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

Investigation of the innate and adaptive immune components in psoriasis

by Ahmad Mohammad Khaleel Khasawneh

Supervisor: Anikó Kapitány, PhD

UNIVERSITY OF DEBRECEN

GYULA PETRÁNYI DOCTORAL SCHOOL OF ALLERGY AND CLINICAL IMMUNOLOGY

DEBRECEN, 2025

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Supervisor: Anikó Kapitány, PhD

Gyula Petrányi Doctoral School of Allergy and Clinical Immunology,
University of Debrecen

Head of the Examination Committee: Péter Antal-Szalmás, MD, PhD, DSci

Members of the Examination Committee: Árpád Lányi, PhD

Kornélia Szabó, PhD

The Examination takes place at the Library of the Department of Dermatology, Faculty of Medicine, University of Debrecen, 10:00, 11. April, 2025

Head of the Defense Committee: Péter Antal-Szalmás, MD, PhD, DSci

Reviewers: Johanna Mihály, PhD

Balázs Mayer, PhD

Members of the Defense Committee: Kornélia Szabó, PhD

Árpád Lányi, PhD

The PhD Defense takes place at the Lecture Hall of Department of Internal Medicine, Building "A", Faculty of Medicine, University of Debrecen, 13:00, 23rd January 2026.

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. 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. 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.

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. Additionally, we have established that AGR and GP skin also exhibits distinct immune characteristics. 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. 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.

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).

2.2. 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. 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. 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.

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. 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. 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. Moreover, parallel with these, topographically distinct immune and barrier features have also been described on these skin areas as described above.

2.2.1. Pathogenesis of psoriasis

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.

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. 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. With this step, they induce the IFNα dependent maturation of mDCs which cells are responsible for further maintenance and amplification of the inflammatory processes by activating and polarising different T cells subsets. The number of $CD11c^+$ mDCs in the dermis of psoriatic skin is 30-fold higher then in uninvolved or healthy skin. In psoriasis they have two distinct populations: the $CD11c^+$ $CD1c^-$ inflammatory DCs (called TIP-DCs) and the $CD11c^+$ $CD1c^+$ resident DCs (dermal DCs - DDCs). TIP-DCs, produce TNFα and iNOS, moreover, together with the DDCs, they are important sources of Th17 activating cytokine IL-23. Moreover $CD1c^+$ DDCs also produce the Th1 polarizing cytokine, IL-12.

Besides producing T cell polarizing cytokines DCs also secrete different chemokines. The level of several chemokines is elevated in psoriatic skin, and skin DCs were found to be important sources of CXCL1, CXCL8, CXCL9, CXCL10 as well as of CCL20. CXCL1, CXCL9 and CXCL10 are chemoattractants for Th1 lymphocytes, CXCL8 recruits neutrophils, while CCL20 attracts Th17 lymphocytes, DCs and monocytes. 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.

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, 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, but later experimental and clinical evidences highlighted the outstanding role of IL-23/IL-17 pathway in its pathogenesis. 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. IL-23 stimulates differentiation, activation, proliferation, and survival of Th17 cells that promote production of effector cytokines such as IL-17A and IL-22.

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, data from in vitro and clinical studies indicate, that among them IL-17A is the cytokine that principally drives changes within affected tissues. 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.

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.

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 (StudyI.)

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 matcing. 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 wer 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. Skin biopsies (Study II)

For the second study 7mm punch biopsies were collected from lesional skin of 12 psoriatic patients (6 biopsies from scalp psoriasis and 6 from classical plaque type psoriasis vulgaris) 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 immunohistochemistry (IHC) investigations, while another part of the biopsies was frozen in RNA later (Qiagen, Hilden, Germany) at -80°C until qRT-PCR investigations.

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 antibiotics (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 3µg/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 0.5mM 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: CD1c+/CD11c+ mDCs (anti-CD1c-APC-Cy7; anti-CD11c-APC or anti-CD11c-FITC); pDCs (anti-CD303-PerCP-Cy5.5 or anti-PerCP-Cy7 and anti-CD304-Pacific Blue); maturation and activation markers of the cells (anti-CD86-PE or anti-CD83-PerCP-Cy5.5).

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 100µl 1x Permeabilization Buffer. This suspension was then stained in the dark, at RT for 20 minutes using assorted combinations of the following intracellular cytokine/chemokine-specific

antibodies: PE-conjugated abs: TGFβ, IL-23p19, IFN-α, CCL17, CXCL1, CXCL10; FITC-conjugated abs: IL-6, IL-12, IL-2, CXCL8; PerCP-Cy5.5 conjugated abs: IL-10, IL-4, TNFα; APC conjugated abs: CXCL9 and CCL20. 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. After staining cells were washed firstly 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.

4.2.3. Flow cytometry

To carry out flow cytometric analyses on the fixed cells a Beckman Coulter Navios cytometer (Beckman Coulter, Brea, CA, USA) was used with 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.

4.2.4. 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, 3.84pg/ml for CXCL9, 1.67 pg/ml for CXCL10, and 0.47pg/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 using pressure coocker (full pressure, 120°C, 3-5 minutes). 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: 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 (human filaggrin; Abcam); human KRT17 (rabbit polyclonal IgG [ab53707]; Abcam). 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 paralelly 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 fulfield, using high capacity cDNA Archive Kit (Invitrogen, Life Technologies, San Francisco, CA, USA) according to the instructions of the manufacturer's. 1ug of total RNA were 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: 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). The qRT-PCR investigation was performed with 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

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

From the peripheral blood of patients with psoriasis and health 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 appearence 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). Furthermore, a substantial portion of the cells in both diseased and healthy groups exhibited the CD86 activation marker. However, a statistically significant increase in fluorescence intensity was observed in the psoriatic group (CD86 Mean Fluorescence Intensity (MFI) 3.68 vs. 3.28, respectively, p=0.0445).

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 investigated: 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). 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).

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). The number of IL-12-producing cells and PASI also exhibited some correlation, although it was not statistically significant.

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 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.

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, 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). 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). 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 and resembled the levels observed in control cells.

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).

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.

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 region 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.

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. 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.

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 gene 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 qPCR data revealed similar mRNA expressions of IL-1β and TNF-α in the investigated groups.

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 gene level. 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 level.

5.2.3. Th17 related AMPs and barrier molecules in psoriasis vulgaris 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 gene or protein level between the two groups except for lipocalin. Through qRT-qPCR, the mRNA expression of LCN2 was found to be significantly higher in scalp psoriasis compared to psoriasis vulgaris.

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 q-RT-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.

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. While it is clearly established that psoriatic skin DCs express CD83 and CD86 maturation/activation markers and release IL-12 and IL-23 cytokines, 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 investigated. 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-investigated 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 are seem to be in a pre-mature state, as suggested by their significantly elevated expression of CD83 and CD86 markers revealed by our investigations.

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

source of this cytokine, since circulating mDCs also produced significantly high levels of IL-12, moreover their production was correlated with the disease severity.

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., 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 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.

In summary, our investigations on the cell surface markers and cytokine/chemokine production in blood precursor DCs in psoriatic patients revealed distinct behaviors. While plasmacytoid dendritic cells (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.

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. From this cytokine set, TNF-α, IL-6, and IFN-α may contribute to the pre-mature state of blood CD1c+ mDCs, 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.

Study II.

In the second part of our investigations we studied the psoriasis subtypes accuring 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 the SGP, SGR, and AGR.

It was observed earlier, that some immune-mediated skin diseases have special region preference, for example, atopic dermatitis (AD) primarily localize 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* gene (encoding lipocalin-2) and the chemokine CCL20, which were elevated in scalp psoriasis. Since these parameters

were elevated even in healthy SGR skin, these differences may reflect the basic immune characteristics of SGR skin region rather than a psoriasis-related feature.

To our knowledge, so far only a few publications investigated the differences in the immune characteristics of psoriasis in different skin areas and only two publications were focusing on the comparison of skin and scalp psoriasis. 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. 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 is quite similar. 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. 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 apparent contradiction between these two studies becomes 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. 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. Therefore, new formulations, such as foam or gel, were developed as new treatment modalities for this region. 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. 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. 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.

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 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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Registry number: DEENK/192/2025.PL
Subject: PhD Publication List

Candidate: Ahmad Khasawneh
Doctoral School: Gyula Petrányi Doctoral School of Allergy and Clinical Immunology

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.
   Acta Derm.-Venereol. 100 (14), 1-7, 2020.
   DOI: https://doi.org/10.2340/00015555-3553
   IF: 4.437

2. **Khasawneh, A.**, Baráth, S., Retzlerné Medgyesi, B., Béke, G., Dajnoki, Z., Gáspár, K., Jenei, A., Pogácsás, L., Pázmándi, K. L., 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. 189, 109-113, 2017.
   DOI: http://dx.doi.org/10.1016/j.imlet.2017.04.005
   IF: 2.436

Total IF of journals (all publications): 6,873
Total IF of journals (publications related to the dissertation): 6,873

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

09 May, 2025