onto skin grafts from sebaceous gland poor regions of the body, we used Raman spectroscopy to determine the extent of penetration and accumulation for each of the lipids.

Our results showed that all of the tested lipids could penetrate through the epidermis without disrupting its structure. While PA could be detected in high concentrations in the full depth of the dermis, SQ and LA accumulated in the upper region. Interestingly STA displayed a prominent enrichment in the intermediate dermal region, while OA was only present in moderate amounts however it showed a scattered distribution throughout the skin. These findings provide evidence that sebum lipids could have a far more complex physiological role than being only topical moisturizing agents.

### **4.3 Alternatively activated macrophages show characteristic distribution beneath the epidermis and around sebaceous glands**

Macrophages have been described in higher numbers beneath the epidermis and around sebaceous glands. In our studies we also confirmed these observations by comparing the number of CD163 positive dermal macrophages around the sebaceous glands, beneath the epidermis and in “neutral areas” in physiological conditions.

Importantly macrophages displayed a characteristic distribution, by lining up almost exclusively parallel with the basal cell layers of the sebaceous glands, indicating that their presence might not be a random occurrence.

In order to further characterize the accumulating macrophages, we used double label immunofluorescence staining for the marker combinations FXIII-A and CD206 or CD209. We found that all detected macrophages in healthy skin were expressing the markers of alternative activation (FXIII-A<sup>+</sup>/CD209<sup>+</sup>/CD206<sup>+</sup>). Notably, classically activated macrophages (CD163<sup>+</sup>/FXIII-A<sup>-</sup>) could be detected only under pathological conditions such as acne.

These results suggest that the local microenvironment might have a role in the accumulation and activation of macrophages around the sebaceous glands to which sebocytes could be potential contributors.

### **4.4 Th17 cells surround the pilosebaceous unit in acne lesions**

Staining of paraffin embedded skin sections of acne lesions also revealed CD4<sup>+</sup>IL-17<sup>+</sup> double-positive T cells accumulating in close proximity to the pilosebaceous unit not only in and acne samples, but also in healthy skin biopsies, indicating a potential crosstalk of Th17 cells and sebocytes not only during inflammation, but also in physiological conditions.

### **4.5 Sebocytes attract immune cells through CXCL-8 release**

The histological findings that macrophages and T cells are lined up around the sebaceous glands in the healthy pilosebaceous unit, called us to assess if sebocytes could actively attract these cells. We analysed supernatants derived from SZ95 sebocytes for the production of 27 cytokines, chemokines and growth factors by Bio-plex technology revealing a robust secretion of chemokines such as CXCL-8, CCL-2, CCL-5 and CXCL-10.

As these chemokines are important migration factors, we analysed the migratory capacity of monocytes, neutrophils and T cells towards the SZ95 sebocyte supernatant. By performing migration assays, we found that all three cell types showed a significant increase in their capacity to migrate towards the sebocyte supernatant in a time dependent manner. To confirm

the possible role of CXCL-8 in the migration, CXCL-8 was neutralized in the SZ95 sebocyte supernatant prior to the migration assay resulting in a restored migratory capacity.

Next, we tested if a proinflammatory environment alters the secretion of proteins as well as the subsequent migration of cells. Therefore, SZ95 sebocytes were pre-stimulated with IL-4, IFN- $\gamma$ , TNF- $\alpha$ , IL-17, LPS, LTA and *P. acnes*. Whereas all conditions lead to increased secretion of CXCL-8, CCL-5 and CXCL-10; IFN- $\gamma$ , IL-17, LPS and LTA were the predominant activators of sebocytes. Also, the migration of neutrophils, T cells as well as monocytes followed the course of induced chemokines.

These data provide evidence that resting sebocytes can attract immune cells *in vitro* in a CXCL-8 dependent manner and that this chemoattractant effect is further raised in a proinflammatory environment.

#### **4.6 SZ95 sebocytes promote alternative polarization of monocyte-derived macrophages**

In order to test a probable communication between sebocytes and macrophages we established an *in vitro* model, where peripheral blood monocytes were differentiated into macrophages in the presence of SZ95 sebocyte supernatant.

First, we characterized the effects of SZ95 sebocyte supernatant on the polarization of macrophages and measured the expression of the widely-accepted markers via Western blotting (FXIII-A) and flow cytometry (CD206 and CD209). An enhancement of the expression of all markers studied was detected when monocytes were differentiated in the presence of the SZ95 sebocyte supernatant. Importantly, when using lipid depleted SZ95 sebocyte supernatant, we found a prominent down-regulation of FXIII-A and an almost complete inhibition in the induced expression of CD206 and CD209 pointing to a possible participation of sebaceous lipids in macrophage differentiation.

Individually replacing the major components of the sebum such as OA, LA, PA, STA and SQ in the lipid-depleted SZ95 sebocyte supernatant used for macrophage culturing, we found LA and OA to be potent inducers of the measured markers of alternative macrophage activation, while SQ, STA and PA had no effect.

Despite the strong polarizing effect towards the alternative activation, the SZ95 sebocyte supernatant treated macrophages conserved their potential to differentiate into classical activation, suggesting that the polarizing effect can be overwritten in case of a danger signal.

#### **4.7 Sebocyte-derived lipids contribute to the potential of macrophages to uptake *P. acnes***

Alternatively activated macrophages have an enhanced potential for phagocytosis as one of their most important functional features. As *P. acnes* has been shown to contribute to the pathogenesis of acne, we next measured the uptake of FITC-labelled *P. acnes* by macrophages cultured with and without the SZ95 sebocyte supernatant. We found that in the presence of sebocyte-produced lipids macrophages gained an increased potential to uptake the bacteria. To address the possible lipids that may contribute to the enhanced phagocytosis, we selectively supplemented single lipids in the lipid-depleted SZ95 sebocyte supernatant and found LA and OA to be potent players in the increased uptake.

#### **4.8 Sebum component lipids modulate the macrophage activating potential of *P. acnes***

*P. acnes* is crucial in the pathogenesis of acne by inducing IL-1 $\beta$  production in macrophages, however, in *in vitro* settings, *P. acnes* itself only induces IL-1 $\beta$  production and secretion at a very moderate level suggesting that other factors are also required for its pathological role. Therefore, we first measured if supplementation with the SZ95 sebocyte supernatant could alter the IL-1 $\beta$  levels in *in vitro* *P. acnes*-treated macrophages. Western blot experiments revealed a prominent induction in the protein levels of IL-1 $\beta$  when *P. acnes* was added in combination with SZ95 sebocyte supernatant to the culturing medium. Interestingly, IL-1 $\beta$  production was further increased in the *P. acnes*-treated macrophages when lipids were depleted from the SZ95 sebocyte supernatant.

To address the contribution of the previously tested lipids to the IL-1 $\beta$  secretion in *P. acnes* activated macrophages, we extended our studies by selectively replacing the different lipids in the lipid-depleted SZ95 sebocyte supernatant. Our ELISA measurements revealed that the lipids had different effects on the IL-1 $\beta$  secretion. Besides confirming the potential of PA, STA, OA and SQ to induce IL-1 $\beta$ , we could also show that PA, OA and STA sensitized and further augmented the macrophage response to *P. acnes*. On the contrary, LA inhibited IL-1 $\beta$  production even in the presence of *P. acnes*.

IL-6 and TNF- $\alpha$  are other characteristic inflammatory cytokines produced by infected macrophages. Measurement of these cytokines in the supernatants showed that the pro-inflammatory effect of STA and PA and the anti-inflammatory effect of LA are not limited to IL-1 $\beta$  secretion, but could also affect TNF- $\alpha$  secretion in a similar manner. Interestingly, OA decreased the levels of both IL-6 and TNF- $\alpha$  but not IL-1 $\beta$ , suggesting that it may have a special



role among sebum lipids by selectively and differentially interacting with the different inflammatory signalling pathways.

These data show that the role of sebocytes in regulating macrophage function is not limited to altering their polarization, but can also be integrated into disease-specific settings, such as acne, where sebocytes may regulate inflammation via the secretion of its biologically active lipids.

#### **4.9 Sebocytes do not influence CD4<sup>+</sup>CD45RO<sup>+</sup> effector T cell cytokine secretion**

Since CD4<sup>+</sup>CD45RO<sup>+</sup> T cells were the largest subset attracted by sebocytes, we questioned whether their function is actively influenced by sebocytes. Therefore, human blood-derived CD4<sup>+</sup>CD45RO<sup>+</sup> cells were stimulated with plate-bound  $\alpha$ CD3 and soluble  $\alpha$ CD28 (T cell receptor stimulation) in the presence of SZ95 sebocyte supernatant or control medium for 72 hours. Here, no significant alteration in the secretion of IL-17, IFN- $\gamma$ , TNF- $\alpha$  and IL-4 could be detected, however, a significant induction of IL-22 secretion was detectable compared to control medium

As in this setting, T cell receptor (TCR) stimulation alone might not be sufficient for inducing alterations in cytokine secretion, we next investigated if sebocytes trigger functional changes in T cell activation *via* DCs. Therefore, CD14<sup>+</sup> monocytes were differentiated into DCs in presence of SZ95 sebocyte supernatant or control medium and stimulated with LPS prior to co-culture with allogenic CD4<sup>+</sup>CD45RO<sup>+</sup> cells (mixed leukocyte reaction). SZ95 supernatant did not have an impact on DC maturation or T cell activation, suggesting that human sebocytes do not affect memory T cell cytokine secretion neither directly nor mediated by DCs.

#### **4.10 Sebocytes trigger a Th17 immune response**

As naïve T cells were also attracted in small numbers by sebocytes, we investigated the influence of sebocytes on T cell differentiation. For this purpose, CD4<sup>+</sup>CD45RA<sup>+</sup> T cells were stimulated with  $\alpha$ CD3/ $\alpha$ CD28 in presence of the SZ95 sebocyte supernatant or control medium and the secretion of effector cytokines was assessed by ELISA. Whereas SZ95 sebocyte supernatant did not induce a Th1 or Th2 immune response, as no significant IFN- $\gamma$ , TNF- $\alpha$  and IL-4 release was detected in the supernatant of differentiated T cells, a significantly higher production of IL-22 and IL-17 was detected after 6 days in culture. In line with that, also DCs that were generated in presence of SZ95 sebocyte supernatant were able to drive naïve T cell polarization towards the Th17 phenotype with significant increased expression of IL-17 and IL-22. Interestingly, IL-17 and IL-22 cytokine levels were even higher compared to the solely

stimulation with  $\alpha$ CD3/ $\alpha$ CD28 whereas levels of IFN- $\gamma$  and TNF- $\alpha$  were significantly reduced in this set-up.

Thus, our *in vitro* data imply that human sebocytes have the capacity to skew immune responses towards a Th17 profile.

#### **4.11 Sebocytes induce Th17 differentiation via secretion of IL-1 $\beta$**

To address the contribution of lipids or proteins to the observed effect on Th17 differentiation, we incubated the SZ95 sebocyte supernatant with proteinase K. CD4<sup>+</sup>CD45RA<sup>+</sup> naïve T cells were stimulated with  $\alpha$ CD3/ $\alpha$ CD28 and cultured in presence of the protein-digested SZ95 sebocyte supernatant resulting in a reduction of IL-17 secretion in absence of the whole protein fraction. This suggests that the sebocyte effect on naïve T cell polarization is primarily protein-mediated.

As in steady-state SZ95 sebocytes secrete cytokines that are known to contribute to Th17 polarization, such as IL-1 $\beta$  and IL-6, we next neutralized these cytokines in SZ95 supernatant and performed a subsequent naïve T cell differentiation. Neutralization of IL-1 $\beta$ , lead to a 35% decrease of IL-17 secretion in differentiated T cells, whereas neutralization of TGF- $\beta$  and IL-6 alone only had marginal effects. Conversely, the depletion of all three cytokines simultaneously abrogated IL-17 production by 44%. Therefore, it is likely that sebocytes drive a Th17 immune response via the production of IL-6, TGF- $\beta$ , and largely IL-1 $\beta$ .

#### **4.12 *P. acnes* does not influence immune cell recruitment, but affects priming capacity of DCs**

To explore whether *P. acnes* synergistically acts with sebocytes to reinforce the local symbiosis and/or immune response, SZ95 sebocytes were pre-incubated for 24h with *P. acnes* 889 sonicate, extensively washed to remove bacteria and further incubated for 24h prior to supernatant collection. In migration assays, *P. acnes* did not alter the chemoattractant potential of sebocytes as compared to SZ95 supernatant, as migration of neutrophils, monocytes or lymphocytes was not altered when sebocytes were pre-incubated with *P. acnes*.

However, when DCs were generated in the presence of *P. acnes* pre-stimulated SZ95 supernatant and subsequently used for differentiation of allogeneic CD4<sup>+</sup>CD45RA<sup>+</sup> naïve T cells, these T cells showed a slight, however not significant, increase in IL-17 and IL-22 production. Interestingly, DCs matured with the SZ95 sebocyte supernatant (both unstimulated or pre-stimulated with *P. acnes*) induced a significant reduction of the Th1 cytokine IFN- $\gamma$  ( $p=0.03$ ), whereas TNF- $\alpha$  and IL-4 levels were not significantly altered.

These data indicate that sebocytes induce Th17 polarization, and *P. acnes* indirectly contributes to this phenomenon by inhibiting Th1 differentiation.

## 5. DISCUSSION

The enrichment of macrophages around the sebaceous glands in healthy dermal skin has been mentioned in previous publications, however these findings have been left at the level of observations with no conclusions or discussion. Moreover, a possible involvement of sebocytes under pathological conditions is also supported by findings in diseases such as acne, which is characterized by the extensive infiltration of the sebaceous glands by immune cells, such as Th17 cells, suggesting that sebocytes indeed could have complex roles in inflammation. A possible explanation for the lack of interpretation of these findings is most likely due to the relative ignorance, which considered SGs to be atavistic remnants of the skin with only one remaining function: sebum production that contributes to the triggering of acne. The studies of the last decade, however, highlighted that sebaceous lipid metabolism is under a complex regulation that can be altered by different inflammatory stimuli. Moreover, upon these stimuli sebocytes are also capable of producing various cytokines and chemokines making sebocytes not only an interesting model to study, but suggesting other potential (patho)physiological roles such as the link between lipid metabolism and inflammation at a cellular level. These features largely resemble adipocytes, which serve the best example for a cell type with a primary function to metabolize lipids, but also having immunomodulatory effects.

The concept of immunologically competent skin cells with no myeloid or lymphoid origin has been a challenging issue in skin biology. After decades of research keratinocytes, primarily via their cytokine production, are now accepted as immune regulators that are important in the pathogenesis of various inflammatory diseases, such as psoriasis and AD. In contrast, sebaceous glands are still widely considered to contribute only to the lipid barrier of the skin by producing the lipid rich sebum. However, as we show in this work, there is an impressive difference already in the dermal lipid content between sebaceous gland rich and poor skin.

The key question whether sebum lipids could also contribute to the dermal lipid content, was first assessed by Butcher in the 1950s, who demonstrated that the sebum component lipids, STA (radioactive) OA and LA (with an increased fluorescence intensity) absorbed readily through rat skin with a notable accumulation around the sebaceous gland in the case of STA, while LA passed even to the blood vessels. In this study, we extended and completed the

characterization on the penetration of sebum component lipids by using Raman spectroscopy and showed that all of the examined lipids (STA, LA, OA, PA and SQ) were able to make their way through the epidermis and accumulated in the dermis to various degrees. While Butcher proposed that lipids are mostly penetrating through the pilosebaceous duct, our results support that secreted sebum lipids from the skin surface could also penetrate and contribute to the dermal lipid milieu.

Besides cytokines and pathogens, lipids also have a key role in the differentiation and activation of various immune cells, therefore, in our studies we aimed to challenge the interaction of sebocytes with macrophages and lymphocytes, the key immune cells in maintaining the immune milieu of the healthy skin and also in initiating various diseases.

In order to provide explanation for the previously described findings of macrophage accumulation around sebaceous glands, we relied on currently available histological methods and markers to distinguish different subsets of macrophages. Depending on tissue environment macrophages can differentiate towards the alternative pathway, in which primarily IL-4/IL-13, but also different lipids might act as inducers. This pathway, best represented by “skin resident macrophages” which express all the characteristic markers such as CD206, CD209 or FXIII-A, is central in the maintenance of the tissue environment by producing extracellular matrix components and contributing to tissue remodelling. On the other end of the spectrum is the classical activation, where IFN- $\gamma$  and TNF- $\alpha$  are the key cytokines. In this work, we characterized the macrophages in the vicinity of the sebaceous glands according to this classification and found that under physiological conditions only alternatively activated macrophages could be detected, while classically activated ones appeared under pathological conditions such as acne. Integrating our findings into the current knowledge regarding macrophage activation pathways, we suggest that under normal conditions the exclusive presence of alternatively activated macrophages may be a sign and consequence of a continuous “non-danger signal” contributing to the homeostasis of the skin, of which sebocytes are a potential source.

In steady-state, SZ95 sebocytes release several chemokines and cytokines. This is line with previously published reports highlighting the *in situ* production of e.g. CXCL-8, IL-6 and IL-1 $\beta$  by sebocytes in the sebaceous gland and underlining the importance of SZ95 sebocytes as *in vitro* model for sebocyte research. Among the steady-state chemokines, CXCL-8 has a key role in recruitment of immune cells such as monocytes and neutrophils to sites of skin inflammation. Although previous reports suggested that neutrophils are the first immune cells in acne lesions, some studies revealed that, along with macrophages, also T lymphocytes

infiltrate sites of evolving inflammatory lesions. Considering the fact that sebocytes undergo holocrine secretion, we used cell free supernatant of SZ95 sebocytes in migration assays and indeed, found that, in a CXCL-8-mediated fashion, sebocytes recruit monocytes, neutrophils and different subsets of T cells such as CD4<sup>+</sup>CD45RO<sup>+</sup> effector, but also CD4<sup>+</sup>CD45RA<sup>+</sup> naïve T cells to the skin. However, during inflammatory responses sebocytes become further activated by proinflammatory cytokines and/or bacterial products leading to enhanced secretion of chemokines and cytokines. In line with that, pre-stimulated SZ95 sebocytes showed an increased chemoattractant potential on immune cells *in vitro* that was mainly following the concentration of CXCL-8 and is reflected *in vivo* by high numbers of immune cells surrounding the sebaceous gland in acne lesions.

These findings strongly suggest that the regulated expression of CXCL-8 by sebocytes could be pivotal in initiating and accelerating the accumulation of immune cells around the sebaceous glands.

To further investigate the probable interaction, we approached the possible effects of sebocytes on macrophages from different aspects such as regulation of the expression of macrophage markers related to the differentiation pathways, phagocytosis and cytokine production of *P. acnes* infected macrophages.

In support of our histological findings we have proved that treatment with SZ95 sebocyte supernatant resulted in the upregulation of all the widely-accepted markers for the alternative macrophage activation pathway (CD206, CD209 and FXIII-A). This suggests that sebocytes can, indeed, affect the polarization of macrophages favouring the alternative pathway and may also provide an answer for the phenomenon of macrophages expressing these detected markers in conditions where IL-4/IL-13 is not involved. In addition, the supernatant-treated macrophages *in vitro* also exerted an increased capacity for phagocytosis of *P. acnes*, a previously defined hallmark of CD209<sup>+</sup> macrophages, suggesting that sebocytes might not only affect marker profiles but also macrophage function. These results support that sebocyte derived lipids could be potent contributors to the homeostasis of the skin which is in line with recent findings, that sebaceous gland rich healthy skin has a distinct non-inflammatory immune surveillance.

Based on the findings, that the lipid composition of sebum is not a steady-state condition but can be changed, as it was observed both in *in vitro* sebocytes in response to various stimuli as well as in the sebum of acne patients where the variations in the quantity and mostly the quality of sebum lipids could significantly affect inflammation by targeting both keratinocytes and sebocytes, we extended our experiments also with *P. acnes*-treated macrophages. Our results

provided convincing data that macrophages could also be possible targets for the altered sebum production in acne. With dissecting the role of sebocyte-produced lipids in regulating the secretion of IL-1 $\beta$  and other inflammatory mediators, such as IL-6 and TNF- $\alpha$  by *P. acnes*-infected macrophages, we found that each of the major lipid components had a well-defined inflammatory property, that has been overlooked so far perhaps not just in acne, but most likely in the pathogenesis of other dermatological diseases as well. Interestingly, PA and STA, which are both major sebum components, were proven to be potent stimulators of inflammatory cytokine production in macrophages themselves even without the presence of *P. acnes*. Moreover, our results that certain lipids, such as LA and OA, had a significant effect on sensitizing macrophage response towards *P. acnes* even when the bacteria only had a minor effect on the secretion of IL-1 $\beta$  itself, addresses and (partially) explains another intriguing question, namely, how an otherwise commensal bacterium *P. acnes*, could still be a potent macrophage activator in diseases such as acne. Our results shed light on the essential role of the sebum lipids in this process. With ratio alterations, lipid fractions could determine the extent of macrophage' response to *P. acnes* and most likely to other pathogens as well. Keeping the limits of our study in mind, we also provide data supporting the biological relevance for the significant decrease in the levels of LA and thus the increased ratio of OA/LA in the sebum of acne patients and suggest that LA may be a key player, not just in comedo formation, but also in the impaired symbiosis between pathogens and macrophages with a primarily anti-inflammatory effect, which is further affected by the changing levels of the potent and selective IL-1 $\beta$  inducer OA. These results altogether fully corroborate that sebaceous lipogenesis does not only contribute to the accumulation of sebum but is also an instrument for several coordinated actions, including the communication with macrophages.

Based on these results we can conclude that sebocytes, similarly to keratinocytes, may contribute to the inflammatory milieu and act as a guard at a "locus minoris resistentiae" within the dermis, especially for the pilosebaceous unit, where the lipids, cytokines and resident bacteria form a unique symbiotic environment that has to be conserved and balanced. Moreover, the analysis of sebum lipid fractions should also be addressed from the scope of their potential immunoregulatory functions, that may give new explanations for the altered lipid profile in sebum of various pathological conditions such as acne and rosacea.

In the second part of this study, we provide evidence for a functional communication between sebocytes and T cells as well, resulting in the induction of a Th17-dominated immune response. On the other hand, we demonstrate an indirect contribution of *P. acnes* via sebocytes and DCs towards acne-associated inflammation.

Due to the important role of T cells in the inflammatory tissue response, we investigated whether sebaceous glands influence T cell differentiation similarly to that observed in macrophages. We could demonstrate that factors released by sebocytes do not alter cytokine secretion of CD4<sup>+</sup>CD45RO<sup>+</sup> effector T cells indicating that sebocytes do not impact on previously determined T cell phenotype. One exception is a slight increase in IL-22 production implying that sebocytes ensure barrier homeostasis by fostering the IL-22/TNF- $\alpha$  axis.

Unlike effector cells, we found that sebocytes impact on the differentiation of CD4<sup>+</sup>CD45RA<sup>+</sup> naïve T cells. As sebocyte secreted various cytokines, and most importantly IL-6 and IL-1 $\beta$ , that represent the key cytokines for *de-novo* differentiation of Th17 cells, we could show that sebocyte supernatants alone are capable to fully induce the Th17 phenotype in naïve T cells and that this interaction is mainly dependent on IL-1 $\beta$  production. As T cell priming does not take place in peripheral tissues, we assume that sebocytes contribute to the generation of a local micro milieu that skews differentiation of naïve T cells towards the Th17 phenotype in skin draining lymph nodes.

The Th17 population bridges innate and adaptive immunity and has a key role in mediating host defence. Alone or in synergy, the Th17 effector cytokines IL-17 and IL-22 induce an array of antimicrobial peptides to produce a robust antimicrobial response. However, Th17 cells can also induce pathological inflammation and are associated with several inflammatory skin conditions such as psoriasis, atopic eczema and allergic contact dermatitis. Moreover, a role for Th17 cells in acne pathogenesis has recently been described, as an enhanced expression of Th17 associated cytokines and differentiation factors were found in lesional skin. In line with our data, Agak *et al.* showed that Th17 cells are present in the perifollicular infiltrate of comedones. However, and in contrast to our findings, the authors hypothesized that the Th17 immune response is mainly regulated by *P. acnes*. Similarly, a recent study showed that *P. acnes* induces a Th1/Th17 response even though acne pathogenesis has been associated with a Th1 type immunity. Our data indicate that sebocytes induce neither a Th1 nor a Th2 cell differentiation, but skew the immune response towards a Th17 profile that is further enhanced by the presence of *P. acnes*.

Furthermore, it has been reported that *P. acnes* efficiently induces IL-1 $\beta$  secretion in sebocytes by activating the NLRP3 inflammasome. We also could detect an increase in IL-1 $\beta$  levels when sebocytes were pre-treated with *P. acnes*, however, we did not find a further increase in Th17 differentiation arguing for a quite high intrinsic production of IL-1 $\beta$  that is totally sufficient for Th17 priming, even in a resting state. Our data argues for a steady-state induction of Th17 cells by sebocytes to maintain skin homeostasis. However, when the pilosebaceous unit is colonized

with *P. acnes* under pathological conditions the Th17 response is further enhanced *in vivo*. In line with this, we could detect CD4<sup>+</sup>IL-17<sup>+</sup> cells only sparsely around sebaceous glands in healthy individuals, whereas these cells were frequently co-localized with sebocytes in acne lesions.

Several *in vitro* studies show that *P. acnes* whole cells or cell fractions stimulate cytokine release from immune cells, keratinocytes and sebocytes through binding to TLR2. However, the mechanism by which *P. acnes* exerts its activity *in vivo* is still unknown. *P. acnes* can reside in the deeper portions of sebaceous follicles, but rarely in the sebaceous gland. When this commensal bacterium proliferates, it can come into contact with DCs and activate their maturation with the consequent immune response crucially depending on presence of local commensals or pathogens, biofilm production and additional signals from tissue cells. It has been reported that DCs stimulated with *P. acnes* show an increased expression of adhesive molecules and cytokines, which is similar to DCs activated by LPS. In presence of naïve T cells, *P. acnes*-matured DCs induced a strong secretion of IFN- $\gamma$  that is comparable to LPS-matured DCs confirming the capacity of *P. acnes* in eliciting a powerful Th1-type immune response. However, presence of sebocyte supernatant reduced the capability to induce Th1 responses and instead drove the symbiotic and/or immune response to *P. acnes* specifically towards a Th17 commitment. Taken together, we assume that sebocytes intend to maintain the epithelial barrier by i) by homeostatic priming of Th17 cells, ii) initiation of effective inflammatory responses and iii) reduction of pathogenic IFN- $\gamma$  production to reach homeostasis after inflammation.

Our study provides evidence that sebocytes actively participate in inflammatory processes in the skin via recruitment of immune cells and a functional cross-talk with T cells leading to a pronounced Th17 differentiation. This interaction might be of importance for the pathogenesis of acne vulgaris; however, further studies have to clarify whether the sebocyte-Th17 axis contributes to a beneficial host defence or the perpetuation of a vicious circle of inflammation. In order to elucidate the conditions and the stimuli that could drive sebocytes to harmonize and balance their production of inflammatory proteins and lipids as well as the identification and characterization of additional lipids that have an immune modulatory role further studies are required. Our findings therefore set the basis and open several new avenues for research of scientific and of therapeutic interest, which can lead to the identification of new players of protein and lipid nature in the inflammatory stimuli/sebocyte/immune cell axis that could be involved in various skin diseases.



## 6. SUMMARY

Sebaceous glands and hair follicles together form the pilosebaceous unit, which primary function is the production of sebum. Sebaceous glands have already been attributed to have a role in inflammatory processes via pattern recognition receptors such as TLR2, TLR4, and TLR6. Furthermore, an altered lipid composition and increased sebum production is a hallmark in the development of acne. Despite these findings, up until recent years, sebaceous glands have been only considered as passive players in inflammation. In this work we challenged this dogma and addressed sebocytes as potential immunomodulators.

Based on our Raman spectroscopy findings we have showed that sebum can penetrate through the epidermis and accumulate in the dermal region of the skin, suggesting a more complex role for sebocytes than only contributing to the lipid barrier of the skin. Utilizing various immunohistochemical techniques we displayed that both macrophages and Th17 cells surround the pilosebaceous unit both in physiological and pathological conditions.

On the basis of these observations we have set up an *in vitro* model, where we used SZ95 sebocyte supernatant to culture either monocytes, DCs or T cells. Using migration assays, we have demonstrated that by CXCL-8 production sebocytes attracted all of the above-mentioned cell types via chemotaxis. Furthermore, we proved that sebum component lipids, primarily LA and OA promoted the alternative activation of monocyte derived macrophages and contributed to the potential of macrophages to uptake *P. acnes*. Moreover, sebum component lipids selectively and differentially altered the macrophage activating potential of *P. acnes*.

When investigating a potential sebocyte – T cell interaction, we have determined that sebocytes trigger a Th17 immune response via the secretion of IL-1 $\beta$ .

Finally, we have demonstrated that *P. acnes* does not influence immune cell recruitment, but affects the priming capacity of DCs.

These results therefore suggest that the proteins (chemokines, cytokines) and even more importantly the sebum component lipids produced by sebocytes, both in amount and in their ratios, should be integrated into our thinking. Further research is required, both in understanding sebocyte function and lipidomics, to provide useful data for therapeutic advances in sebaceous gland-related skin diseases. Therefore, placing (sebum) lipids in the centre of research provides a vast and intriguing field for further studies to come.

## 7. PUBLICATIONS



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Registry number: DEENK/13/2018.PL  
Subject: PhD Publikációs Lista

Candidate: Marianna Lovászi  
Neptun ID: JOW8FK  
Doctoral School: Doctoral School of Health Sciences  
MTMT ID: 10053302

### List of publications related to the dissertation

1. \*Mattii, M., \***Lovászi, M.**, Garzorz, N., Atenhan, A., Quaranta, M., Lauffer, F., Konstantinow, A., Küpper, M., Zouboulis, C. C., Kemény, L., Eyerich, K., Schmidt-Weber, C. B., Törőcsik, D., Eyerich, S.: Sebocytes contribute to skin inflammation by promoting the differentiation of Th17 cells.  
Br. J. Dermatol. [Epub ahead of print], 2017.  
DOI: <http://dx.doi.org/10.1111/bjd.15879>  
IF: 4.706 (2016)
2. **Lovászi, M.**, Mattii, M., Eyerich, K., Gácsi, A., Csányi, E., Kovács, D., Rühl, R., Szegedi, A., Kemény, L. V., Stähle, M., Zouboulis, C. C., Eyerich, S., Törőcsik, D.: Sebum lipids influence macrophage polarization and activation.  
Br. J. Dermatol. 177 (6), 1671-1682, 2017.  
DOI: <http://dx.doi.org/10.1111/bjd.15754>  
IF: 4.706 (2016)



\*These authors contributed equally to this work.



List of other publications

3. Szentkereszty-Kovács, Z., **Lovászi, M.**, Zatik, Z., Duró, E., Kovács, D., Törőcsik, D.: Perspektívák a faggyúmirigy kutatásban.  
*Bőrgyógyász. Venerol. Szle.* 93 (3), 126-135, 2017.  
DOI: <http://dx.doi.org/10.7188/bvsz.2017.93.3.7>
  
4. **Lovászi, M.**, Szegedi, A., Zouboulis, C. C., Törőcsik, D.: Sebaceous-immunobiology is orchestrated by sebum lipids.  
*Dermatoendocrinol.* 2017, 1-11, 2017.  
DOI: <http://dx.doi.org/10.1080/19381980.2017.1375636>
  
5. Kovács, D., **Lovászi, M.**, Póliska, S., Oláh, A., Bíró, T., Veres, I., Zouboulis, C. C., Stähle, M., Rühl, R., Remenyik, É., Törőcsik, D.: Sebocytes differentially express and secrete adipokines.  
*Exp. Dermatol.* 25 (3), 194-199, 2016.  
DOI: <http://dx.doi.org/10.1111/exd.12879>.  
IF: 2.679
  
6. Törőcsik, D., Kovács, D., Camera, E., **Lovászi, M.**, Cseri, K., Nagy, G. G., Molinaro, R., Rühl, R., Tax, G., Szabó, K., Picardo, M., Kemény, L., Zouboulis, C. C., Remenyik, É.: Leptin promotes proinflammatory lipid profile and induces inflammatory pathways in human SZ95 sebocytes.  
*Br. J. Dermatol.* 171 (6), 1326-35, 2014.  
IF: 4.275

Total IF of journals (all publications): 16,366

Total IF of journals (publications related to the dissertation): 9,412

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

12 January, 2018



## 8. ACKNOWLEDGEMENTS

First of all, I would like to thank my PhD supervisor, Dr. Dániel Törőcsik for his patience, help and immense scientific guidance over these past five years.

I would like to thank Dr. Stefanie Eyerich and Dr. Martina Mattii for their immense efforts on behalf of our joint projects, as well as their colleagues at the ZAUM center especially Professor Kilian Eyerich, Dr. Natalie Garzorz-Stark, Anne Atenhan and Jana Sängler.

I am grateful to Dr. Dóra Kovács, without her help and encouragement, this work could not have been realized.

I would like to express my gratitude towards our collaborational partners from Szeged, Dr. Erzsébet Csányi and Dr. Attila Gácsi for their help and expertise in completing this work.

Special thanks to Professor Christos C. Zouboulis and Professor Lajos Kemény for their scientific knowledge and constructive suggestions.

For their outstanding technical support, I would like to thank Tünde Toka-Farkas, Mrs. Ágnes Bana Sándor, Erzsébet Vágóczki and Mrs. Ildikó Sandra Csapó.

I owe thanks to Professor Éva Remenyik for providing me with the opportunity to work in the Department of Dermatology.

I would like to express my gratitude towards all of my colleagues at the Department of Dermatology for providing a positive working atmosphere during these years.

Finally, I would like to thank my family and friends for their love, support and patience and for always standing by me.

This work was supported by the project of TÁMOP 4.2.2.A-11/1/KONV-2012-0031, the Hungarian National Research Fund (OTKA NN117020), the New National Excellence Program of the Ministry of Human Capacities, the Marie Skłodowska-Curie actions (627547 RNAsebgland FP7-PEOPLE-2013-IEF), the Fondation Acteria and the Helmholtz Association. The work is also supported by the GINOP-2.3.2-15-2016-00005 project.