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DISTRIBUTION OF HUMAN B-DEFENSIN 2, TNF-ALPHA, IL-1 ALPHA, IL-6 AND IL-8 IN PSORIATIC SKIN

E. Mozeika[1,2], M. Pilmane[1], J. Kisis[2]

[1] Institute of Anatomy and Anthropology, Riga Stradins University
[2] Department of Infectology and Dermatology,
Riga Stradins University, Latvia

ABSTRACT

Psoriasis is a chronic, inflammatory, proliferative condition of the skin, clinically characterized by red, scaly plaques. Psoriasis has a large and heterogenous genetic and immunologic background with the dysregulation of the host defense system. The human keratinocytes obtained from psoriasis lesions are a very rich source to various antimicrobial peptides. Both Th 17 and Th 1 pathways play a role in the pathogenesis of psoriasis. Our aim of the study was to evaluate the expression of human beta defensin 2 and TNF alpha in correlation with interleukins 1 alpha, 6 and 8 in the skin biopsies of psoriatic lesions.

We evaluated 14 *Psoriasis vulgaris* patients' skin samples. Skin biopsies were obtained using a routine punch method. All the tissue specimens were stained with hematoxylin and eosin and by immunohistochemistry for human beta defensin 2, TNF-alpha, IL-1 alpha, IL-6 and IL-8. We graded the intensity of staining semiquantitatively.

We observed intraepithelial lymphocytes, marked the diffuse intradermal infiltrates of inflammatory cells, the inflammatory cells in the hair follicle, surrounding sweat glands and subepithelial blood vessels. Defensin-containing and TNF-alpha positive cells were found in all the skin samples: defensin-containing cells varied from few to abundant positive structures in the visual field and TNF-alpha positive cells varied from few to numerous positive structures in the visual field. IL-1 alpha expressed poorly, while IL-6 positive cells were found in the range from few positive to the abundance of positive structures in the visual field and IL-8 positive structures varied from numerous positive structures to the abundance of positive structures in the visual field.

We conclude that IL-6, IL-8 and TNF-alpha are most common cytokines for psoriatic skin lesions. A moderate number of structures expresses the antimicrobial protein defensin in the psoriatic skin.

***Key words*:** *antimicrobial peptides; cytokines; human keratinocytes; psoriasis.*

INTRODUCTION

Psoriasis is a chronic, inflammatory, proliferative and enormously variable in the extent, duration and periodicity condition of the skin, in which both genetic and environmental factors play an important role. Psoriatic skin lesions are characterized by red, scaly, sharply demarcated and indurated plaques [5]. The classical histological picture of psoriasis includes parakeratosis with neutrophils, a thin granular layer, acanthosis, focal spongiosis, increased mitotic figures, dilated blood vessels in papillary dermis and the perivascular infiltrate of lymphocytes in the early disease course. At a later stage also elongated rete ridges and absent granular layer can be found. Although psoriasis has many histological features, the only truly diagnostic criteria are Munro micro-abscesses and the spongiform pustules of Kogoj [10].

The most common clinical variant *Psoriasis vulgaris* affects 85 to 90% of all the psoriasis patients and is most studied in scientific researches. Psoriasis has a large and heterogenous genetic and immunologic background with the dysregulation of the innate immune system [11]. The human keratinocytes obtained from psoriasis lesions are a very rich source to various antimicrobial peptides such as human alpha and beta defensins, cathelicidin LL-37, psoriasin. The role of the ultraviolet B (UVB) irradiation of vitamin D has been studied and was found to be an important part in the activation of antimicrobial immunity, particularly cathelicidin pathway [1]. T helper (Th) 17 cells have a conclusive role in the host defense and any alterations in its pathway can lead to the inflammatory conditions of the skin such as psoriasis. While interleukin-6 (IL-6) is responsible for the initial Th17 cell differentiation, interleukin-1 (IL-1) possibly finalizes the differentiation [3, 13]. Another key regulator of the innate immune system is tumor necrosis factor (TNF) alpha, which takes part in Th1 cell pathway and has industrially designed antagonists as therapeutic agents [12].

Our aim of the study was to evaluate the expression of human beta defensin 2 (HBD-2) and TNF alpha in correlation with interleukins 1 alpha, 6 and 8 in skin biopsies of psoriatic lesions.

MATERIAL AND METHODS

Patients

Patient selection criteria were created to exclude possible affecting side factors. Our target patient was at least 18 years old, suffering from psoriasis at least 6 weeks, having visible characteristic psoriatic eruptions in typical localization sites, without a fierce tan. All the patients were diagnosed by a dermatologist. We selected fourteen psoriasis patients with the histologically confirmed diagnosis of *Psoriasis vulgaris*. Skin biopsies were obtained using the routine 3 mm punch biopsy method and local lidocaine anesthesia. At the time of biopsy all the patients were off any topical or systemic psoriasis medication for at least one month.

The study was approved by the Ethics Committee at Riga Stradins University, the permit issued on 10 September 2009.

Methods

Skin biopsies were first fixed in the Stefanini's solution, dehydrated and embedded in paraffin. Further, four micrometer thick sections were prepared and stained routinely with hematoxylin and eosin.

The immunohistochemical method (IMH). Human beta defensin 2 (cat No AF 2758, LOT VJU015051, obtained from the goat, 1:100 dilution, R&D Systems, Germany), TNF-alpha (code ab 6671, obtained from the rabbit, 1:100 dilution, Abcam, Cambridge, UK), IL-1 alpha (B7: sc-9983, obtained from the mouse, 1:50 dilution, Santa Cruz Biotechnology, Inc., USA), IL-6 (NYRhIL6: sc-73319, obtained from the mouse, 1:50 dilution, Santa Cruz Biotechnology, Inc., USA) and IL-8 (C-19: sc-1269, obtained from the goat, 1:50 dilution, Santa Cruz Biotechnology, Inc., USA) were used by biotin – streptavidin IMH (Hsu et al., 1981).

Our findings were illustrated using Leica DC 300F camera and the image processing and analysis software Image Pro Plus.

The intensity of immunostaining was graded semiquantitatively:

few positive structures in the visual field were labelled with +,
a moderate number of positive structures in the visual field was labeled with ++,
numerous positive structures in the visual field were labeled with +++,
and the abundance of positive structures in the visual field was marked with ++++.

RESULTS

Intraepithelial lymphocytes and the marked diffuse intradermal infiltrates of the inflammatory cells were observed in all the patients (Figure 1). Similarly infiltration of inflammatory cells were also found in the hair follicle, the surrounding sweat glands and the subepithelial blood vessels. Arteriole sclerosis and sweat gland cell vacuolization were detected, as well as the Munro microabscesses were observed.

Defensin-containing cells varied from few (+) to abundant (++++) positive structures in the visual field (Figure 2). Particular increase of defensin-positive structures was observed in the sites of well defined inflammation.

TNF-alpha positive cells were found in all the skin samples – mainly subepithelium and their number varied from few (+) to numerous (+++) positive structures in the visual field (Figure 3).

IL-1 alpha findings were poor and varied from negative (–) to few (+) positive structures in the visual field (Figure 4), meanwhile IL-6 and IL-8 both showed explicit expression. IL-6 positive cells were found in the range from few (+) positive to abundance (++++) of positive structures in the visual field (Figure 5). IL-8 positive structures mostly varied from numerous (+++) positive structures to abundance (++++) of positive structures in the visual field. IL-8 expressed in epidermis and the connective tissue, inflammatory infiltrates, the hair follicle external root sheet and around blood vessels (Figure 6–8). All the results are summarized in Table 1.

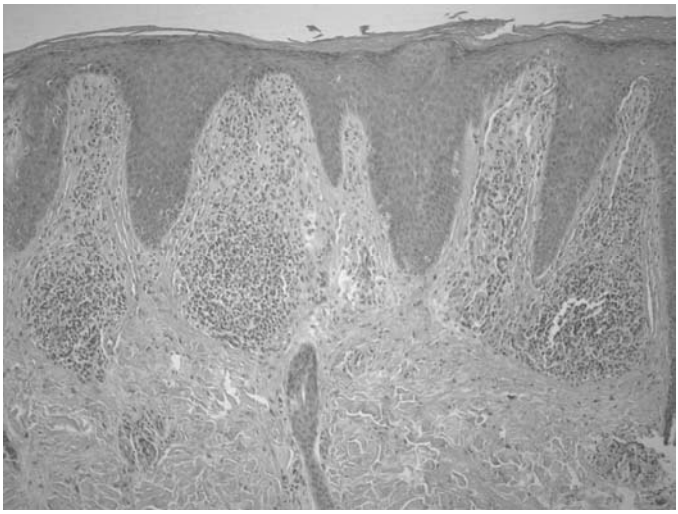

Figure 1. Explicit inflammatory cell infiltrates in the dermal layer of the skin. Hematoxylin and eosin, X 100.

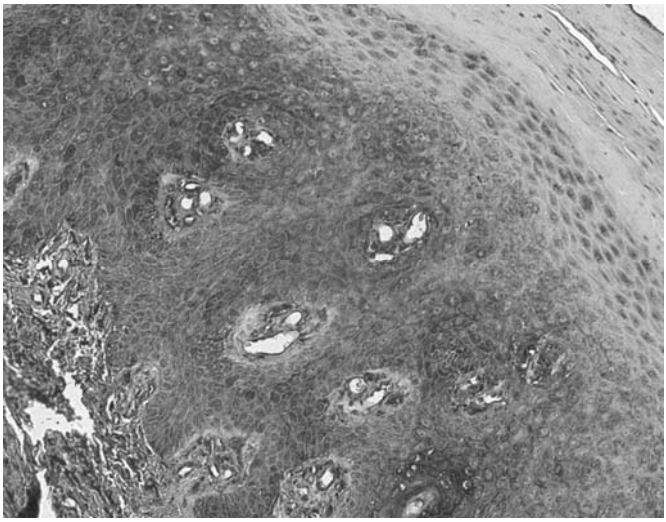

Figure 2. Abundance of defensin-containing cells in the psoriatic skin lesion with a marked border between *stratum spinosum* and *stratum granulosum*. Human beta defensin 2 IMH, X 200.

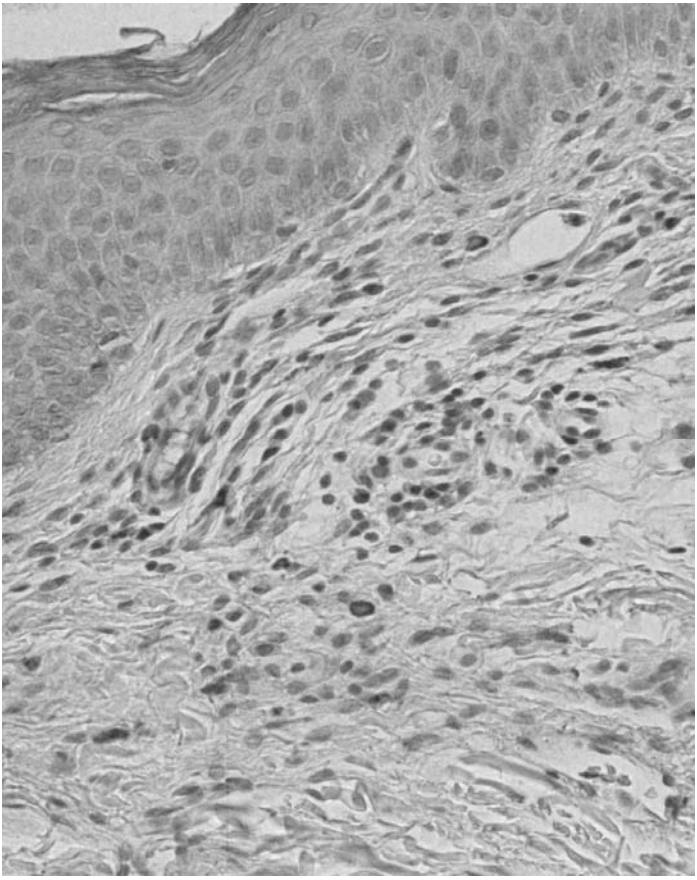

Figure 3. Numerous TNF-alpha positive structures in subepithelium. TNF-alpha IMH, X 400.

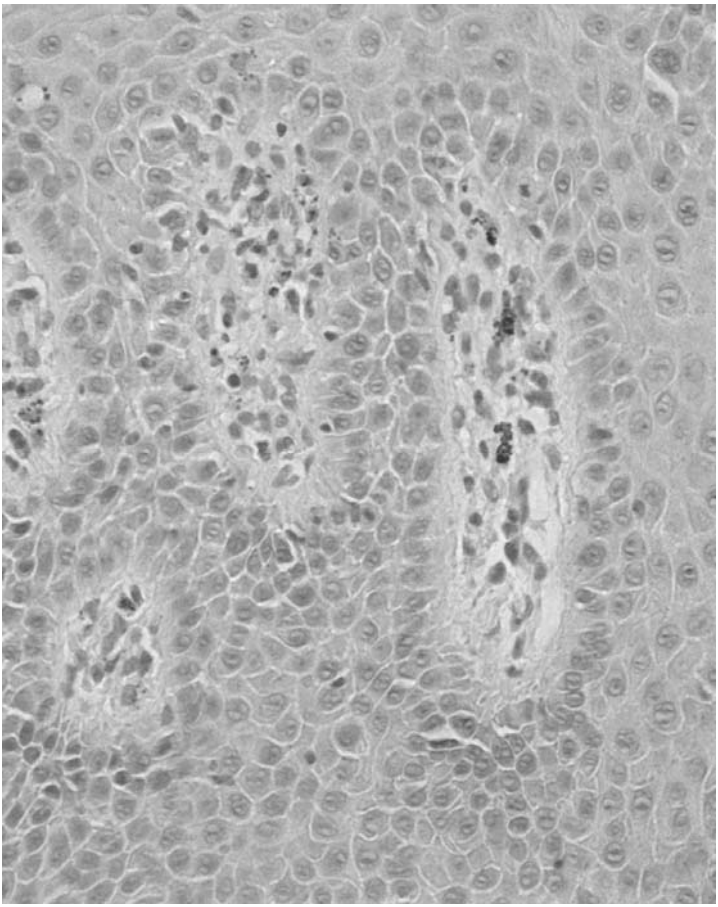

Figure 4. Sparse IL-1 alpha positive cells in the psoriasis patient's skin. IL-1 alpha IMH, X 400.

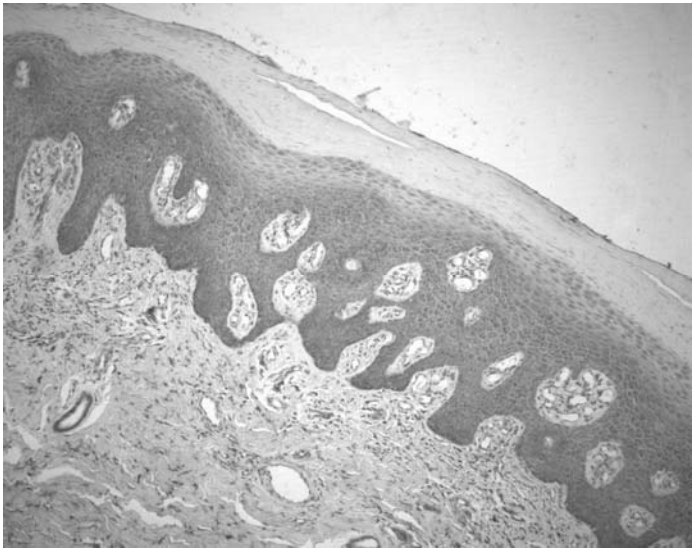

Figure 5. Pronounced expression of IL-6 in the skin from a psoriasis patient with a long progress of the disease. IL-6 IMH, X 100.

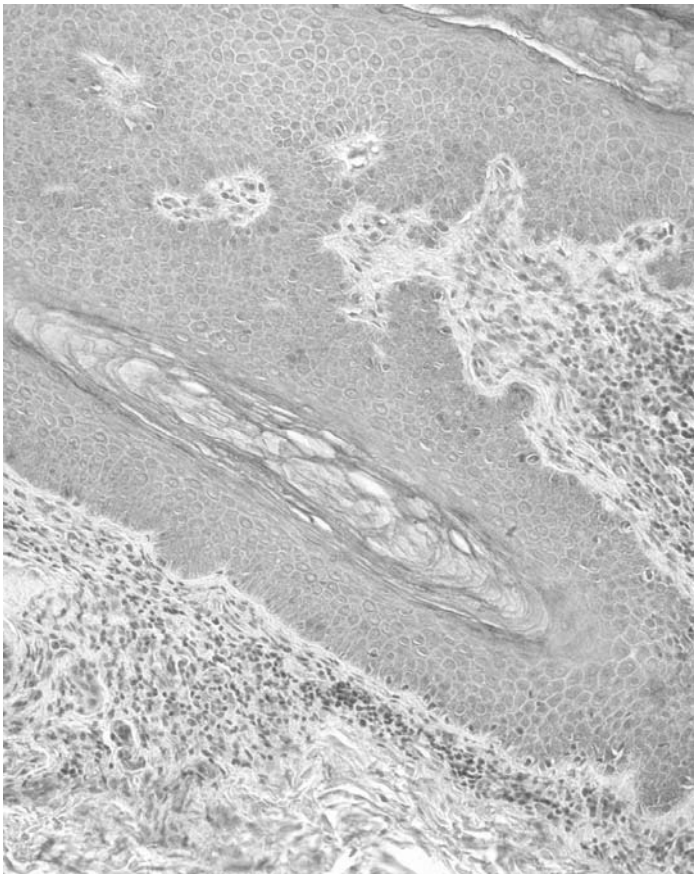

Figure 6. Even distribution of IL-8 through epidermal layer of the psoriatic skin. IL-8 IMH, X 200.

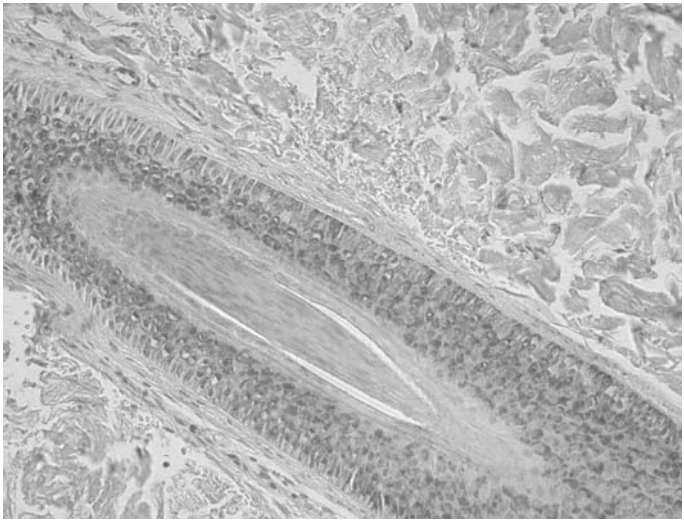

Figure 7. Marked expression of IL-8 in the external root sheath of the hair follicle. IL-8 IMH, X 200.

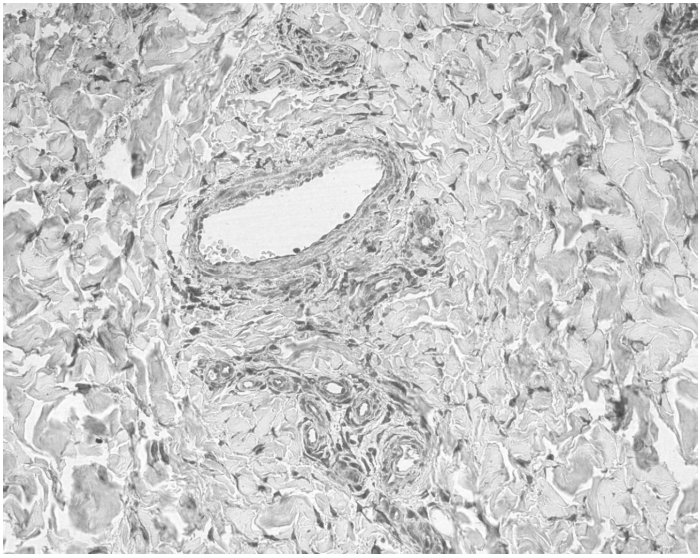

Figure 8. IL-8 positive cells surrounding dermal blood vessels. IL-8 IMH, X 200.

Table 1. Semiquantitatively evaluated expression of HBD2, TNF-alpha, IL-1 alpha, IL-6 and IL-8 positive structures in psoriasis lesions

Patient	Human beta defensin 2	TNF-alpha	IL-1 alpha	IL-6	IL-8
No. 1	++	+	–	+++	++
No. 2	+++	+++	+	+++	+++
No. 3	+++	+++	–	+++	++++
No. 4	+++	+++	+	+++	++++
No. 5	+++	+++	+	++	++++
No. 6	++	+	+	++	++++
No. 7	++++	++	–	++++	++++
No. 8	++	++	+	++	+++
No. 9	+	+++	+	++	+++
No. 10	+	++	–	++	+++
No. 11	+	++	+	++	++++
No. 12	+	+++	–	+	+++
No. 13	+	++	–	++	++++
No. 14	+	++	–	++	+++

To obtain statistical data we used non-parametric statistics and the Spearman's rank correlation coefficient was calculated. A correlation between defensin and IL-1 alpha or TNF-alpha and IL-1 alpha was not obtained. We found statistically significant correlation between human beta defensin 2 and IL-6 – the Spearman's rank correlation coefficient was 0.7745. Between defensin and IL-8 the Spearman's rank correlation coefficient was 0.3629 (weak), while between TNF-alpha and IL-6 (0.0054) and TNF-alpha and IL-8 (0.1123) statistically we could not find a relevant correlation.

DISCUSSION

This study reflects the complicated nature of psoriasis. The information obtained from our study can help fill gaps in the still very incompletely understood pathogenesis of classic psoriasis.

Antimicrobial peptides have been evaluated in many studies regarding various skin conditions. Skin antimicrobial peptides include an enormous group of various host defense agents, such as dermcidin, secreted by eccrine sweat glands, psoriasin S100A7, RNase 7 and

RNases from eosinophils, cathepsin G, bactericidal/permeability increasing protein and related proteins, the secretory leukocyte protease inhibitor, elafin, and trappin-2, eppin, antimicrobial sperm proteins, histatins, platelet microbicidal proteins, kinocidins [14]. In our study we chose to evaluate human beta defensin 2 in the tissue and it expressed in all the skin samples with more pronounced distribution in inflammatory areas. The recent novel study has found a close relation between human beta defensin 2 and inflammatory cytokines in the serum of patients with psoriasis. Human beta defensin 2 acted as a stimulator by enhancing interferon gamma, TNF-alpha, IL-10, IL-1 beta, IL-6 and IL-22 production and regulator by suppressing IL-17 or stimulating IL-10 production [8].

The role of TNF-alpha in the pathogenesis of psoriasis is substantial and TNF-alpha inhibitors have been used in the therapy of many inflammatory diseases including psoriasis. Therefore we chose to include TNF-alpha in our study. Distribution in tissue samples varied mostly between a moderate number of positive structures and numerous positive structures in the visual field and was found to be more expressed than human beta defensin 2 even in the skin with week findings of defensin.

We evaluated the expression of IL-1 alpha, IL-6 and IL-8. IL-1 alpha in our patients' skin varied from a completely negative finding to few positive structures. In contrast several other studies have found the overexpression of IL-1 family cytokines and even the new signaling system in psoriasis [6, 7]. IL-6 and IL-8 both were found to be positive in our skin tissue and it accords with previously known data as these interleukins have been found in high levels in active psoriasis patients, both skin and serum samples. The serum level of IL-6 has also been described as a good predictor for the successful topical therapy outcome as it prevents from immunosupression [2, 4, 9].

We conclude:

1. IL-6, IL-8 and TNF-alpha are most common cytokines for psoriatic skin lesions.
2. A moderate number of structures expresses the antimicrobial protein defensin in the psoriatic skin.

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Address for correspondence:
Elga Mozeika,
Riga Stradins University
The Institute of Anatomy and Anthropology
Ozolu str. 11, Adazi, LV-2164, Latvia
E-mail: elga.mozeika@yahoo.com