DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of innate and adaptive immune components in psoriasis

by

Ahmad Mohammad Khaleel Khasawneh

UNIVERSITY OF DEBRECEN
GYULA PETRÁNYI DOCTORAL SCHOOL OF ALLERGY AND CLINICAL IMMUNOLOGY

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Table of content

1. Introduction 6
2. Background 7
   2.1. The anatomy of the human skin 7
   2.2. Barrier function of the skin 8
      2.2.1. The physico-chemical barrier of the skin 8
      2.2.2. The immunological barrier of the skin 9
         2.2.2.1. KCs in the skin 9
         2.2.2.2. DCs in the skin 10
         2.2.2.3. T cells in the skin 12
   2.3. Immunological differences in the the topographically different healthy skin regions 14
   2.4. Psoriasis 15
      2.4.1. Classification of psoriasis 16
      2.4.2. Pathogenesis of psoriasis 21
      2.4.3. The therapy of psoriasis 26
3. Objectives 27
4. Patients and methods 28
   4.1. Patients 28
      4.1.1. Patients and healthy controls for cellular investigations (Study I.) 28
      4.1.2. Patients for skin biopsies (Study II) 28
   4.2. Cellular investigations (Study I.) 29
      4.2.1. Cell isolation and cell culturing 29
      4.2.2. Cell surface marker and intracytoplasmic cytokine staining 30
      4.2.2. Flow cytometry 31
      4.2.3. Chemokine investigations by ELISA 31
   4.3. Investigations on skin biopsies (Study II.) 31
      4.3.1. Immunohistochemistry 31
      4.3.2. RNA Isolation, Reverse Transcription and Real-Time Quantitative PCR 33
   4.4. Statistical analysis 34
5. Results 35
   5.1. Examination of psoriatic blood $CD1c^+$ mDCs and pDCs focusing on their activation and maturation stages, their cytokine and chemokine release (Study I.) 35
      5.1.1. Activation/maturation state of psoriatic $CD1c^+$ mDCs 35
      5.1.2. Cytokine production of psoriatic $CD1c^+$ mDCs 36
      5.1.3. Characteristics of psoriatic blood pDCs 37
      5.1.4. Chemokine production of psoriatic blood $CD1c^+$ mDCs and pDCs 37

5.1.4.1. Investigation of the intracytoplasmic chemokine production by flow-cytometry 37
5.1.4.2. Investigation of the chemokine secretion by ELISA 38
5.2. Comparison of the immune and barrier characteristics in psoriasis vulgaris on SGP skin and scalp psoriasis (Study II.) 40
5.2.1. T cells and DCs in psoriasis vulgaris on SGP skin and scalp psoriasis 40
5.2.2. Investigation of Th1 and Th17 cytokines and Th17 related chemokines in psoriasis vulgaris on SGP skin and scalp psoriasis 42
5.2.3. Th17 related AMPs and barrier molecules in psoriasis vulgaris on SGP skin and scalp psoriasis 45
6. Discussion 47
7. Summary 53
8. References 54
9. Keywords 64
10. Acknowledgements 65
11. Appendix 66

Abbreviations

AD	Atopic Dermatitis
AGR	Apocrine Gland-rich
AMP	Antimicrobial Peptides
BSA	Bovine Serum Albumin
CTLs	Cytotoxic T Lymphocytes
DCs	Dendritic Cells
dDCs	Dermal Dentritic Cells
DLQI	Dermatology Life Quality Index
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
FLG	Filaggrin
FSC	Forward Light Scattering
GP	Gland Poor
HS	Hidradenitis Suppurativa
IHC	Immunohistochemistry
inf DCs	Inflammatory DCs
IDEC	Inflammatory Dendritic Epidermal Cell
KC	Keratinocytes
KRT17	Keratin 17
LCs	Langerhans Cells
LCN2	Lipocalin
LOR	Loricrin
Mac	Macrophage
mDCs	Myeloid DCs
mo-Mac	Monocyte-derived Macrophage
mono	Monocytes
NKT	Natural Killer T cells
NLRs	Nod-like Receptors
PASI	Psoriasis Area Severity Index
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate-buffered Saline
pDCs	Plasmacytoid DCs

PPIA	Peptidylprolyl Isomerase A
preDCs	Precursor DCs
pTreg	Peripherally Induced regulatory T cell
SB	Stratum Basale
SC	Stratum Corneum
SD	Standard Deviation
SG	Stratum Granulosum
SGP	Sebaceous Gland-Poor
SGR	Sebaceous Gland-Rich
SS	Stratum Spinosum
SSC	Side Light Scattering
Tc	Cytotoxic T Cells
Tcm	Central Memory T cells
Tem	Effector Memory T cells
Th1	Type 1 Helper T Cells
TIP-DC	TNF-α, iNOS Producing Inflammatory Dermal Dendritic Cells
TJ	Tight Junctions
TLRs	Toll-like Receptors
Trm	Tissue-resident Memory T
tTreg	Thymus-derived regulatory T cell

1. INTRODUCTION

Psoriasis is a very common inflammatory, immune-mediated skin disease, affecting millions of people worldwide. Both innate and adaptive immunity play a role in its pathogenesis, since psoriasis is a Th1/Th17-related disease and the role of dendritic cells (DCs) in the initiation and maintenance of the disease is also well established. Among DCs plasmacytoid DCs (pDCs), activated by LL-37/self-DNA complexes, have been identified as the key cell type in the initiation phase of inflammation, which cells, due to their high IFNα production, in turn induces the maturation of myeloid DCs (mDCs). The number of mDCs in the dermis of psoriatic skin lesions is found to be significantly increased with these cells being responsible for further amplification and sustenance of the T cell-mediated inflammatory processes. Although DCs in the psoriatic skin are well characterised, less is known about peripheral blood DCs which can serve as precursors of skin DCs according to the literature. Therefore, the aim in the first study of my PhD work was to explore the characteristics of circulating DCs, to investigate the phenotype as well as the function (the cytokine and chemokine production) of $CD1c^+$ mDCs and pDCs in the blood of psoriatic patients.

In the second part of my research we investigated DCs in patients' skin, supplemented by examining further components of the innate and adaptive immune systems. Psoriasis can be notably heterogenous and can involve all skin regions (scalp, folds, palmo-plantar areas and body skin). The skin areas on which the different forms develop [psoriasis vulgaris occurs on sebaceous gland poor (SGP) skin, while scalp psoriasis presents on sebaceous gland rich (SGR) regions], have different anatomical features and bear distinct microbiota and chemical milieu, moreover, in recent years distinct immune and barrier characteristics have also been described in SGR and SGP skin areas. SGR regions are characterised by a significantly higher T cell and (non-activated) DC count and a non-inflammatory IL-17/IL-10 citokine milieu together with the presence of Th17 related chemokines, antimicrobial petides. Therefore, in our second study, we compared the immune and barrier features of psoriasis vulgaris (on SGP areas) and scalp psoriasis (on SGR areas) to determine if the basic immune milieu of healthy skin influences the immune characteristics and, consequently, the treatment of psoriasis on SGP and SGR areas. We investigated the immune cell counts and the expression of Th1/Th17 cytokines, Th17-related chemokines, antimicrobial peptides, and barrier molecules in the two psoriatic groups.

2. BACKGROUND

2.1. The anatomy of the human skin

The skin is the largest organ of our body, in which 3 main layers can be distinguished regarding its anatomy: the epidermis, dermis and hypodermis (subcutis) (Figure 1.). Epidermis is the outermost layer which provides a waterproof barrier. It consists of four layers, the lowermost is the *stratum basale* (SB), which is renewing continuously. Basal keratinocytes (KC) and continuously dividing cells built up the single cell-line of this layer. Above the SB the *stratum spinosum* (SS) can be found. The polygonal KC-s of this layer undergo differentiation and maturation processes. Keratin synthesis is already taking place in this layer. The next layer is the *stratum granulosum* (SG), where the cells contain dark nodules, called keratohialin granules. These cells actively produce keratin proteins and lipid molecules. The outermost cell layer of the epidermis is the *stratum corneum* (SC), which is made up of dead KCs devoid of organelles. In some areas of the skin, a fifth layer, the stratum lucidum, can be detected. In addition to KCs, other cells are also present in the epithelium, including epidermal melanocytes responsible for melanin production, a subset of DCs called Langerhans cells (LCs), Merkel cells and CD8+ cytotoxic T cells (Tc) in the SB and SS layers (1, 2). The SC together with the tight junctions (TJ), as the cell-connecting elements of the SG layer are primarily responsible for the skin's physico-chemical barrier function.

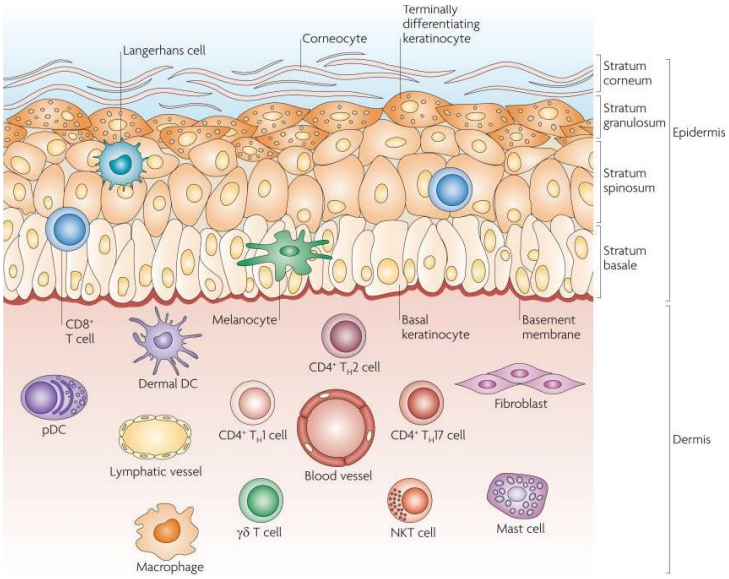

Figure 1. The anatomy of the skin, and its main cell types
Source: Nestle, F.O., et al., Skin immune sentinels in health and disease., Nat. Rev. Immunol., 2009. 9(10):679-91. (1)

Beneath the epidermis the fibrous connective tissue layer of the dermis can be found, composed mainly of connective tissue fibres (paralell bundles of collagen fibers), extracellular matrix molecules and the fibroblasts that produce them. The collagen and elastic fibers of the dermis ensure the elasticity of the skin. The capillary and lymphatic vessels in the dermis supply the cells of the dermis and epidermis with oxygen and nutrients and serve as entry and exit points for immune cells (3). The dermis shows high cell diversity. This layer contains more professional immune cells than the epidermis, mainly different populations of DCs such as dermal DCs (dDCs), and plasmacytoid DCs (pDCs), CD4+ type 1 helper T cells (Th1), Th2, Th17, Th22, γδT cells, natural killer T cells (NKT), macrophages, mast cells and fibroblasts (1, 3, 4). The hypodermis is the deepest layer of the skin, which consists of fatty tissue and connective tissue. Fat cells in the subcutis help to insulate the body from the outside world and protect the body from heat loss (3).

2.2. Barrier function of the skin

Our skin barrier, together with the mucosal membranes, is considered to be the first line of protection, it defends our body from the harmful effects of the external environment: from pathogens and from physical and chemical damages. It actively fulfils its role through different cells, humoral mediators, and enzymes. Skin barrier consists of two major barrier systems: the physico-chemical and the immunological barriers (5, 6), which barriers cannot be separated from each other, they function together to maintain the homeostasis. In the skin, a variety of cells perform innate immune functions: besides KCs, which is part of both the physical and the immunological barrier, the skin contains numerous innate and adaptive immune cells as mentioned above (e.g. DCs, macrophages, T cells) (4, 7).

2.2.1. The physico-chemical barrier of the skin

The KCs are differentiated in an ordered manner while moving from SB to corneal layer. The physico-chemical barrier consists of the upper cell layer of SG, and the 2-3 profound cell layers of SC (8). This barrier consists of cells (KCs, corneocytes), intracellular structural proteins (filaggrin, involucrin, loricrin, keratins etc.) and extracellular materials (intercellular lipids, proteases, protease inhibitors) (9). The brick-mortar hypothesis may describe its structure accurately, as the bricks are represented by the cells, and the intercellular lipids are considered as mortar (10).

2.2.2. The immunological barrier of the skin

Our skin, to preserve the body's integrity, exerts not only physico-chemical, but also immunological functions. The main resident immune cells of the normal human skin are the KCs, DCs and T cells.

2.2.2.1. KCs in the skin

KCs considered as sentinels of the skin, since they can sense the skin microbiota and its microenvironment (sebum, eccrine and apocrine gland secretion, etc.) via their cell surface and intracytoplasmatic receptors. They reside in the epidermis playing a crucial role in innate immunity. The microbial elements originated from pathogens are mainly sensed by Toll-like receptors (TLRs), while other danger molecules are recognized by Nod-like receptors (NLRs) or other sensors of these cells. Their receptor-ligand binding induces the activation of signaling pathways and the production of special mediators. KCs are sources of different cytokines, chemokines, colony-stimulating factors, growth factors, lipid mediators and antimicrobial peptides (AMP) (11).

All molecules produced by epithelial cells including KCs, that can fundamentally influence the innate and adaptive immune cell functions, are referred to „epimmunome" in the literature (12). IL-1α, IL1β, IL-6, IL-8, IL-18, IL-24, IL-25, IL-33, IL-23, IL-17C, IL-36 receptor antagonist (IL-36RA), IL-38, and AMPs like TSLP (thymic stromal lymphopoietin), S100 family, beta-defensins and LL37 are considered to be the most important epimmunome molecules (12-14). Some of them are produced under homeostatic conditions and responsible for maintaining „steady state", whilst proinflammatory epimmunomes are produced after the sensation of danger signals. The epimmunome molecules have significant impact on both the innate and the adaptive immune cells of the skin, principally on DCs and T cells (12).

Antimicrobial peptides

AMPs are one of the crucial subgroups of the epimunome molecules. To protect our body from microbial pathogens, immune and epithelial cells secrete AMPs (15, 16). Mainly local skin cells like KCs, sebocytes, and eccrine gland cells produce them (17), but infiltrating immune cells like neutrophils and NK cells also add to the skin's reservoir of AMPs (18, 19). The members of the AMPs' class are very diverse. These peptides were initially grouped together based on their antimicrobial activity rather than their structural properties. Currently the most

information is available regarding defensin and cathelicidin AMP families, but there are many other cutaneous AMPs (16, 20).

We can distinguish AMPs, wich are produced constitutively in the skin, while others are expressed in much higher amounts when there is a risk of infection or skin injury (21). AMP expression and activity are both transcriptionally and posttranscriptionally regulated. The majority of AMPs are produced as pro-peptides and become active after being cleaved from their precursor molecules by proteolysis (22).

A large number of AMPs were initially discovered connected to their function in eradicating microbes and constructing a chemical barrier on the skin. Recently, it became evident that several AMPs are not just endogenous antibiotics, but also immune response-initiating and -coordinating molecules (23). Some AMPs were given the name "alarmins" in recognition of their additional immune roles as the induction of immune responses (24). Since disturbed AMP production and/or functions may lead to a weakened antimicrobial barrier or may negatively impact epithelial cells, therefore the proper regulation of their expression and activation is essential.

2.2.2.2. DCs in the skin

The next important cell type of the immunological barrier is the DC. In steady state there are two main groups of myeloid DC-s in the skin, which can be classified according to their location and to their characteristic cell surface molecules: the Langerhans cells (LCs) ($CD207^+$ ($Langerin^+$), $CD1a^+$, $Fc\varepsilon RI^+$)) in healthy epidermis and $CD11c^+$ dermal DCs ($CD11c^+$, $CD1c^+$, $Fc\varepsilon RI^+$) in the dermal compartment (11, 25). According to other classifications, LCs does not belong directly to the DCs. $CD11c^+$ DCs can be divided into two major subgroups: $CD14^+$ and $CD1c^+$ DCs. Over the last decade their group was supplemented with a smaller, $CD141^{hi}$ DCs population (Figure 2.). This population hardly expresses CD11c, and their further characteristic is the lack of CD14 (26). Beside myeloid DCs, plasmacytoid DCs can also be detected in healthy skin, although their numbers are very low since they leave the blood stream mainly during inflammatory conditions. During inflammation other DCs also appear in the skin. In the epidermis of atopic dermatitis (AD) patients the so-called inflammatory dendritic epidermal cells (IDEC) develop, while in psoriasis the TNF-α, iNOS producing inflammatory dermal dendritic cells (TIP-DC) can be found in the dermal compartment (Figure 2.) (27, 28).

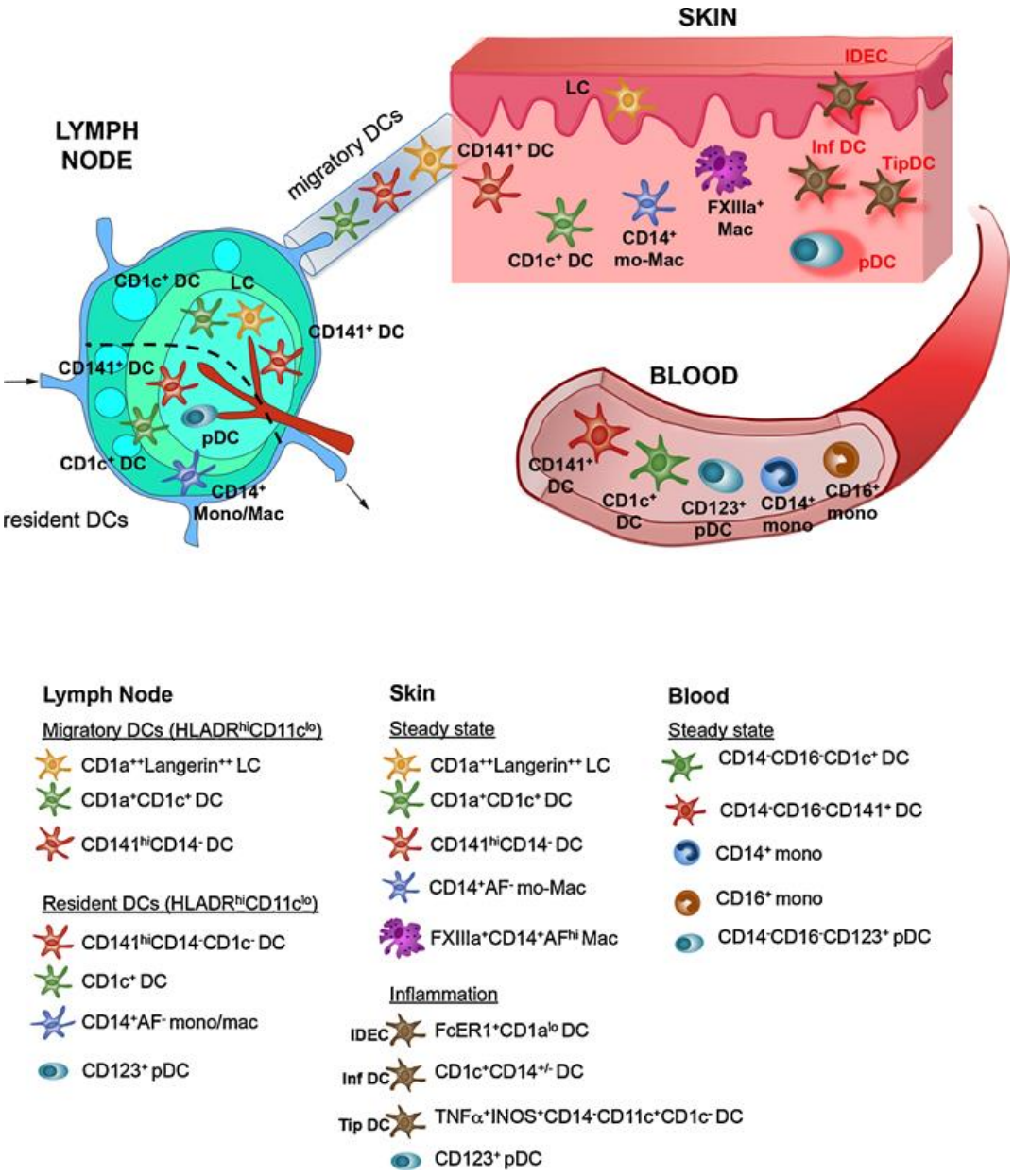

Figure 2. The different DC subtypes, monocytes and macrophages in lymph nodes, skin, and blood *Cells appearing in inflammation are marked with red letters. IDEC = inflammatory dendritic epidermal cell, inf DC = inflammatory DCs, Mac = macrophage, mono = monocytes, mo-Mac = monocyte-derived macrophage, pDC = plasmacytoid DCs, TipDC = TNFa and iNOS producing DC. Source: Haniffa et al. Journal of Dermatological Science 2015 7785-92DOI: (10.1016/j.jdermsci.2014.08.012)*

DCs are so called antigen presenting cells, they also act as immune sentinels in the skin. The tissue microenvironment closely controls their activation. They present antigens for T cells in the draining lymph nodes to induce immune response against microorganisms. DCs influence naïve T cells also through the production of different T cell polarising cytokines, since these cytokines can initiate the differentiation and the lineage commitment of several Th subpopulations. Among different T cell polarising cytokines, IL-12 indicates Th1 polarisation; while IL-2, IL-4 and CCL17 are responsible for the induction of Th2 cells (29, 30). Furthermore, IL-6 together with TNFα induce Th22 cells (31, 32); TGFβ1, IL-6 and IL-23 induce Th17 cells (33); while TGFβ1 and IL-10 take part in the polarisation of regulatory T cells (34).

Although monocytes are thought to be their main source, blood DCs are also considered as precursors of skin DCs (referred to as precursor DCs- preDCs) (35-38). In AD, pre-DCs from peripheral blood have been emerged to serve not only as precursors of skin DCs but also as precursors of inflammatory DCs of the skin (35-38). Although extensive data are available on the properties of skin DCs, their peripheral blood precursors are less characterized.

2.2.2.3. T cells in the skin

In the normal human skin app. $2x10^{10}$ T lymphocytes can be detected, and that is two-fold of the T cell number in the blood (39, 40). Most of these skin T cells are long-lived memory T cells. They can be divided into 3 groups: central memory T (Tcm), effector memory T (Tem), and tissue-resident memory T (Trm) cells. αβT cells in the skin are memory cells, and 2 main lineages of them can be distinguished: $CD4^+$ T cells and $CD8^+$ T cells. The majority of αβT cells are located in the dermis, and most of them are $CD4^+$ T cells, while only a few αβT cells can be found in the epidermal part of the skin, and they are fundamentally $CD8^+$ tissue-resident memory T (Trm) cells. Natural killer T (NKT) cells can also be classified as αβT cells (39).
In the last twenty years several subsets of $CD4^+$ T cells has been identified. The most finely characterised are the Th1, Th2, Th17, Th22, Th9 and regulatory T cells (Treg). These cell populations can be detected either in healthy skin or in immune-mediated skin diseases. Th1 and Th2 cells were identified first, and then the IL-17 cytokine-producing Th17 cells were described. Th9, Th22 cells and follicular T helper (Tfh) cells have only been isolated in the last 10-15 years. In steady-state, mainly Treg and Th17(β) cells has been detected, in contrast to the inflamed skin, which is infiltrated by Th1, Th2, Th17(23) or Th22 cells (40). In immune-

mediated skin diseases the number of T cells are highly increased and the subtypes are characteristic for the inflammatory disease itself.

Th1 cells produce mainly type I cytokines: IFNγ, TGFβ and IL-2. They have a role in the defence against intracellular pathogens (viruses, Mycobacteria). They express IL-12 and IL-18 receptors. The differentiation of naive T cells towards Th1 is promoted by IL-12, which activates the T-bet transcription factor (40, 41). Th1-type immune responses are characteristic for example, in psoriasis and in the early phase of allergic contact eczema (40).

Th2 cells can be connected strongly to atopic diseases such as atopic dermatitis (AD) allergic bronchial asthma, allergic rhinoconjunctivitis or food allergies. They regulate early and late phase allergic responses. Th2 cells are characterised by production of cytokines such as IL-4, IL-5, IL-13, IL-24, IL-25 and IL-31 (40, 42, 43).

Th17 cells are playing role in the defence mechanisms against bacteria and fungi. Two subsets of Th17 cells can be distinguished: the non-pathogenic or "Th17(β)" cells and pathogenic Th17 cells or "Th17(23)" cells (44). Non-pathogenic Th17-Th17(β)-cells can produce IL-17A, IL-17F, IL-10, CCL20, while the pathogenic Th17 subset produces IL-17A, IL17F together with IFNγ, IL-22 and CCL9. IL-6 cytokines together with TGF-β1 take part in the differentiation of the non-pathogenic subset, while pathogenic Th17-Th17(23)-cells are induced by the IL-1β, IL-6, and IL-23. Upon exposure to IL-23, non-pathogenic Th17 cells can turn into pathogenic Th17 cells (40, 44).

Th9 cells in the blood and tissues are skin-tropic memory cells producing IL-9, TNF-α and granzyme B (45). Th9 cells are induced from naïve T cells (or from Th2 cells) by the combination of IL-4 and TGF-β (46). The numbers of IL-9-producing cells are increased in AD and psoriasis (45).

Two types of $CD4^+$ Treg cells can be distinguished: the $Foxp3^+$ and the $Foxp3^-$ Tregs. $Foxp3^+$ Treg cells have immunosuppressive caracteristics and are divided into two subtypes: thymus-derived (tTreg) and peripherally induced (pTreg) cells (47). Deficiency of Treg cells can cause various skin disorders as seen in a genetic disorder called IPEX, which is caused by Foxp3 mutation (48). $Foxp3^-$ Tregs can be further devided to Tr-1 and Th3 cells. Among the two subtypes of Foxp3- Tregs Tr1 produce IL-10 while Th3 cells release TGF-β (44, 49, 50).

$CD8^+$ T cells are the main effector cells, which recognize antigens on MHC-I. These $CD8^+$ killer or cytotoxic T lymphocytes (CTLs) in steady-state are essentially Trm cells, they reside in epidermis without entering the circulation (51). $CD8^+$ Trm cells can also attract circulating memory T cells to the site of inflammation besides exerting their effector functions (52).

Human innate-like αβT cells are natural killer T (NKT) cells, and mucosal associated invariant T cells. The CD1d-restricted NKT cells develop in thymus, and share the characteristics with natural killer (NK) cells and T cells (53). They produce several cytokines and chemokines that play role in DC maturation, and enhance Th1- and Th2-type responses.

2.3. Immunological differences in the the topographically different healthy skin regions

The different regions of healthy skin exhibit variations in their anatomical features (such as sebaceous and apocrine gland quantities), chemical milieu, and microbiota composition (54). According to these differences at least three main regions can be distinguished: sebaceous or sebaceous gland-rich (SGR), moist or apocrine gland-rich (AGR), and dry, or sebaceous and apocrine gland-poor (GP) regions. Considering the significant impact of microbiota and chemical environment on skin immune function, our research group has recently investigated whether these regional distinctions also manifest in the skin's immune response (55-57).

In the previous studies of our research group we have detected notable differences in the amount and functionality of DCs and T cells in SGR regions compared to GP skin areas (55, 56). Additionally, we have established that AGR and GP skin also exhibits distinct immune characteristics (57). Specifically, in SGR and AGR regions a non-inflammatory IL-17/IL-10 environment is present, linked with elevated levels of T cells and DCs (although they are non-activated), along with elevated levels of IL-17-associated chemokines and AMPs (55-57). Furthermore, we have identified variations in the physical barrier characteristics across the different regions. Main components of this barrier, such as tight junction and desmosome elements, display reduced expression in SGR and AGR skin regions compared to GP areas (58).

These differences in different healthy skin regions potentially elucidate the characteristic localization of region-specific immune-mediated skin disorders. Certain inflammatory skin conditions exhibit a nearly exclusive localization to a particular skin area. For instance, rosacea primarily emerges on sebaceous skin, hidradenitis suppurativa (HS) tends to develop in moist, AGR regions, and atopic dermatitis primarily affects dry, GP areas. Conversely, some immune-mediated skin disorders can develop across all skin regions. For example, psoriasis has the capability to affect both sebaceous (scalp psoriasis) or apocrine gland-rich (inverse psoriasis) areas as well as gland-poor regions (psoriasis vulgaris).

2.4. Psoriasis

Psoriasis is a chronic inflammatory, immune-mediated skin disease. It is a very frequent disorder; the prevalence is about 2% in the Caucasian population (59-61). There are some racial differences in the prevalence of psoriasis, with Faroese people having the highest incidence (2.8%) and Japanese people having the lowest (0.2%) (62, 63). In Europe and North-America the incidence of psoriasis is about 2%, the lowest in childhood showing increased level with age (64). In women, the disease can appear at a younger age than in men, with an onset mainly occuring between the ages of 16–22 years and 55–60 years. Based on the immunological and genetic differences between these two age groups, two different subtypes can be distinguished: early-onset (before the age of 40; 75% of cases), and late onset (after the age of 40) (65). About 80% of patients with psoriasis have mild to moderate forms of disease, and 20% suffers from moderate to severe disease that affects more than 5% of body surface area (BSA) or vital body parts (e.g. face, hands, feet or genitals (59, 66, 67).

The pathogenesis of psoriasis is heavily influenced by genetic factors proven by the observations, that monozygotic twins are 2-3 times more likely to develop psoriasis than dizygotic twins, and 30% of people with psoriasis have an affected first-degree relative (68, 69). Several susceptibility loci have already been found (70), to date more than 40 PSORS (psoriasis susceptibility regions) were identified. Most important among them is the PSORS1, which is responsible for the 30-50% of the genetical susceptibility (71). Furthermore, the HLA-C*06:02 allele, identified within the PSORS1 locus, is the most dominant risk allele for the development of psoriasis, carried by about 60% of patients with early-onset psoriasis, but surprisingly, no connection between this allele and late onset psoriasis could be detected (72). Environmental factors, such as trauma, infection and stress, are also important contributors to the development of psoriasis (73). Moreover climate, exposure to the sun and ethnicity can affect the severity of the disease, which gets worse in winter and less severe in summer (74).

Among immune-mediated skin diseases psoriasis is extremely important, since this very common, chronic skin disease is incurable, only symptomatic treatment is available, and its negative impact on the quality of life is profound. In 2014, the World Health Organization declared psoriasis a serious noninfectious disease and also declaired it a major global public health burden on the world's population (75). Psoriasis is characterized clinically by raised, scaly, red plaques that are most common on the scalp, knees, and elbows. Acanthosis is visible in the epidermis, the granular layer is diminished, the rete ridges are lengthened, the cornified layer is increased (hyperkeratosis), and both the dermis and the epidermis have immune cell

infiltrates due to increased KC proliferation (76). Psoriasis patients frequently require lifelong treatment because it frequently coexists with other conditions such as depression, psoriatic arthritis, and cardiovascular disease. In psoriasis it has been postulated that the severe, untreated forms of the disease will generate a systemic inflammation that further promote the progression of other co-morbidities with a decreasing life expectancy.

The primary appearance of psoriasis can be notably heterogenous (chronic stable plaques, eruptive papules or even pustules) and can involve all skin regions (scalp, folds, palmo-plantar areas and body skin). The clinical classification usually depends on these characteristics e.g. chronic plaque type, which is often mentioned as psoriasis vulgaris, guttate psoriasis, erythrodermic psoriasis, eruptive papular type and pustular psoriasis, while according to the special localization: scalp psoriasis, inverse psoriasis, nail psoriasis and palmo-plantar psoriasis (65, 77). The most frequent forms among them are chronic plaque type psoriasis, affecting mainly the trunk and extremities (psoriasis vulgaris or skin psoriasis) and scalp psoriasis. Although both forms have strong negative impact on the quality of patients' life, scalp psoriasis is usually more visible, and its treatment is even more difficult due to the special localization (78-81). These two forms can occur separately, but patients often have both. The two distinct skin areas (gland poor and sebaceous gland-rich areas) on which psoriasis vulgaris and scalp psoriasis develop not only have different anatomical characteristics, but bear distinct microbiota and chemical milieu (82-85). Moreover, parallel with these, topographically distinct immune and barrier features have also been described on these skin areas as described above (55, 56).

2.4.1. Classification of psoriasis

Traditionally, the morphologic descriptions of psoriasis have served as the foundation for phenotyping (86). Although phenotyping is very helpful for classification, clinical findings in specific patients frequently overlap in more than one category (77).

Plaque psoriasis

About 80 to 90 percent of patients have plaque psoriasis, which is the most prevalent type. Patients suffering from this type of psoriasis have been involved in most of the clinical trials or in experimental studies. In plaque psoriasis the size of erythematous plaques range from 1 to several cm, which are clearly demarcated, and well-defined (Figure 3. and 4.). Histologically, psoriasiform epidermal hyperplasia, parakeratosis, hypogranulosis, spongiform pustules, an infiltrate of lymphocytes and neutrophils in the dermis and epidermis, as well as an expanded

dermal papillary vasculature reflect these clinical findings. Patients may be affected in varying degrees, from having just a few plaques to having many lesions that nearly cover their whole skin surface. The round or oval plaques typically develop on the trunk, scalp, buttocks, or limbs, with an affinity for extensor surfaces like the elbows and knees. Particularly on the legs and trunk, smaller plaques and papules may combine to form more extensive lesions. Lesions over joint lines or on the palms and soles can cause painful fissuring within plaques. Due to regional anatomical variations, psoriatic plaques frequently have a dry, thin, silvery-white or micaceous scale and tend to be symmetrically distributed throughout the body (Figure 3. and 5.) (65, 77, 87).

Erythrodermic psoriasis

Erythrodermic psoriasis can develop abruptly or gradually from chronic plaque disease with little to no pre-existing psoriasis. Nearly the entire body surface exhibits generalized erythema, with scaling (Figure 3. E). Chills and hypothermia could result from the erythrodermic skin's altered thermoregulatory capabilities, and dehydration could result from fluid loss. Malaise and fever are frequent ailments (65, 77, 87).

Pustular psoriasis

The stratum corneum of all psoriasis subtypes contains more or less neutrophils. It is known as "pustular psoriasis" when the neutrophil collections are significant enough to be visible clinically. Pustular psoriasis can be either generalized or localized. The acute generalized form, also known as the "von Zumbusch variant," is a rare, but severe type, characterized by widespread pustules on an erythematous background (Figure 3. D, and Figure 4. C). Before, during, or after an acute pustular episode, cutaneous lesions typical of psoriasis vulgaris may appear. The localized pustular form usually appears on the palms and soles, with or without indications of the classic plaque-type disease (65, 77, 87).

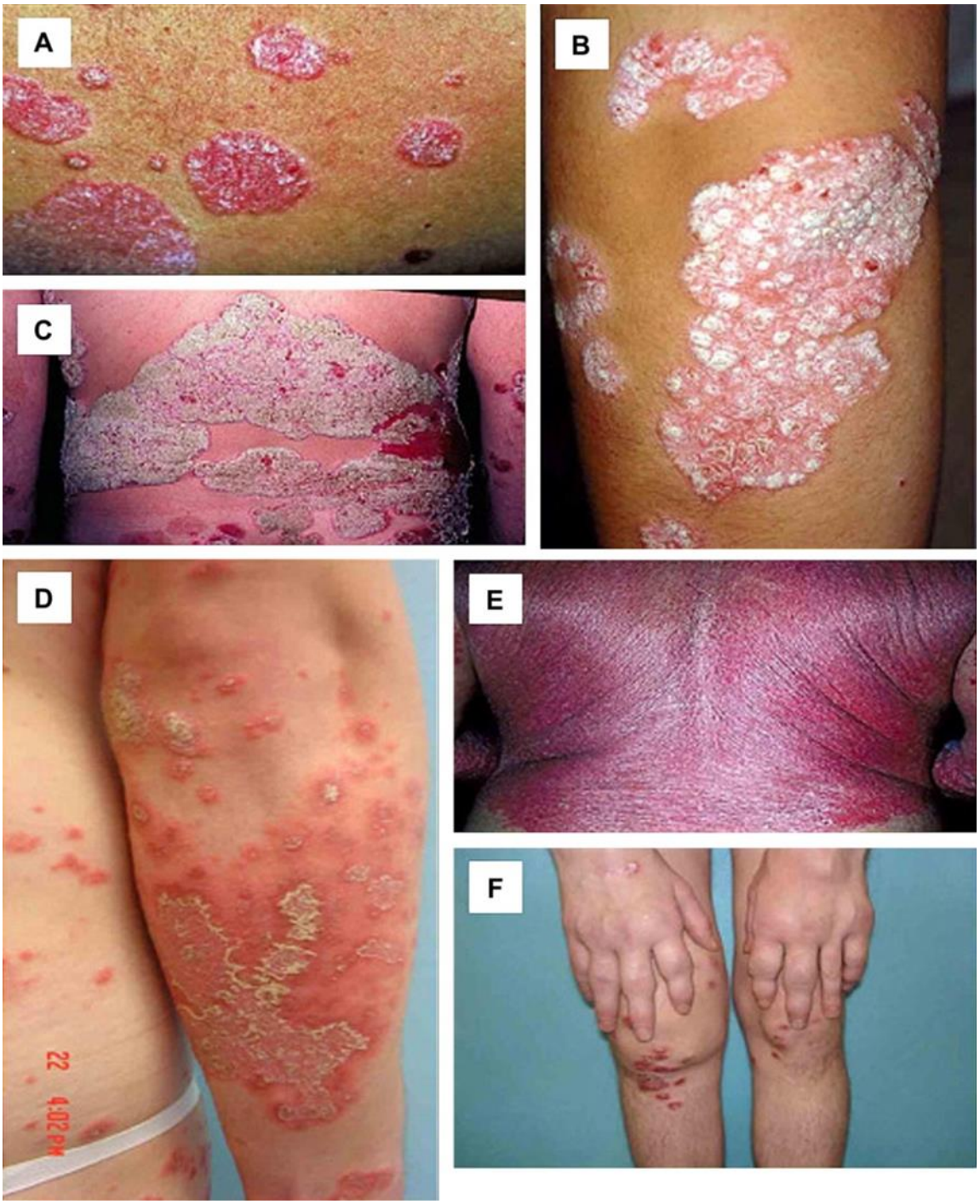

Figure 3. Different appearence of psoriasis. *(A) Small plaque psoriasis. (B) Localized thick plaque type psoriasis. (C) Large plaque psoriasis. (D) Inflammatory localized psoriasis. (E) Erythrodermic psoriasis. (F) Psoriasis and psoriatic arthritis. Source: Menter et al. Journal of the American Academy of Dermatology. 2008;58(5):826-50 (77)*

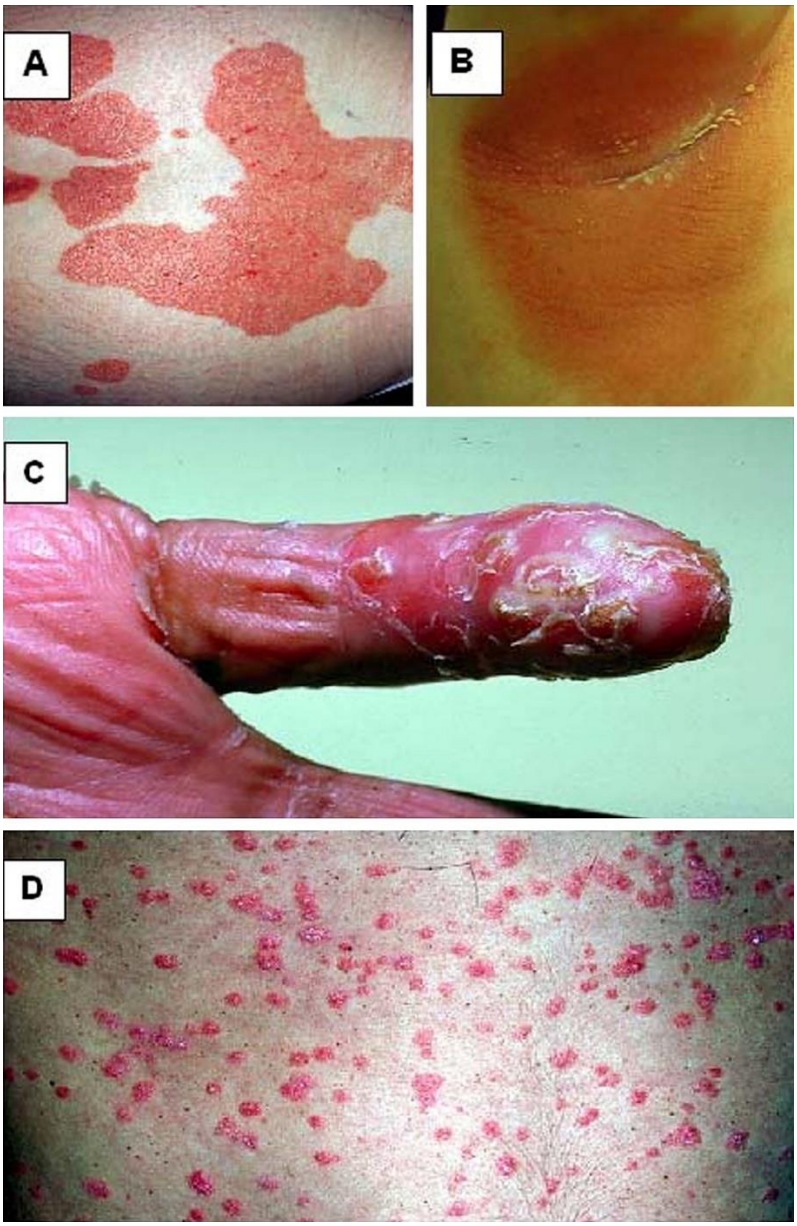

Figure 4. Different appearence of psoriasis. *(A) Thin plaque type psoriasis. (B) Inverse type psoriasis. (C) Pustular type psoriasis. (D) Guttate type psoriasis. Source: Menter et al. Journal of the American Academy of Dermatology. 2008;58(5):826-50 (77)*

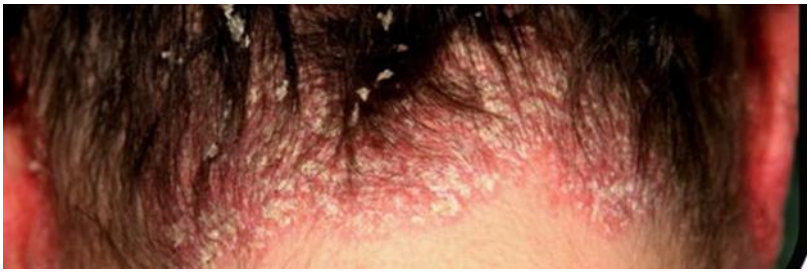

Figure 5. Scalp psoriasis *Source: photo by the Dept.of Dermatology, University of Debrecen*

Guttate psoriasis

In this type of psoriasis the dewdrop-like, salmon-pink papules appears on the trunk and proximal extremities, and are typically finely scaled. This type of the disease is not so frequent, and it affects about 2% of patients with psoriasis being more common in younger ages (under 30 years). Particularly in younger patients, guttate psoriasis frequently develops 2 to 3 weeks prior to upper respiratory infection with group A beta-hemolytic streptococci. The sudden onset of papular lesions may be the first sign of psoriasis in someone who had never had it before or it may be an acute exacerbation of long-standing plaque psoriasis (Figure 4. D) (65, 77, 87).

Psoriasis with special localizations

Scalp psoriasis

Scalp psoriasis is a very frequent form of psoriasis, which can affect the entire scalp or it can appear in smaller or bigger patches. It can also reach the hairline, behind or inside the ears, and also the upper part of the neck. The causes of scalp psoriasis are similar to those of psoriasis on other parts of the body, but the treatment of scalp psoriasis is more challenging (Figure 5.) (65, 77).

Inverse psoriasis

Lesions in the folds of the skin are a feature of inverse psoriasis. These areas tend to be moist, so the lesions are typically erythematous plaques with little scale. The inframammary, axillary, genital, perineal, and intergluteal regions are typical sites. Comparable lesions can be seen on flexural surfaces like the antecubital fossae (Figure 4. B) (65, 77).

Nail disease (psoriatic onychodystrophy)

All psoriasis subtypes can cause nail psoriasis. A little over half of all psoriatic patients have affected fingernails, and 35% of them have affected toenails. Pitting, onycholysis, subungual hyperkeratosis, and the oil-drop sign are a few of these modifications. Nail changes may occur in up to 90% of psoriatic arthritis patients. The therapy of nail psoriasis is a challenge (65, 77).

Psoriatic arthritis - as a comorbidity of psoriasis

An inflammatory arthropathy linked to psoriasis is called psoriatic arthritis. (Figure 3. F) (65, 77).

2.4.2. Pathogenesis of psoriasis

Psoriasis is an immune mediated skin disease suggested by the results of genome based studies identifying immune related genes among the DEGs. Moreover, immunoregulatory genes have been shown to be central to the development of psoriasis (88, 89). Although there are still several gaps in our understanding of the definite pathomechanism of psoriasis, there is an ample literature that explored the crucial role of adaptive immunity in its immune processes, moreover in the last 15 years there has been an emphasis on the investigation of innate immunity as well. These studies revealed, that the elements of both the innate and the adaptive immune system are essential in its pathogenesis with highlighing inflammatory DCs, T cells and KCs as the main trigger and effector cells (Figure 6.) (90-96).

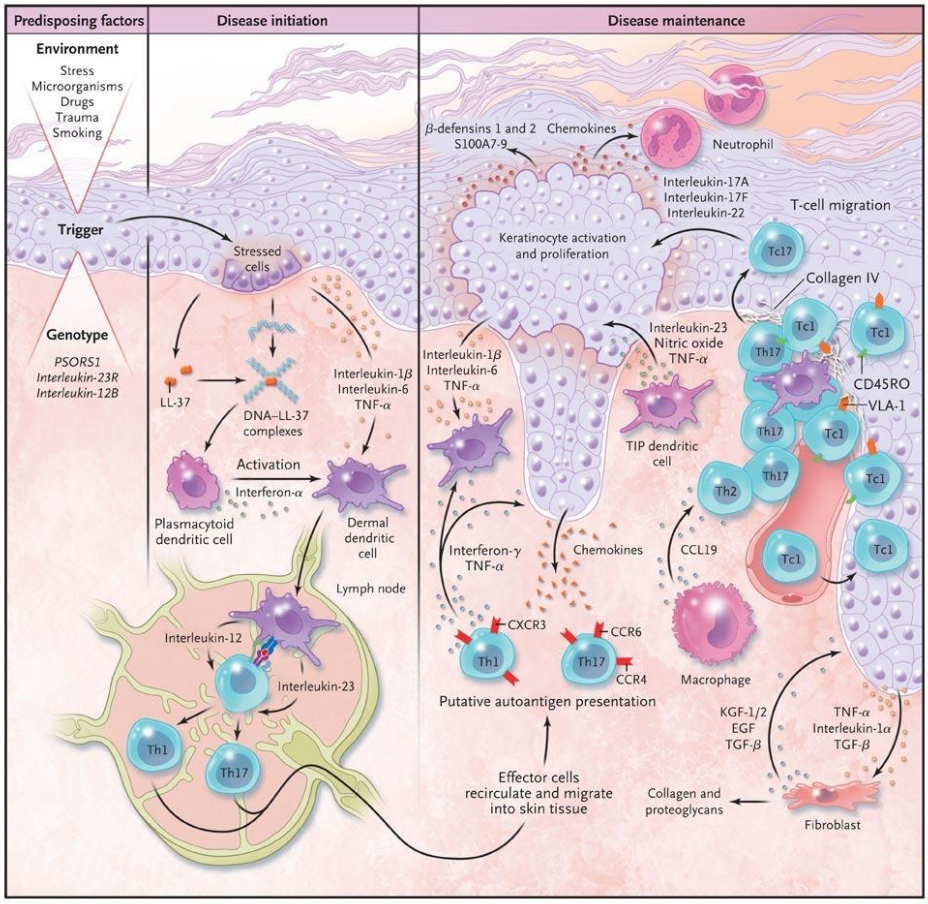

Figure 6. Evolution of a Psoriatic Lesion from Initiation to Maintenance of Disease.
Source: Nestle FO, et al. N Engl J Med. 2009;361(5):496-509

The role of DCs in psoriasis

Among innate immune cells, skin DCs play a crucial role in the pathogenesis of psoriasis, by contributing both to the initiation steps and to the maintenance of the disease (93). In the initiation of inflammation pDCs are thought to play key role, since they start to produce IFNα after being activated by LL-37/self-DNA complexes (97). With this step, they induce the IFNα dependent maturation of mDCs (98) which cells are responsible for further maintenance and amplification of the inflammatory processes by activating and polarising different T cell subsets (99). The number of $CD11c^+$ mDCs in the dermis of psoriatic skin is 30-fold higher then in uninvolved or healthy skin (100). In psoriasis they have two distinct populations: the $CD11c^+$ $CD1c^-$ inflammatory DCs (called TIP-DCs) and the $CD11c^+$ $CD1c^+$ resident DCs (dermal DCs - DDCs) (27). TIP-DCs, produce TNFα and iNOS (100-103) moreover, together with the DDCs, they are important sources of Th17 activating cytokine IL-23 (27, 104). Moreover $CD1c^+$ DDCs also produce the Th1 polarizing cytokine, IL-12 (30, 100, 103).

Besides producing T cell polarizing cytokines DCs also secrete different chemokines. The level of several chemokines is elevated in psoriatic skin (35, 105, 106), and skin DCs were found to be important sources of CXCL1, CXCL8, CXCL9, CXCL10 as well as of CCL20 (30). CXCL1, CXCL9 and CXCL10 are chemoattractants for Th1 lymphocytes, CXCL8 recruits neutrophils, while CCL20 attracts Th17 lymphocytes, DCs and monocytes (107). The precise contribution of skin DC subsets to the chemokine secretion is not clearly defined, but many of their representatives, like Langerhans cells, DDCs, TIP-DCs and also pDCs are considered to release them (30, 105, 107, 108).

The above evidences highlight the important role of skin DCs in the development of skin inflammation in psoriasis, on the other hand the role of their blood precursors is less explored, and moreover their phenotypic characterisation is also incomplete. Since it has emerged that peripheral blood pre-DCs can serve as precursors of not just DDCs, but also of TIP-DCs (35-38), the relevance of their characterization is essential.

The role of T cells in psoriasis: the IL-17/IL-23 pathway

Previous studies have initially indicated that psoriasis is a Th1 mediated disease (90-93), but later experimental and clinical evidences highlighted the outstanding role of IL-23/IL-17 pathway in its pathogenesis (94-96). It is clearly established now, that in the pathogenesis of psoriasis IL-23 and IL-17A are the most crucial cytokines, and the IL-23/IL-17 immunologic pathway is essential both in the initiation and the perpetuation of the disease (95, 96, 109). IL-23 stimulates differentiation, activation, proliferation, and survival of Th17 cells that promote

production of effector cytokines such as IL-17A and IL-22 (109). Among others Risso et al revealed that IL-23 is “upstream” of IL-17A, whereas IL-17A, acting “downstream,” directly affects tissue on murine disease models. Rizzo et al demonstrated that in wild-type mice IL-23 injection can initiate psoriasis-like disease, but in IL17 knockout mice this effect of IL-23 failed. Moreover the effect of IL-23 injection in wild-type mice could be blocked by anti-IL-17A antibodies (109).

Although T cell activation in psoriasis is associated with the secretion of proinflammatory cytokines including TNF-α, IL-17A, IL-22, and interferon IFN-γ (110, 111), data from in vitro and clinical studies indicate, that among them IL-17A is the cytokine that principally drives changes within affected tissues (112-116). Direct evidence supporting the central role of IL-17A in psoriasis includes upregulation of IL-17A and related genes in lesional and non-lesional skin of patients with psoriasis and production of IL-17A by cells associated with psoriasis (113, 116, 117). In an in vitro study using reconstituted human epidermal sheets, IL-17A stimulated greater transcriptional activation than IL-22 or IFN-γ, correlating with the psoriasis transcriptome (118). Moreover, therapies targeting IL-17A alone are known to modulate gene expression of various cytokines and chemokines, and effectively clear psoriatic lesions (119, 120). More specifically, 2 weeks of IL-17A inhibition resulted in normalization of 765 genes, whereas TNF-α inhibition resulted in the normalization of far fewer genes (121, 122).

The Th1/Th17 cytokine milieu leads to the characteristic exaggerated proliferation of KCs, since one of the main role of the cytokines released by Th1 and Th17 cells (TNFα, IL17, IL21 and IL22, but especially IL17A) is to convert KCs into an activated state, which thus produce AMPs and various cytokines, chemokines. These cytokines maintain the mDCs in activated state, thus developing the chronic amplification of the inflammatory cycle which is characteristic of psoriasis (1, 123).

The role of KCs in psoriasis

Earlier KCs were considered to bystender elements in the pathomechanism of psoriasis, assisting in the functions of immune cells (124, 125), but recently it was revealed, that KCs are also key players in early immune defence mechanisms (126), playing a fundamental role in both the initiation and maintenance of psoriasis. KCs activated by different pro-inflammatory cytokines (produced by DCs and T cells) secrete significant amounts of cytokines and chemokines (e.g. CXCL1/2/3, CXCL8, CXCL9/10/11, CCL2 and CCL20), which mediators, besides the above mentioned role on mDCs, attract leukocytes to the site of initiation. KCs also

produce AMPS (e.g. S100A7/8/9/12, hBD2 and LL37) which shape the functions of immune cells, in addition, they have role in tissue reorganization through activation of endothelial cell proliferation and inducing changes in extracellular matrix (1, 127). The interaction between KCs and Th17 cells contributes to the initiation and maintenance of psoriasis, which includes hyperproliferation and aberrant differentiation of KCs.

The role of cathelicidin in the pathogenesis of psoriasis

Cathelicidine (or LL37) is an AMP, which has an outstanding role in the pathomechanism of psoriasis. There is only one cathelicidin found in humans, and its gene, CAMP, is located on chromosome 3. Cathelicidin expression in healthy skin is barely perceptible, only KC lamellar bodies and neutrophil granules exhibit positive immunostaining (128). On the other hand, cathelicidin expression is strongly induced by injury, infection, or inflammation (129, 130). KCs considered as significant cathelicidin producers, and advancing neutrophil cells deliver further amount of cathelicidin to the infected area.

Cathelicidin has essential role in the triggering of a chain of immune mechanisms which eventually lead to the development of psoriasis. As mentioned earlier, dDCs and pDCs are activated in the inflamed skin in the early steps of psoriasis pathomechanism with the help of the LL-37/self-DNA complexes. Physiologically, pDCs detect viral nucleic acids through TLRs 7 and 9 and they start producing type I IFN, which activate a protective immune response against viruses. Normally, host-derived (self) nucleic acids released by dying cells are not able to connect to intracellular TLR7 and 9 receptors, but in psoriasis, through forming complex with endogenous LL-37 peptide, they become able to connect to those receptors (131).

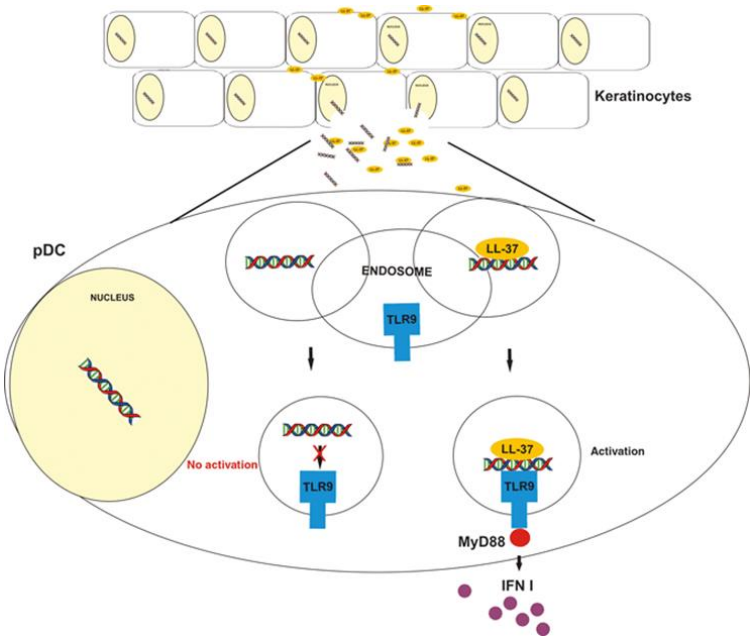

Figure 7. LL-37 peptide / DNA complexes activate pDCs in psoriasis
Source: Dombrowski and Schauber Exp Dermatol 2012; 21(5):327-330, DOI: (10.1111/j.1600-0625.2012.01459.x

The anionic self-DNA in complex with the cationic LL-37 peptide after entering to pDCs, subsequently trigger a TLR9 MyD88 IRF7 mediated IFNα response (Figure 7). By activating self-reactive T cells, IFNα then triggers immune response, which leads to the development of a psoriatic lesion (131). LL-37 can similarly alter self-RNA, which stimulates mDC activation through TLR7 and 8. (132). In psoriasis, extracellular DNA and RNA released from dying cells combined with an increase in LL-37 induce a pro-inflammatory trigger that starts and intensifies inflammation. The dendritic cell activated this way releases inflammatory cytokines IFN-α, TNF-α, IL-1B and IL-23 as mentioned earlier. These cytokines activate T cells as well as sustain the maturation of IL-23, resulting in T cells differentiating into Th17 and Th22 cells. According to their names, they produce IL-17 and IL-22, the elevated concentration of which cytokines are responsible for the development and maintenance of chronic inflammation. Activation of KCs initiates intense cell proliferation and increases the expression of cell adhesion molecules (133). Hyperproliferation and processes of keratinocytes cause characteristic histological changes in the affected skin area.

2.4.3. The therapy of psoriasis

As psoriasis is a chronic disease, patients usually require long-term treatment. Traditional treatment options for psoriasis include topical therapies: corticosteroid-containing creams, vitamin D analogues and phototherapy. In more severe cases systemic drug therapy (methotrexate, retinoids and cyclosporine) is applied, but their long-term use is limited. Nowadays TNF blockers are widely used, moreover many new medications target IL-23 and IL-17 cytokines because of the critical importance of IL-23/IL-17A axis in the pathogenesis of psoriasis (134). These drugs enhance the efficacy of psoriasis therapies, they dramatically improve skin and joint symptoms in patients with moderate-to-severe psoriasis and psoriatic arthritis (135, 136), moreover they can have notable clinical differences related to dosing and safety profiles (137). Therapies targeting cytokines further upstream in this pathway require less-frequent dosing to maintain efficacy than drugs targeting more downstream cytokines and receptors (138). IL-23 and IL-12/23 inhibitors (furthest upstream) require maintenance dosing every 8–12 weeks, whereas maintenance dosing with approved IL-17A inhibitors (midstream) is required every 4 weeks, and the IL-17 receptor A antagonist brodalumab (furthest downstream) is administered every 2 weeks (137).

3. OBJECTIVES

Study I. Skin DCs are important in the development of psoriatic skin inflammation, at the same time their pheripheral blood precursors, their phenotypic and functional characteristics in psoriasis are scarcely explored. Since it has emerged that peripheral blood pre-DCs can serve as precursors of not just dermal DCs, but also of TIP-DCs, the relevance of their characterization is essential. Therefore, in the first part of our work we aimed to examine the characteristic features of psoriatic blood $CD1c^+$ mDCs and pDCs:

- their activation and maturation stages (with CD83 and CD86 maturation and activation markers) by flow-cytometry
- their cytokine and chemokine production [Th1 (IL-12), Th2 (IL-2, IL-4, CCL-17), Th17 (IL-6, IL-23, TGFβ), Th22 (IL-6, TNFα), Treg (TGFβ, IL-10) cytokines, and Th1/Th17 chemokines (CXCL1, CXCL8, CXCL9, CXCL10, CCL20)] by flow-cytometry
- chemokine secretion of $CD1c^+$ mDCs (CXCL8, CXCL9, CXCL10, CCL20) by ELISA method

Study II. In the second part of my research we went on with the investigation of DCs in psoriasis, supplemented with the examination of further innate and adaptive immune components. In this study we aimed to clarify whether the immune mediated inflammation is similar in scalp psoriasis (psoriasis on SGR skin) and psoriasis vulgaris (classical plaque type psoriasis on SGP region), or the inflammation developed in the 2 subtypes are influenced by the primarily distinct immune milieu of the different healthy skin areas where they develop. Therefore, we investigated the cellular and molecular immune characteristics in the two form of psoriasis:

- the disease-specific innate (CD11c+ mDCs, LCs) and adaptive immune cells (CD4+ T cells) by immunohistochemistry (IHC)
- the representative cytokines of the Th1 pathway (IFNγ, TNFα, L-12) by IHC and qPCR
- the Th17 related cytokines (IL-17, IL-23), chemokines (CCL2, CCL20), AMPs (S100A7/8/9, DEFB4B, LCN2) by IHC and qPCR
- and the barrier-related molecules (KRT6, KRT17, LOR, FLG) by IHC and qPCR

4. PATIENTS AND METHODS

4.1. Patients

4.1.1. Patients and healthy controls for cellular investigations (Study I.)

To investigate the characteristics of psoriatic blood DCs, peripheral blood was collected from patients suffering from psoriasis (n=21, 8 females, 13 males, mean age: 51.5±13.8 years,) as well as from healthy volunteers (n=17) with gender and age matching. To accommodate the limited number of extractable cells from each patient, different group of patients were randomly chosen to be involved either in the investigations of $CD1c^+$ mDC or pDC as well as in ELISA based experiments. Specifically, 12 patients and 9 healthy volunteers were selected to the cytokine examination of $CD1c^+$ mDCs, 5 patients and 4 controls for the pDCs' investigations along with the chemokine investigations of $CD1c^+$ mDCs, while in the ELISA experiments 4 patients and 4 healthy controls were involved. Recruited patients were suffering from moderate-to-severe psoriasis, as determined by the Psoriasis Area and Severity index (PASI) calculation (mean PASI: 23.9±10.1) and had refrained from receiving systemic treatment or phototherapy for at least 4 weeks prior to blood sampling. The study was performed in accordance with the Declaration of Helsinki principles. Written informed consent was obtained from all participants. The study was approved by National Ethics Committee, Hungary (50935/2012/EKU, 7879/2013/EKU).

4.1.2. Patients for skin biopsies (Study II)

For the second study 7 mm punch biopsies were collected from lesional skin of 12 psoriatic patients (6 biopsies from scalp psoriasis and 6 from classical plaque type psoriasis vulgaris, see patients' characteristics in Table 1.) after obtaining written informed consent from the patients. The study was performed according to the Declaration of Helsinki principles. The National Medical Research Council approved the study (50935/2012/EKU, 7879/2013/EKU). One part of the biopsies was paraffin-embedded for IHC investigations, while another part of the biopsies was frozen in RNA later (Qiagen, Hilden, Germany) at -80°C until Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) investigations.

Patients	Sex	Age	Localisation	PASI score	Local severity	Local PASI	Duration time (years)	DLQI
PsV-SGP (n=6)								
PsV-SGP 1	F	59	forearm	13.3	9	18	4	N/A
PsV-SGP 2	M	57	shin	13	9	18	19	N/A
PsV-SGP 3	M	46	forearm	17.6	8	24	28	7
PsV-SGP 4	M	28	shin	10.6	8	16	7	8
PsV-SGP 5	M	52	forearm,crook of the arm	38.4	11	33	32	29
PsV-SGP 6	M	34	forearm,crook of the arm	27.6	10	40	27	23
Mean ± SD		46.0 ± 12.6		20.1±10.8	9.2±1.2	24.8±9.7	19.5±11.7	16.7±10.9
Scalp Ps (n=6)								
Scalp Ps 1	F	59	scalp	13.3	8	16	4	N/A
Scalp Ps 2	F	35	scalp	11	9	18	10	17
Scalp Ps 3	M	62	scalp	13.4	6	12	12	18
Scalp Ps 4	M	52	scalp	38.4	10	30	32	N/A
Scalp Ps 5	F	54	scalp	25.8	10	30	35	13
Scalp Ps 6	M	36	scalp	13.1	6	12	14	N/A
Mean ± SD		49.7 ± 11.5		19.2±10.8	8.2±1.8	19.7±8.3	17.8±12.6	16.0±2.6

Table 1. Summary of psoriatic patients' characteristics (in Study II.)
Scoring of psoriasis skin was performed according to the severity of the disease. All patients were moderate to severe. Abbreviations: DLQI, Dermatology life Quality Index; F, female, M, male; N/A, not available; PASI, Psoriasis Area Severity Index; Ps, psoriasis; SD, standard deviation

4.2. Cellular investigations (Study I.)

4.2.1. Cell isolation and cell culturing

Peripheral blood mononuclear cells (PBMCs) were acquired through gradient centrifugation using Ficoll-Paque Plus (GE Healthcare Bio-Science AB, Uppsala, Sweden), from the peripheral blood of both individuals with psoriasis and healthy volunteers. Blood DCs (including both CD1c+ myeloid DCs and pDCs) were isolated from the PBMCs by utilizing the Blood Dendritic Cell Isolation Kit (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany) according to the manufacturer's guidelines. To perform ELISA experiments, CD1c+ DCs were separated with the use of CD1c+ Blood DC isolation kit (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany). Blood DCs were cultured in RPMI 1640 medium (Miltenyi Biotech GmbH), supplemented with 1% antibiotic-antimycotic solution (100 U/ml penicillin, 100 µg/ml streptomycin and amphotericin B, PAA Laboratories GmbH, Pasching, Austria) and 10% FBS (Lonza Group Ltd, Basel, Switzerland), for a duration of 6 hours. For chemokine ELISA investigations, purified CD1c+ myeloid DCs were cultured for 24 hours followed by the collection of their supernatants. A total of 1x 10^5 dendritic cells were cultured per one well of a 96-well plate with a flat bottom, using 200 µl of cell culture medium.

4.2.2. Cell surface marker and intracytoplasmic cytokine staining

To investigate the cytokine/chemokine production, DCs were incubated for 6 hours with a solution of 3 μg/ml Brefeldin A (eBioscience Inc, San Diego, CA, USA) to inhibit their secretion. Then cells were gathered and washed in phosphate-buffered saline (PBS) containing 5% FBS and 0.5 mM EDTA. Cells, resuspended in FACS buffer (PBS enriched with 1% bovine serum albumin) were aliquoted into tubes (one for the negative control, four for staining purposes) and stained in the dark for 30 minutes, at 4°C with the following fluorescent dye-conjugated monoclonal antibodies:

Cell type	**Marker 1**	**Marker 2**
CD1c+/CD11c+ mDCs	anti-CD1c-APC-Cy7	anti-CD11c-APC or anti-CD11c-FITC
pDCs	anti-CD303-PE-Cy7 and anti-CD303 PerCP-Cy5.5	anti-CD304-Pacific Blue
Maturation and activation of the cells	anti-CD86-PE	anti-CD83-PerCP-Cy5.5

Table 2. Identification of different DC populations and their maturation/activation markers

Subsequently, the stained cells were washed with FACS buffer and were fixed in the dark at room temperature (RT) for 20 minutes by using Intracellular (IC) fixation buffer (eBioscience, San Diego, CA, USA). Afterwards, the cells were washed with 1x Permeabilization Buffer (eBioscience), and the pellet that resulted from centrifugation was suspended in 100 μl 1x Permeabilization Buffer. This suspension was then stained in the dark, at RT for 20 minutes. After staining cells were washed with 1x Permeabilization Buffer, followed by another wash with FACS Buffer. The pellet resulting from centrifugation was resuspended in 200 μl FACS buffer and maintained at 4°C until measurement. As negative controls unstained cells were used.

During the experiments the following combinations of the antibodies were applied:

CD11c-APC, CD1c-APC-Cy7, CD303-PE-Cy7, CD304-Pacific Blue	TGFB1-PE, IL-10 PerCP-Cy5.5, IL-6-FITC
CD11c-APC, CD1c-APC-Cy7, CD303-PE-Cy7, CD304-Pacific Blue	IL-23p19-PE, IL-4PerCP-Cy5.5, IL-12-FITC
CD11c-APC, CD1c-APC-Cy7, CD303-PE-Cy7, CD304-Pacific Blue	CCL17-PE, TNFα-PerCP-Cy5.5, IL2-FITC
CD11c-APC, CD1c-APC-Cy7, CD303-PE-Cy7, CD304-Pacific Blue	CD83-PerCP-Cy5.5, CD86-PE

CD11c-APC, CD1c-APC-Cy7, CD303-PerCP-Cy5.5, CD304-Pacific Blue	CXCL8-FITC
CD11c-FITC, CD1c-APC-Cy7, CD303-PerCP-Cy5.5, CD304-Pacific Blue	CXCL9-APC, CXCL10-PE
CD11c-FITC, CD1c-APC-Cy7, CD303-PerCP-Cy5.5, CD304-Pacific Blue	CCL20-APC, CXCL1-PE

Table 3. Antibodies used for cytokine and chemokine staining

Most of these antibodies were procured from Biolegend, San Diego, CA, USA, except for TGFβ, CCL17, CXCL1, and CCL20, which originated from R&D Systems, Minneapolis, MN, USA.

4.2.2. Flow cytometry

To carry out flow cytometric analyses on the fixed cells a Beckman Coulter Navios cytometer (Beckman Coulter, Brea, CA, USA) was used applying eight colour flow cytometric protocol. Typically, 100,000–500,000 cells were acquired, depending on the yield of cell isolation. To detect cells and to exclude debris and clustered cells forward (FSC) and side light scattering (SSC) was applied. The different subtypes of DCs were gated according to their characteristic cell surfice markers: CD11c, CD1c, CD304, CD303, and then the expression of activation markers and the cytokine/chemokine production was investigated in the gated $CD1c^+$ mDC and pDC populations. List mode data (LMD) files were analyzed using Navios software v. 1.1 and Kaluza™ software version 1.2 (Beckman Coulter). Histograms were plotted on 'logicle' axes in Kaluza software.

4.2.3. Chemokine investigations by ELISA

The secretion of CCL20, CXCL8, CXCL9 and CXCL10 (as psoriasis related chemokines) was measured by Quantikine ELISA Kits (CXCL8/IL8, CXCL9/MIG, CXCL10/IP10 and CCL20/MIP-3α kits, R&D Systems) according to the instructions of the manufacturer from the supernatants of $CD1c^+$ mDCS (4 patients and 4 controls were investigated). The detection limits were the following: 10 pg/ml for CXCL1, 3.5 pg/ml for CXCL8, 3.84 pg/ml for CXCL9, 1.67 pg/ml for CXCL10, and 0.47 pg/ml for CCL20.

4.3. Investigations on skin biopsies (Study II.)

4.3.1. Immunohistochemistry

For IHC investigations paraffin-embedded sections from scalp and skin psoriasis samples were used. Subsequent to deparaffinization and rehydration of the samples, a solution of 3% H_2O_2 was applied for 15 minutes to neutralize endogenus peroxidase activity. Following this, antigen retrieval by heat induction was executed in a pressure cooker (full pressure, 120°C,

3-5 minutes) followed by cooling and washing procedures. After blocking with a 1% solution of bovine serum albumin (BSA), the sections were incubated overnight at 4°C with the corresponding primary antibodies.

The following primary antibodies were applied:

Primary antibody	**Identifier**	**Manufacturer**
human CD4	rabbit monoclonal IgG [ab133616]	Abcam, Cambridge, UK
human CD11c	rabbit monoclonal IgG [ab52632]	Abcam
human CD83	mouse monoclonal IgG [ab123494]	Abcam
human CD1a	mouse monoclonal IgG [AM33361PU-T]	Acris, Rockville, MD, USA
human IL-17	rabbit polyclonal IgG [bs-2140R]	Bioss Antibodies, Woburn, MA, USA
human IL-23	rabbit polyclonal [PA5-20239]	Thermo Fisher, Rockford, IL, USA
human IFN-gamma	rabbit polyclonal [NBP1-19761]	Novus Biologicals, Littleton, CO, USA
human TNF-alfa	mouse monoclonal IgG [SAB1404480-100UG]	Sigma-Aldrich, St. Louis, MO, USA
human CCL2/MCP1	mouse monoclonal IgG1 [NBP2-22115]	Novus Biologicals
human CCL20/MIP-3-α	mouse monoclonal IgG [LS-B7409]	LifeSpan Biosciences, Seattle WA, USA
human lipocalin/NGAL	rabbit polyclonal IgG [PA5-32476]	Invitrogen, Life Technoligies, San Fransisco, CA, USA
human S100A8	rabbit polyclonal IgG [HPA024372]	Sigma-Aldrich
human loricrin	rabbit monoclonal IgG [NBP1-33610]	Novus Biologicals
human filaggrin	mouse monoclonal IgG	Abcam
human KRT17	rabbit polyclonal IgG [ab53707]	Abcam

Table 4. Antibodies used for immunohistochemistry staining

Afterwards, anti-mouse/rabbit (Dako from Agilent Technologies, Santa Clara, CA, USA) HRP-conjugated secondary antibody was applied. Before and after incubating with antibodies, samples were washed with TBST for 5 minutes, 3 times in each step. For signal detection Vector® VIP and ImmPACT™ NovaRED™ Kit (VECTOR Laboratories, Burlingame, CA, USA) was used. Methylene green was applied for the background staining of the sections. Detection of each protein was carried out parallelly on all sections.

4.3.2. RNA Isolation, Reverse Transcription and Real-Time Quantitative PCR

Skin samples previously stored in RNA later were homogenized in TriReagent solution (Sigma Aldrich) with Tissue Lyser (QIAGEN, Hilden, Germany) applying autoclaved metal beads (QIAGEN), then total RNA was isolated. The concentration and purity of RNA were assessed on a NanoDrop spectrophotometer (Thermo Scientific, Bioscience, Budapest, Hungary), the quality was checked using Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA, USA). After DNase I treatment (Applied Biosystems, Foster City, CA, USA) a reverse transcription step was fulfilled, using high capacity cDNA Archive Kit (Invitrogen, Life Technologies, San Francisco, CA, USA) according to the instructions of the manufacturer's. 1 ug of total RNA was reverse transcribed into complementary DNA (cDNA). qRT-PCR measurements were carried out in triplicate using pre-designed FAM-MGB assays as well as TaqMan® Gene Expression Master Mix from Applied Biosystems (Life Technologies).
The following primers were used:

Molecules	**Identifier**
PPIA	(Hs99999904_m1),
IL-17A	(Hs00174383_m1),
IL-1β	(Hs00174097_m1),
IL-12B	(Hs01011518_m1)
IL-23	(Hs00900829_g1)
IFNγ	(Hs00174143_m1)
TNFα	(Hs00174128_m1)
CCL2	(Hs00234140_m1)
CCL20	(Hs00355476_m1)
S100A7	(Hs00161488_m1)
S100A8	(Hs00374264_g1)

S100A9	(Hs00610058_m1)
DEFB4B (hBD-2)	(Hs00175474_m1)
LCN2	(Hs01008571_m1)
FLG	(Hs00856927_g1)
KRT17	(Hs00356958_m1)
KRT6A	(Hs01699178_g1)

Table 5. Primers used in qRT-PCR investigations

The q-RT-PCR investigations were performed using a LightCycler® 480 System (Roche, Basel, Switzerland). Using either the comparative Ct method or based on a standard curve relative mRNA levels were calculated, and normalized to the expression of Peptidylprolyl Isomerase A (PPIA) mRNA.

4.4. Statistical analysis

The distribution of the data was analysed with Kolmogorov–Smirnov test. We determined mean ± standard error of mean (SEM) values. In case of normal distribution, independent t-test, in other cases Mann-Whitney U test were used for statistical comparison of two experimental group. Analysis of correlations was performed by Spearman r test.

The p values <0.05 were considered statistically significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Statistical analyses were performed using GraphPad Prism software version 7 (GraphPad Software Inc., San Diego, CA, USA) and SPSS 25 (SPSS package for Windows, Release 25; SPSS Inc., Chicago, Illinois, USA).

5. RESULTS

5.1. Examination of psoriatic blood CD1c$^+$ mDCs and pDCs focusing on their activation and maturation stages, their cytokine and chemokine release (Study I.)

5.1.1. Activation/maturation state of psoriatic CD1c$^+$ mDCs

From the peripheral blood of patients with psoriasis and healthy volunteers CD1c+ mDCs and pDCs were isolated simultaneously, then CD1c+/CD11c+ mDCs were gated from this mixed DC population to investigate the phenotypic characteristics and cytokine/chemokine production of these cells.

The appearance of the CD83 maturation marker was notably higher on CD1c+ mDCs derived from the blood of patients in comparison with control cells (74.9% vs. 30.42%, respectively, p=0.0103, Figure 8.A). Furthermore, a substantial portion of the cells in both diseased and healthy groups exhibited the CD86 activation marker. However, a statistically significant increase in its fluorescence intensity was observed in the psoriatic group (CD86 Mean Fluorescence Intensity (MFI) 3.68 vs. 3.28, respectively, p=0.0445, Figure 8.B).

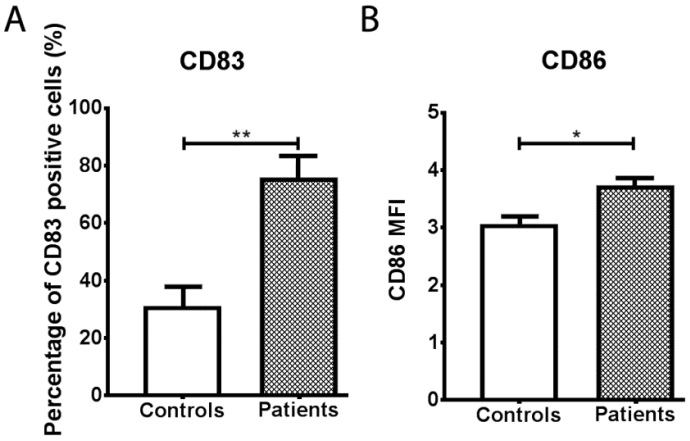

Figure 8. Activation and maturation markers in psoriatic blood mDCs A. *The CD83 maturation marker was expressed significantly more frequently on the surface of psoriatic blood mDCs compared to control cells.* ***B.*** *Significantly increased CD86 fluorescence intensity could be detected in the psoriatic group. Columns represent the mean ± SEM of 12 patients and 9 controls. * $p<0.05$, ** $p<0.01$*

5.1.2. Cytokine production of psoriatic CD1c$^+$ mDCs

The cytokine production of psoriatic blood CD1c$^+$ mDCs were investigated by flow-cytometry using an 8-color staining method. The following cytokines were simultaneously studied: IL-12 as Th1, IL-2 and CCL17 as Th2, TGFβ, IL-23 and IL-6 as Th17, IL-6 and TNFα as Th22, and TGFβ and IL-10 as Treg polarising cytokines.

A significantly higher percentage of CD1c+ mDCs from psoriatic patients exhibited IL-12 production when compared to control cells (68.9% vs. 12.8%, respectively, p=0.013, as illustrated in Figure 9.A). Moreover, not only a higher number of cells produced IL-12 in the psoriatic group, but the quantity of IL-12 within these cells was also significantly elevated (IL-12 MFI: 1.01 vs. 0.75 in psoriatic CD1c+ mDCs vs. control cells, p=0.008, Figure 9.B).

Furthermore, a notable correlation could be observed between the mean fluorescence intensity of IL-12 and the clinical severity index of patients (PASI) (Spearman test, r=0.6110; p=0.0494; Figure 9.C). The number of IL-12-producing cells and PASI also exhibited some correlation, although it was not statistically significant (data not shown).

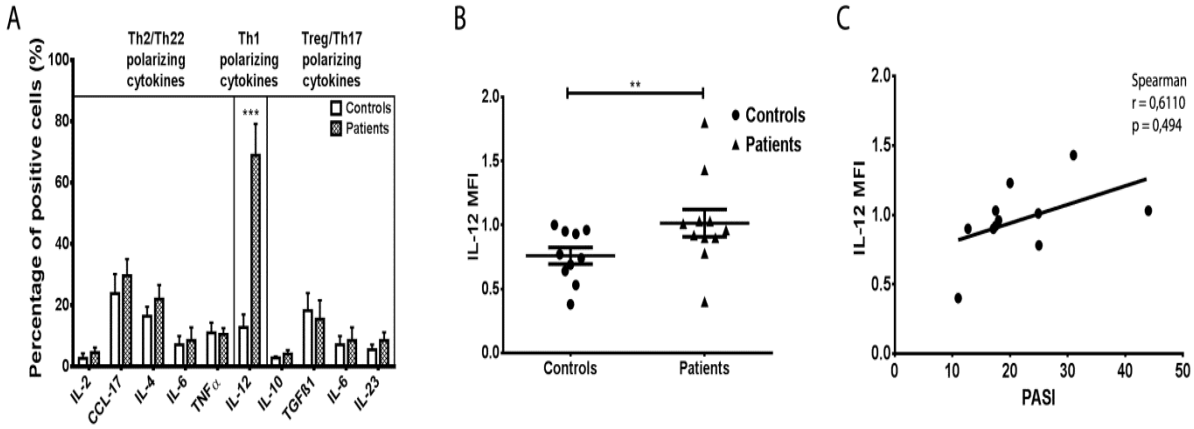

Figure 9. Cytokine production of psoriatic blood CD1c+ mDCs A. *In psoriasis, a significantly higher percentage of mDCs with IL-12-producing ability could be observed then in the control group. The percentages of positive cells are presented as mean ± SEM* ***B****. Both the number of IL-12-positive cells and the quantity of IL-12 produced by these cells were significantly elevated in psoriatic mDCs compared to control cells (MFI: 1.01 vs. 0.75 respectively, p=0.008).* ***C.*** *The IL-12 MFI of mDCs exhibited a significant correlation with the Psoriasis Area and Severity Index (PASI). (Spearman test, r=0,6110; p=0,0494). Number of patients n=12; controls n=9; **p<0.01; *** p<0.001*

5.1.3. Characteristics of psoriatic blood pDCs

Blood pDCs were selected from the "DC cocktail" obtained by the Blood DC isolation kit with the following gating strategy: CD11c-/CD1c-/CD303+(BDCA2+)/CD304+ (BDCA4+), and their maturation/activation state along with their cytokine production were investigated.

According to our results pDCs displayed an inactive phenotype, as they were failed to exhibit either the CD83 maturation or the CD86 activation markers. When the function of pDCs was investigated, our focus was on the detection of key cytokines associated with psoriasis, namely IL-6, IL-12 and IL-23 as well as IFNα, which is thought to be the primary cytokine released by pDCs in the skin of psoriatic patients. However, none of these cytokines were detectable in the blood pDCs of patients with psoriasis (data not shown).

5.1.4. Chemokine production of psoriatic blood CD1c$^+$ mDCs and pDCs

5.1.4.1. Investigation of the intracytoplasmic chemokine production by flow-cytometry

First, we assessed the chemokine production of both CD1c+ mDC and pDCs from the blood of psoriatic patients by flow cytometry. We investigated chemokines that had been previously reported to be produced by psoriatic skin DCs [15], including CXCL1, CXCL8, CXCL9 and CXCL10 as Th1 chemokines, and CCL20 as a Th17 recruiting chemokine.

While the frequency of chemokine-producing DCs was nearly similar in patients and controls, our study unveiled that psoriatic blood CD1c+ mDCs exhibited a significantly higher capacity to produce CXCL9 and CCL20 (CXCL9 MFIs: 3.83 vs. 1.72, $p<0.0001$; CCL20 MFIs: 3.14 vs. 1.36, $p=0.0275$ in patient and control groups, respectively, Figure 10). Additionally, CD1c+ mDCs from patients produced slightly higher amounts of CXCL8 and CXCL10, although these differences were not statistically significant (CXCL8 MFIs: 2.85 vs. 2.56; CXCL10 MFIs: 0.85 vs. 0.78; Figure 10.). CXCL1 production was nearly identical in both mDC groups (CXCL1 MFIs: 1.05 vs. 1.01, not significant). The chemokine amount produced by psoriatic blood pDCs was minimal when compared to CD1c+ mDCs (Figure 10.) and resembled the levels observed in control cells.

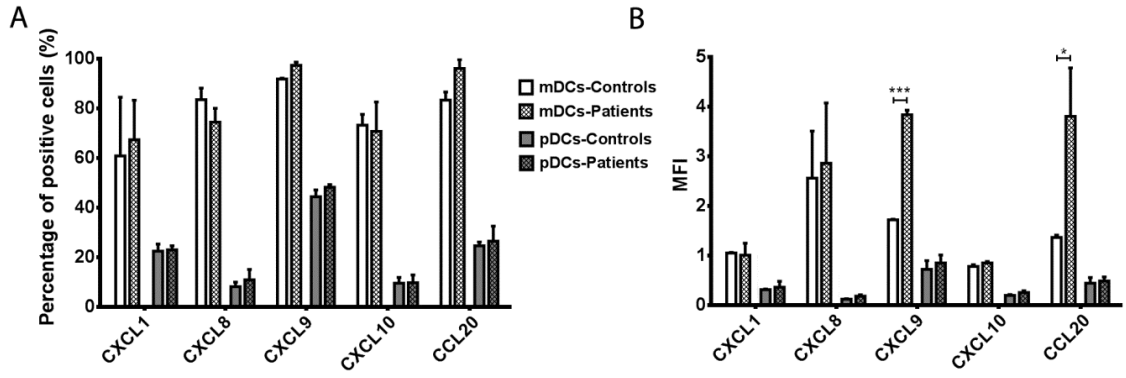

Figure 10. Chemokine production of blood mDCs and pDCs investigated by flow-cytometry *Blood CD1c+ myeloid dendritic cells (mDCs) demonstrated a notably higher production of CXCL9 and CCL20, and this difference was statistically significant. In contrast, the production of chemokines by plasmacytoid dendritic cells (pDCs) was minimal in comparison to mDCs. The columns in the figure represent the mean ± SEM of results obtained from 5 patients and 4 control individuals. * p<0.05, *** p<0.001*

5.1.4.2. Investigation of the chemokine secretion by ELISA

Since our investigations revealed, that CD1c+ mDCs are able to produce different Th1/Th17 recruiting chemokines, we conducted further investigations to quantify their release by ELISA from their supernatant.

We have found that psoriatic blood CD1c+ mDCs could release a significantly higher amount of CCL20, along with noticeably higher quantities of CXCL8 and CXCL10 compared to control cells (CCL20 mean concentrations: 47.2 pg/ml vs. 4.9 pg/ml, p=0.0042; CXCL8 mean concentrations: 29.9 ng/ml vs. 14.01 ng/ml, not significant; CXCL10 mean concentrations: 92.07 pg/ml vs. 25.2 pg/ml, not significant, Figure 11.).

Surprisingly, in contrast to the high CXCL9 production revealed by the flow cytometry investigations, we could not detect CXCL9 at a notable level in the supernatant of either psoriatic or control CD1c+ mDCs by ELISA (data not shown).

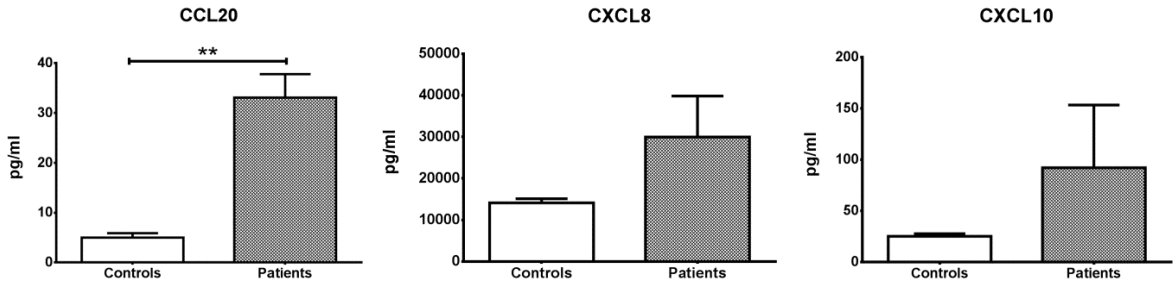

Figure 11. Chemokine production of blood CD1c+ mDCs investigated by ELISA

*Psoriatic CD1c+ mDCs produced significantly higher amounts of CCL20 and markedly higher amounts of CXCL8 and CXCL10 compared to control DCs. The columns in the figure represent the mean ± SEM of results obtained from 4 patients and 4 controls, with ** indicating statistical significance at $p<0.01$.*

5.2. Comparison of the immune and barrier characteristics in psoriasis vulgaris on SGP skin and scalp psoriasis (Study II.)

In the second part of our investigations we compared the immune characteristics of psoriasis vulgaris on SGP skin and scalp psoriasis on SGR skin to determine whether the inflammation developed in the 2 subtypes of psoriasis are influenced by the primarily distinct immune milieu of the different healthy skin areas. Since psoriasis is a Th1/Th17-mediated disorder, therefore the presence of different immune cells (T cells, DC, Langerhans cells), the Th1- and Th17-related immune alterations, like representative cytokines of Th1 pathway (IFNγ and IL-12) together with the production of Th17 related cytokines (IL-17, IL-23), chemokines (CCL2 and CCL20) and antimicrobial peptides (S100A7/8/9, LCN2, DEFB4B) were investigated in the two groups of psoriatic skin samples. The most common pro-inflammatory cytokines (IL-1β and tumor necrosis TNFα) and barrier molecules (LOR, FLG, KRT) were also studied. The lesional skin samples of patients with psoriasis vulgaris on SGP skin and scalp psoriasis (each n=6) were examined by immunohistochemistry and/or qRT-PCR.

5.2.1. T cells and DCs in psoriasis vulgaris on SGP skin and scalp psoriasis

In skin samples from psoriasis vulgaris on SGP region and scalp psoriasis the presence of different immune cells such as $CD4^+$ T cells, $CD11c^+$ mDCs and $CD1a^+$ LC were analysed by IHC methods. Immunostaining found no significant differences in the presence of $CD4^+$ T cells and $CD1a^+$ LCs between the two psoriatic groups. Although the number $CD11c^+$ dermal DCs were higher in scalp psoriasis compared to psoriasis vulgaris, the difference was not significant (Figure 12.).

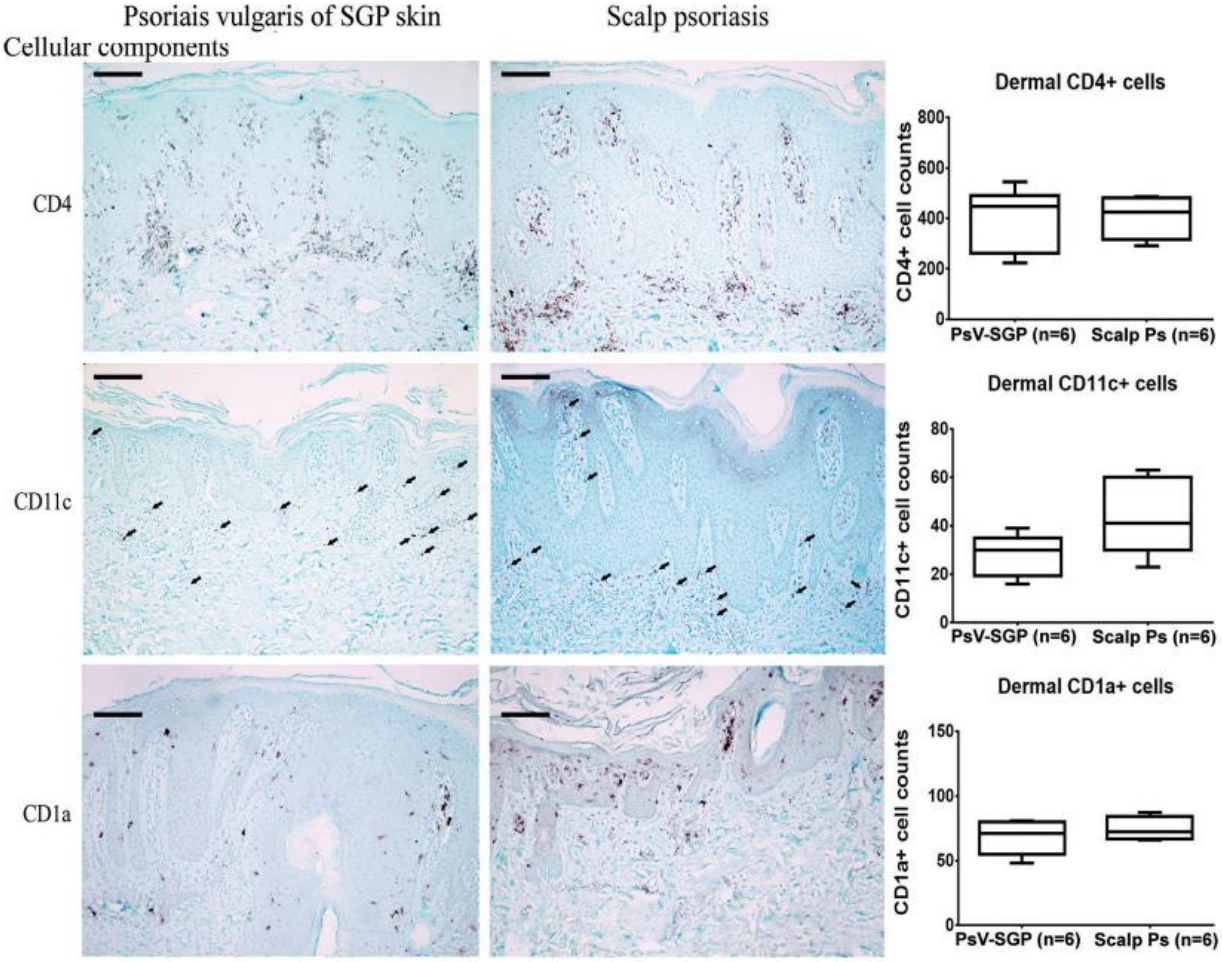

Figure 12. Representative images and quantification of the IHC stainings regarding the cellular components in psoriasis vulgaris and scalp psoriasis samples *In both groups, we examined a total of 6 skin samples for all the investigated cell types. Cell counts were analyzed blindly using Pannoramic Viewer software. Size bars=50 μm. Arrows indicate the positive cells. The graphs display the mean values, along with the corresponding 95% upper and lower confidence intervals, as well as the maximum and minimum values. (*p < 0.05; as determined by independent t-test). Abbreviations: Ps psoriasis; SGP sebaceous gland poor*

5.2.2. Investigation of Th1 and Th17 cytokines and Th17 related chemokines in psoriasis vulgaris on SGP skin and scalp psoriasis

Immunostaining of Th17 related cytokines IL-17 and IL-23 and the Th1 related IFNγ showed similar expression pattern in psoriasis vulgaris and scalp psoriasis (Figure 13.).

qRT-PCR analyses of the IL-17/IL-23 and IFNγ/IL-12 cytokines indicated similar patterns to that we found at the protein level, their expression did not differ significantly between the two groups (Table 6.).

As the next step, we examined the expression of well-known pro-inflammatory cytokines, specifically IL-1β and TNF-α. TNF-α was subjected to investigation both at the protein and the mRNA levels, while IL-1B was determined at the mRNA level only. IHC demonstrated the presence of TNF-α producing cells in similar numbers in both psoriatic groups, moreover qRT-PCR data revealed similar mRNA expressions of IL-1β and TNF-α in the investigated groups (Figure 13. and Table 6.).

Furthermore, we investigated the Th17-related chemokines, CCL2 and CCL20. In the case of CCL2 we could not detect differences between the two groups either at the protein, or at the mRNA level (Figure 13., Table 6.). Although, when IHC was employed, the expression of CCL20 was found to be significantly higher in scalp psoriasis than in psoriasis vulgaris, no significant differences were observed between the two investigated groups at the mRNA level (Figure 13., Table 6.).

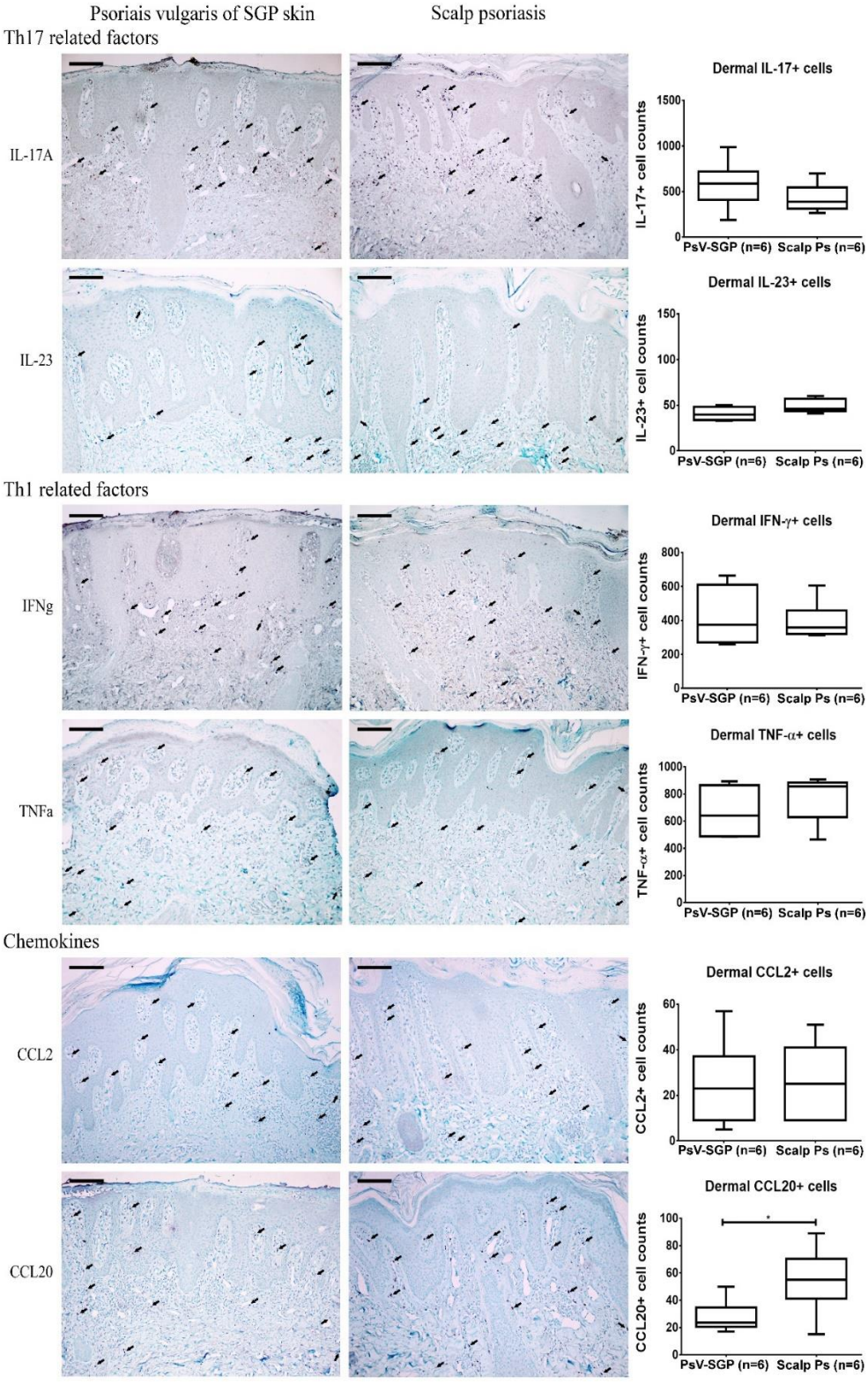

Figure 13. Representative images and quantification of the IHC staining regarding the Th1/ Th17-related cytokines and Th17 chemokines in psoriasis vulgaris and scalp psoriasis samples *All the investigated molecules were examined in 6 samples in both of the two groups. Cell counts were analyzed blindly using Pannoramic Viewer software. Size bars=50 μm. Arrows indicate the positive cells. The graphs display the mean values, along with the corresponding 95% upper and lower confidence intervals, as well as the maximum and minimum values of protein levels (*p < 0.05, as determined by an independent t-test). Abbreviations: CCL, Chemokine (C-C motif) ligand; IFN-γ, interferon gamma; IL, interleukin; Ps psoriasis; TNF, tumor necrosis factor*

Th1 markers	qRT-PCR		IHC	
	p	FC Scalp Ps/PSV-SGP	p	FC Scalp Ps/PSV-SGP
IFNG	0.26	1.59	0.91	1.42
IL-12	0.23	1.48	nd	
TNFA	0.20	1.08	0.56	1.46
Th17 markers	qRT-PCR		IHC	
	p	FC Scalp Ps/PSV-SGP	p	FC Scalp Ps/PSV-SGP
IL-17A	0.99	0.99	0.31	0.74
IL-23	0.83	0.66	0.17	1.52
IL-1B	0.22	2.03	nd	
Innate immune/ proinflammatory molecules	qRT-PCR		IHC	
	p	FC Scalp Ps/PSV-SGP	p	FC Scalp Ps/PSV-SGP
IL-1B	0.22	2.03	nd	
TNFA	0.20	1.08	0.56	1.46
Chemokines	qRT-PCR		IHC	
	p	FC Scalp Ps/PSV-SGP	p	FC Scalp Ps/PSV-SGP
CCL2	0.62	0.96	0.74	0.67
CCL20	0.12	2.31	**0.02 (*)**	**1.96**
Antimicrobial peptides	qRT-PCR		IHC	
	p	FC Scalp Ps/PSV-SGP	p	FC Scalp Ps/PSV-SGP
S100A7	0.13	1.17		
S100A8	0.37	1.01	0.70	1.26
S100A9	0.47	1.27	nd	
DEFB4B	0.69	1.37	nd	
LCN2	**0.005**	**1.95**	0.25	0.44
Barrier molecules	qRT-PCR		IHC	
	p	FC Scalp Ps/PSV-SGP	p	FC Scalp Ps/PSV-SGP
LOR	0.53	0.96	0.48	1.06
FLG	0.14	1.45	0.79	1.04
KRT6A	0.7	0.57	nd	
KRT17	0.34	0.69	0.31	0.7

Table 6. Comparison of immune and barrier components' expression in psoriasis vulgaris and scalp psoriasis

Statistical analyses on mRNA and protein levels were carried out using independent t-test or Mann-Whitney U test. The significantly different data are highlighted in bold letters. A total of 6 samples were examined in each group for all the investigated molecules.

Abbreviations: CCL, Chemokine (C-C motif) ligand; DEFB4B, human beta defensin2; FC, fold change; FLG, filaggrin; IFNG, interferon gamma; IL, interleukin; KRT, keratin; LCN, lipocalin; LOR, loricrin; nd, not determined; qRT-PCR, quantitative real-time PCR; TNFA, tumor necrosis factor alpha

5.2.3. Th17 related AMPs and barrier molecules in psoriasis vulgaris on SGP skin and scalp psoriasis

In the next part of our experiments, we investigated the Th17-related AMPs as further components of the innate immune response, and also the barrier molecules. We aimed to assess the mRNA levels of AMPs (S100A7/8/9, DEFB4, LCN2), moreover for lipocalin (LCN2) and S100A8, immunostaining was also conducted.

The expression of the investigated AMPs showed no significant differences either at the mRNA or protein level between the two groups except for lipocalin (Table 6., Figure 14.). By the means of RT-qPCR, mRNA level of LCN2 was found to be significantly higher in scalp psoriasis compared to psoriasis vulgaris (Table 6.).

In the final step, we examined the principal molecules that play roles in the formation and maintenance of the epidermal barrier (LOR, FLG, KRT6, KRT17) using qRT-PCR. Additionally, loricrin (LOR), filaggrin (FLG), and keratin 17 (KRT17) were also evaluated at the protein level by IHC. According to our results, no significant differences can be observed between the two groups in the expressions of the investigated molecules either at the mRNA or at the protein level (Table 6., Figure 14.).

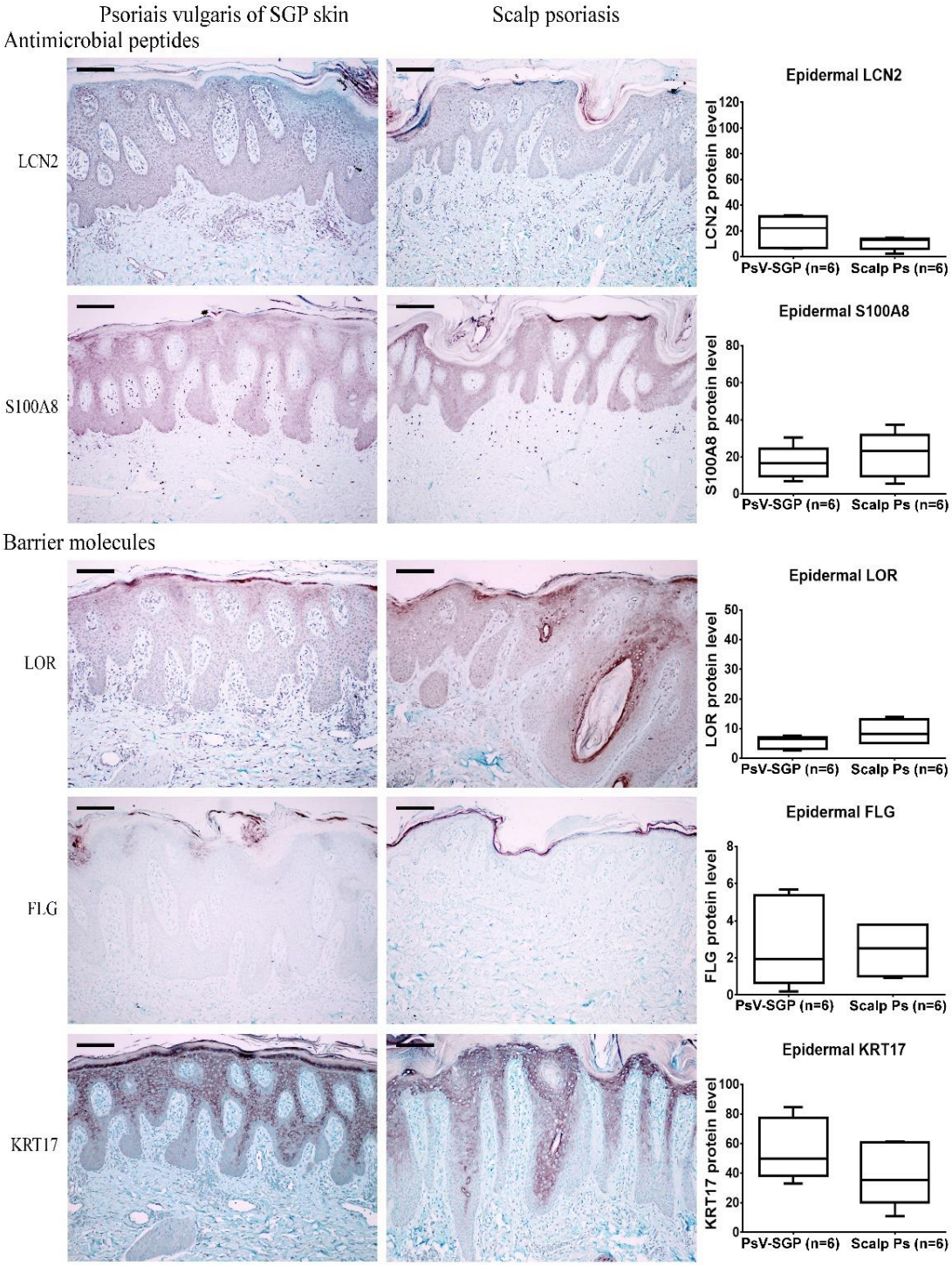

Figure 14. Representative images and quantification of the immunostaining regarding the Th17 related AMPs and barrier molecules in psoriasis vulgaris and scalp psoriasis *6 samples were examined in each group regarding all the investigated molecules. Cell counts were analyzed using Pannoramic Viewer software, in a blind manner. Size bars=50 μm. The graphs depict the mean values, along with the corresponding 95% upper and lower confidence intervals, as well as the maximum and minimum values of protein levels. Abbreviations: AMP, antimicrobial peptide; FLG, filaggrin; KRT, keratin; LCN, lipocalin; LOR, loricrin; Ps psoriasis*

6. DISCUSSION

Study I.

Since psoriasis is one of the most common immune-mediated skin diseases, it is essential to explore every step in its pathogenesis. In the last decade the characteristics, and role of the different skin DC subpopulations in the psoriasis pathogenesis were well explored due to their extensive investigation (103). While it is clearly established that psoriatic skin DCs express CD83 and CD86 maturation/activation markers (139) and release IL-12 and IL-23 cytokines (27, 100, 103, 104), the phenotype and cytokine production of their peripheral blood precursors have been scarcely explored, the contribution of blood DCs, as potential precursors of skin DCs, in the pathogenesis of psoriasis is less studied (140). Therefore, in our first study we investigated the characteristics of blood DCs (CD1c+ mDCs and pDCs) from psoriatic patients. Our findings may contribute to fill this knowledge gap.

By investigating blood precursor DCs, we aimed to reveal whether psoriatic DCs are already influenced in the bloodstream, by certain disease-specific factors or they acquire their specific characteristics exclusively within the skin microenvironment. These questions can be answered by direct investigations of DCs separated from peripheral blood, rather than using monocyte derived DCs differentiated in vitro. While this approach may result in a smaller number of cells from the same volume of blood, it can provide more information about the physiological condition of these cells.

In our study we were focusing on the investigation of the phenotype and function (cytokine/chemokine production) of these less-characterised cells, therefore first, the expression of CD83 activation and CD86 maturation markers of blood CD1c+ mDCs were investigated simultaneously with their Th polarising capacity. While usually DCs in the circulation are inactive, psoriatic blood CD1c+ mDCs seem to be in a pre-mature state, as suggested by their significantly elevated expression of CD83 and CD86 markers revealed by our investigations (Figure 15.).

IL-12, one of the principal cytokines in psoriasis, is known to be produced by skin mDCs. However, according to our results, their blood precursors can also serve as potential sources of this cytokine, since circulating mDCs also produced significantly high levels of IL-12, moreover their production was correlated with the disease severity (27, 100, 103, 104).

Not only the cytokine production, but their chemokine secretion can also prove the pre-activated status of psoriatic CD1c+ mDCs. According to our results, they can produce CXCL9 and CCL20 in significantly higher, while CXCL8 and CXCL10 in a markedly higher amounts

than the control cells. These findings suggest that blood CD1c+ mDCs in psoriatic patients possess an increased potential to produce Th1/Th17 recruiting chemokines. Our results are in line with that of Fujita et al. (30), who could also observe a similar production of Th1/Th17 recruiting chemokines in psoriatic skin DCs.

Our investigations on the phenotypic and functional characteristics of blood pDCs revealed a different picture. In contrast to CD1c+ mDCs, pDCs exhibited an inactive phenotype, since neither CD83 maturation nor CD86 activation markers could be detected on their surface, moreover these cells did not produce psoriasis-related cytokines, or a significant amount of chemokines. These findings support the hypothesis proposed by Nestle et al. that, the peripheral blood precursors of pDCs are in an inactive state despite of the fact, that skin pDCs are one of the most crucial cell types in the initiation of psoriasis. It is probable, that pDCs only become activated in the psoriatic skin microenvironment, locally (Figure 15.) (97).

In summary, our investigations on the cell surface markers and cytokine/chemokine production in blood precursor DCs in psoriatic patients revealed distinct behaviors. While pDCs appear to be functionally inactive in the bloodstream, CD1c+ mDCs are in a pre-mature state and have the capacity to produce disease-specific mediators already in the peripheral blood. The absence of maturation/activation markers and the functional inactivity of pDCs suggest that their activation primarily occurs within the skin microenvironment. In contrast, the pre-mature status of psoriatic CD1c+ mDCs, as proven by their phenotypic and functional properties, suggests that their maturation is markedly influenced not only by the skin milieu but also by the proinflammatory blood milieu, which is likely a consequence of the primarily occurring skin inflammation (Figure 15.).

A special combination of different inflammatory cytokines is characteristically present in the circulation of patients with severe psoriasis, involving TNF-α, IFNγ, together with interleukins such as IL-6, IL-8, IL-12, and IL-18 (141). From this cytokine set, TNF-α, IL-6, and IFN-γ may contribute to the pre-mature state of blood CD1c+ mDCs (61), and upon entering the skin the activation state of these pre-activated cells can be further rapidly enhanced, potentially leading to a vicious cycle.

Novel biological agents, such as anti-TNF-α therapy, could have a dual beneficial impact by modifying the effects of these cytokines both in the skin and in the bloodstream of patients. This could influence the maturation and function of pre-DCs in the blood.

To summarise our study, our results suggest that besides skin-derived mDCs, peripheral blood CD1c+ mDCs from psoriatic patients also have the potential to produce disease-specific

Th1/Th17 cytokines and chemokines, while pDCs seem to primarily function within the skin microenvironment.

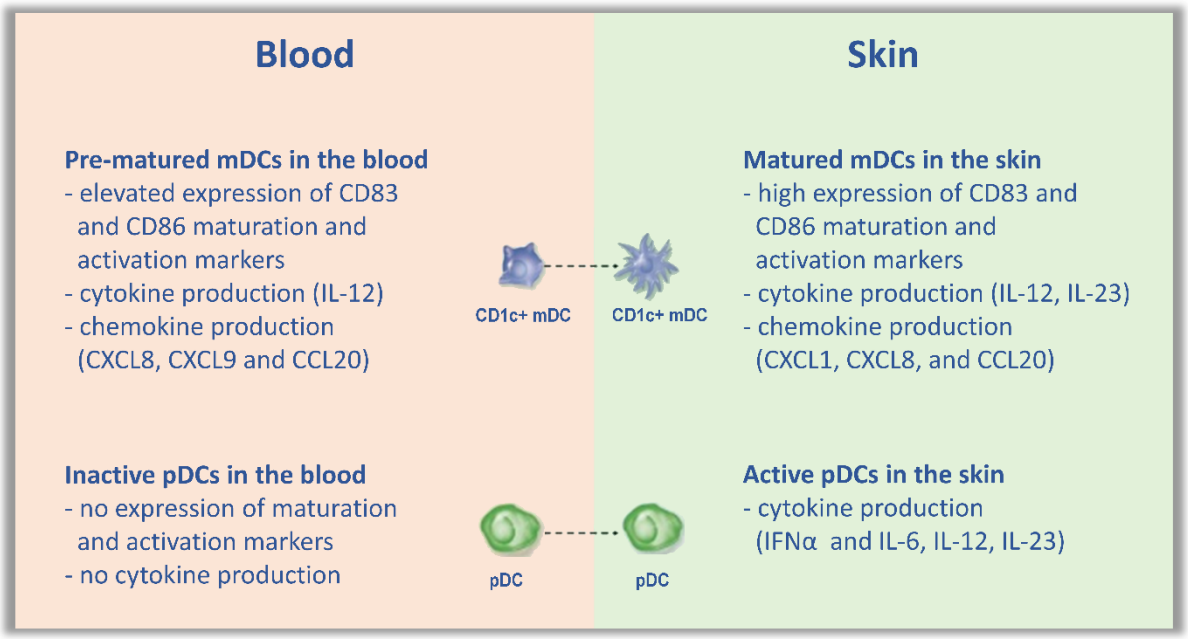

Figure 15. Psoriatic blood myeloid DCs are in a pre-mature state while pDCs become activated only in the skin *Abbreviations: CCL, Chemokine (C-C motif) ligand; CXCL chemokine (C-X-C motif) ligand; IFN, interferon; IL, interleukin*

Study II.

In the second part of our investigations we studied the psoriasis subtypes occuring in different skin regions. In the last decade it was revealed, that beside the different anatomical properties and chemical milieus, variations in microbiota composition and distinct immune and barrier properties characterise different skin areas (55-57, 82, 83) the sebaceous gland poor (SGP), sebaceous gland rich (SGR), and apocrine gland rich skin regions (AGR) (55, 82).

It was observed earlier, that some immune-mediated skin diseases have special region preferences, for example, atopic dermatitis (AD) primarily localizes in SGP, acne and rosacea appear mostly in SGR, and hidradenitis suppurativa occurs in AGR skin areas. In contrast to these region-specific inflammatory skin diseases, other skin diseases, like psoriasis can develop in any skin areas. Psoriatic plaques can manifest in all skin regions, since lesions can appear in SGP skin areas (psoriasis vulgaris or skin psoriasis), SGR areas (scalp psoriasis), and AGR regions (inverse psoriasis).

In our study, we compared the cellular and molecular immune characteristics of psoriasis vulgaris in SGP skin and scalp psoriasis (developing in SGR skin regions) to determine whether the immune characteristics of the two subtypes of psoriasis are influenced by the specific immune milieu of the respective skin regions. In addition to the investigation of

disease-specific innate ($CD11c^+$ mDCs, LCs) and adaptive immune cells ($CD4^+$ T cells), we compared the expression of Th17 related cytokines (IL-17, IL-23), chemokines (CCL2, CCL20), AMPs (S100A7/8/9, DEFB4B, LCN2), and barrier-related molecules (KRT6, KRT17, LOR, FLG). Moreover, the representative cytokines of the Th1 pathway (IFNγ, IL-12) and the most common inflammatory cytokines (IL-1β, TNFα) were also studied.

According to our results, the mediators of both innate immune responses and Th1/Th17 adaptive immune pathways were similarly expressed in psoriasis vulgaris and scalp psoriasis. Similarly, no significant differences in the expression of barrier molecules could be detected. Significant differences were only found in the expression of the *LCN2* mRNA (encoding lipocalin-2) and the chemokine CCL20, which were elevated in scalp psoriasis. Since these parameters were elevated even in healthy SGR skin (55), these differences may reflect the basic immune characteristics of SGR skin region rather than a psoriasis-related feature (Figure 16.).

To our knowledge, so far only a few publications investigated the differences in the immune characteristics of psoriasis in different skin areas (142-145) and only two publications were focusing on the comparison of skin and scalp psoriasis (142, 143). However, they applied different methods and the focus of their research was also different from ours; furthermore, barrier components were not examined by these research groups at the protein level either (142, 143). Moreover, these two publications have drawn contradictory conclusions. Generally, our results are aligning with Ruano' result, since in spite of some minor differences in transcriptomic level, they concluded that the immune characteristics of the two psoriasis subtypes are quite similar (142). In the other publication, Ahn at al. compared scalp, palmoplantar, and conventional plaque psoriasis by RNA Seq and flow cytometry. In this investigation, subtype-specific signalling pathways were identified by Ingenuity Pathway Analysis between the distinct psoriatic subtypes, and, according to their flow-cytometric analysis the IL-17, IFN-γ and IL-22 production of the different subtypes is also different (143). Although these results seem to be contrary to ours, these differences in cytokine production were only significant when plaque-type and palmoplantar psoriasis were compared, or when psoriasis subtypes were compared to the control group. The direct comparison of scalp and conventional plaque psoriasis did not reveal significant differences. In light of our results, the appearent contradiction between these two studies becomes is clearer. In a third study, chronic plaque psoriasis and inverse psoriasis characteristic of AGR skin were compared by immunohistochemistry. In this case, IL-17 was identified as the major shared pathway linking the investigated manifestations of psoriasis (146). These results support our findings showing that psoriasis localised to different skin parts share similar IL-17 related immune characteristics.

In clinical practice, the treatment of scalp psoriasis is considered more difficult than skin psoriasis, since the high density of hair follicles and pilosebaceous units makes the application of local therapy and phototherapy technically complicated (78-81). Therefore, new formulations, such as foam or gel, were developed as new treatment modalities for this region (147, 148). However, the application of different active ingredients in the treatment of scalp psoriasis and skin psoriasis has not emerged. Clinical practice and studies show that biological treatments (ustekinumab, adalimumab, secukinumab, and ixekizumab) have the same efficacy for psoriasis vulgaris of SGP skin and scalp psoriasis (149-154). These findings are supported by our study showing that in spite of the significant differences between healthy SGR and SGP skin immune milieu, psoriatic plaques developing in these distinct areas bear similar cellular (T cell, DC), molecular (cytokine, chemokine), and barrier characteristics (Figure 16.). Furthermore, these data suggest that although the formulation of the local therapy needs to be different for psoriasis localized to the scalp versus skin areas, there is no indication that active ingredients with different mechanisms-of-action for scalp and skin psoriasis need to be developed.

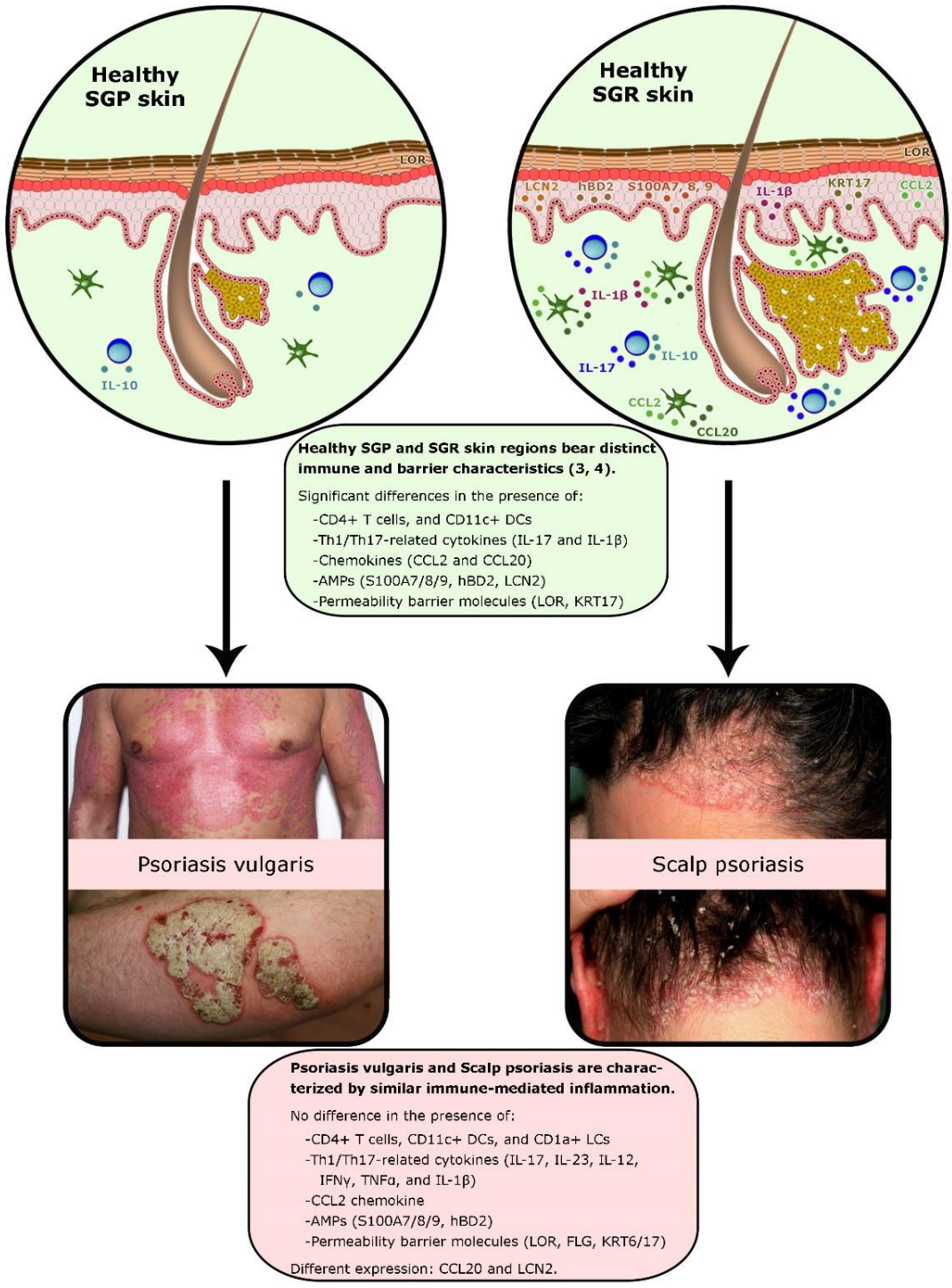

Figure 16. Psoriasis localized to distinct skin regions share similar cellular, molecular and barrier characteristics *Abbreviations: AMP, antimicrobial peptide; CCL, Chemokine (C-C motif) ligand; hBD2, human beta defensin2; FLG, filaggrin; IFNG, interferon gamma; IL, interleukin; KR, keratin; LCN, lipocalin; LOR, loricrin; SGP, sebaceous gland poor; SGR, sebaceous gland rich; TNFA, tumor necrosis factor alpha*

7. SUMMARY

Psoriasis is one of the most common immune-mediated skin diseases. Although its pathomechanism has been extensively studied, there are still unexplored areas. The crucial role of skin-resident dendritic cells (DCs) in the development and maintenance of the disease has long been recognized. However, the characteristics and functions of one of their presumed precursors - the blood-circulating DCs - have not yet been fully elucidated. Therefore, we examined the activity of circulating DCs (myeloid and plasmacytoid DCs) in the blood of patients with psoriasis, as well as their cytokine and chemokine production. We have found that psoriatic blood mDCs are in an early maturation phase and are capable of producing Th1 type cytokines (IL-12) and inflammatory chemokines (CXCL9, CCL20, CXCL8 and CXCL10). These findings suggest that not only skin DCs, but also their blood precursors can serve as potential source of cytokines and chemokines, since CD1c+ mDCs in psoriatic patients possess an increased potential to produce disease-specific mediators even in the peripheral blood. On the other hand, pDCs appear to be functionally inactive in the bloodstream, suggested by the lack of maturation/activation markers and their functional inactivity. According to these results blood pDCs' activation primarily occurs within the skin microenvironment.

The sebaceous gland-rich and gland-poor regions of healthy skin are characterised by different physical, chemical, microbiological and immunological barriers. This may contribute to the fact that certain immune-mediated skin diseases are localised to certain skin regions, such as atopic dermatitis, which is a disease of the gland-poor regions, or rosacea, which is a disease of the sebaceous gland-rich regions. There are also skin diseases such as psoriasis, which affects both gland poor (psoriasis vulgaris) and sebaceous (scalp psoriasis) regions. We compared the cellular and molecular immune characteristics of psoriasis vulgaris in SGP skin and scalp psoriasis to investigate, whether the immune characteristics of the two subtypes of psoriasis are influenced by the specific immune milieu of the skin regions where they develop. We studied the disease-specific innate and adaptive immune cells, the expression of Th1/Th17 related cytokines, chemokines, AMPs, and barrier-related molecules at mRNA and protein levels.

We have found that both innate immune responses, Th1/Th17 adaptive immune pathways and the expression of barrier molecules are similar in psoriasis vulgaris and scalp psoriasis, since we could detect only some minor differences between the two conditions.

According to our results psoriasis localised to different skin parts share similar IL-17 related immune characteristics, therefore to develop active ingredients with different mechanisms-of-action for psoriasis vulgaris and scalp psoriasis is unnecessary.

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List of publications related to the dissertation

1. Gáspár, K., Jenei, A., **Khasawneh, A.**, Retzlerné Medgyesi, B., Dajnoki, Z., Janka, E. A., Szabó, I. L., Hendrik, Z., Méhes, G., Szegedi, A., Kapitány, A.: Comparison of Immune and Barrier Characteristics in Scalp and Skin Psoriasis.
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9. KEYWORDS

psoriasis vulgaris, scalp psoriasis, dendritic cells, skin-regions, Th17 related molecules, therapy

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11. APPENDIX

1. Khasawneh A, Baráth S, Medgyesi B, Béke G, Dajnoki Z, Gáspár K, Jenei A, Pogácsás L, Pázmándi K, Gaál J, Bácsi A, Szegedi A, Kapitány A.: Myeloid but not plasmacytoid blood DCs possess Th1 polarizing and Th1/Th17 recruiting capacity in psoriasis. *Immunol Lett.* 2017 Sep;189:109-113. doi: 10.1016/j.imlet.2017.04.005.

2. Gáspár K, Jenei A, Khasawneh A, Medgyesi B, Dajnoki Z, Janka EA, Szabó IL, Hendrik Z, Méhes G, Szegedi A, Kapitány A.: Comparison of Immune and Barrier Characteristics in Scalp and Skin Psoriasis. *Acta Derm Venereol*. 2020 Jul 2;100(14):adv00203. doi: 10.2340/00015555-3553.