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Chosen IL-17 family proteins in neutrophils of patients with oral inflammation

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ABSTRACT

Purpose: Recent studies have indicated that human neutrophils (PMN), belonging to the first line of cellular defence against various infectious agents and inflammation can produce cytokines of the interleukin 17 (IL-17) family. The relation between pro-inflammatory IL-17A and anti-inflammatory IL-17E may be responsible for the development and maintenance of the inflammatory process. The study objective was to determine the expression of IL-17E and IL-17BR in confrontation with IL-17A and IL-17R in neutrophils of patients with oral cavity inflammation and to compare protein expressions in autologous peripheral blood mononuclear cells (PBMC).

Material and Methods: Expressions of IL-17E, IL-17A, IL-17BR and IL-17R was assessed by the Western blot method. IL-17E and IL-17A levels was determined by the ELISA method.

Results: The expressions of IL-17E, IL-17A, IL-17BR and IL-17R in PMN and PBMC in patients were higher in the cells of patients, as compared to the controls. The expressions of IL-17E and IL-17BR in both kinds of cells were lower than the levels of IL-17A and IL-17R. The levels of IL-17E and IL-17A were increased in cell supernatants and blood serum of patients, as compared to the control group.

Conclusions: The preliminary examinations indicate the predominance of pro-inflammatory effects of IL-17A controlled by PMN, as well as PBMC in patients with oral inflammation. Results obtained also suggest a more significant involvement of PMN in the IL-17E and IL-17A dependent reactions.

Key words: interleukin 17, neutrophils, peripheral blood mononuclear cells, oral inflammation

INTRODUCTION

The IL-17 family appears to be structurally different from all known human cytokines and proteins. Members of this group have a similar molecular weight, 30-35 kDa, but they differ in molecular structure and biological properties [1]. They are dimmers, built up of 150-180 amino acids, with the highest affinity for C-terminal 70-amino acid segments [2].

IL-17A and IL-17E, biologically activated through conjugation with the specific receptors IL-17R [1] and IL-17BR, play a special role among the IL-17 family ligands [3].

As shown by the available data, IL-17A, known as CTLA-8, is produced by a number of human immune cells, involving neutrophils, exerting the expression of receptor IL-17R [4]. Neutrophils belonging to the first line of cellular defence against various infectious agents, also play a significant role in inflammation [5]. The ability of these cells to secrete pro-inflammatory cytokine, such as IL-17A, may amplify the inflammatory response. In contrast, IL-17E has anti-inflammatory properties and might balance the pro-inflammatory properties of IL-17A. However, there are no data concerning the presence of IL-17E and its specific receptor IL-17BR in neutrophils.

Previous studies have revealed that neutrophils and their ability to produce cytokines play a role in patients with oral inflammatory diseases [6,7]. The inflammatory process is controlled by pro- and anti-inflammatory factors. It has also been found that the changes in the secretion of IL-1β and sIL-1RII are characteristic of the cells from inflammation [7]. Therefore, in this study, we examined the expression of IL-17E

Table 1. **Characteristic of patients with oral inflammation (sex, site of inflammation).**

Sex	Number	Dental abscess	Submandibular abscess	Cheek abscess	Dental phlegmon	Submandibular phlegmon	Inflammatory infiltration of dental	Inflammatory infiltration of submandibular	Inflammatory infiltration of cheek mucosa
M	12	1	2	2	1	2	2	1	1
F	8	1	1	-	1	1	1	2	1

and IL-17BR in relation to IL-17A and IL-17R expression in this patient group. For the purpose of comparison, the expression of the above proteins was determined in autologous peripheral blood mononuclear cells (PBMC). Taking into consideration the profile IL-17A and IL-17E activities, the relations between their expression and production by neutrophils may be helpful to understand the role of these cells in reactions mediated by IL-17 family proteins in patients with oral inflammation and explain the immuno-inflammatory mechanism. Furthermore, the important question is whether the changes in expression of these proteins are more characteristic for PMN or PBMC.

MATERIALS AND METHODS

The study involved a group of 15 healthy subjects, volunteer blood donors aged 20-50 years old, and a group of 20 patients with oral inflammation, aged 23-54, hospitalized in the Department of Maxillofacial Surgery, at the Medical University of Bialystok. Characteristics of patients with inflammation of oral cavity was presented in *Tab. 1*.

In all patients leukocytosis ranging from 6.3 to 17.3 x 10^3/ml consisting predominantly of PMNs.

Cells were isolated from whole blood collected for heparin (10 IU/ml-Heparin, Polfa-Lodz, Poland) using Gradisol G (1.115g/ml, Polfa-Lodz, Poland) according to Zeman *et al*. [8]. This method enables simultaneous separation of two highly purified leukocyte fractions: PBMC (containing 94% lymphocytes) and PMN (containing 91% PMN). The purity of isolated PMN and PBMC were determined by May-Grunewald-Giemsa-staining.

Sera were obtained from blood samples collected without anticoagulant agents.

The two leukocyte fractions obtained, *i.e.* polymorphonuclear cells and mononuclear cells, were suspended in a culture medium containing RPMI-1640 and Hanks' fluid (BIOMED-Lublin, Poland), the subjects' own serum, 100U/ml penicillin and 50ng streptomycin (Polfa Trachomin S.A., Poland), reaching a concentration of 5 x 10^6 cells/ml. Then, the cells were incubated in microplatelets (Microtest III-Falcon, BD Biosciences, Bedford, USA) at 37°C, in an incubator with a flow of 5% CO_2 (NUAIRE™). The following culture, the viability of PMN and PBMC were > 93% as determined by trypan blue exclusion.

After 20 hours, the supernatant was collected from each well and stored at - 20°C.

Informed written consent was obtained from all participants and the Ethics Committee of the Medical University of Bialystok approved the study.

Expressions of IL-17E, IL-17A, IL-17BR and IL-17R assessed by the Western blot method

PMN and PBMC were subjected to lysis by means of sonification in the presence of protease inhibitors (Sigma-Aldrich, Steinheim, Germany). The lysate was suspended in Laemli's buffer (Bio-Rad Laboratories, Herkules CA, USA). The cytoplasmic fraction of proteins underwent electrophoresis on SDS-PAGE (Bio-Rad Laboratories, Herkules CA, USA). The protein fractions were transferred onto nitrocellulose (Bio-Rad Laboratories, Herkules CA, USA). Then, nitrocellulose was incubated with suitable polyclonal anti-IL-17E, anti-IL-17A, anti-IL-17BR and anti-IL-17R antibodies (R&D Systems, Minneapolis, USA). After rinsing with 0.1% TBS-T, nitrocellulose was incubated with alkaline phosphatase-labelled antibody against IgG (Victor Laboratories, Burlingame, CA, USA). Immunoreactive protein bands were obtained by adding the BCIP/NBT Liquid Substrate System (Sigma-Aldrich, Steinheim, Germany).

IL-17E and IL-17A levels assessed using the ELISA method

IL-17E concentrations in blood serum and cell supernatants were determined by the ELISA method using a PeproTech kit (Rocky Hill, USA) according to the instructions enclosed. Human recombinant IL-17E was used as a standard.

IL-17A levels in the serum and cell supernatants were measured by the ELISA method using R&D Systems kits (Minneapolis, USA) according to the instructions provided. Human recombinant IL-17A was used as a standard.

Statistical analysis

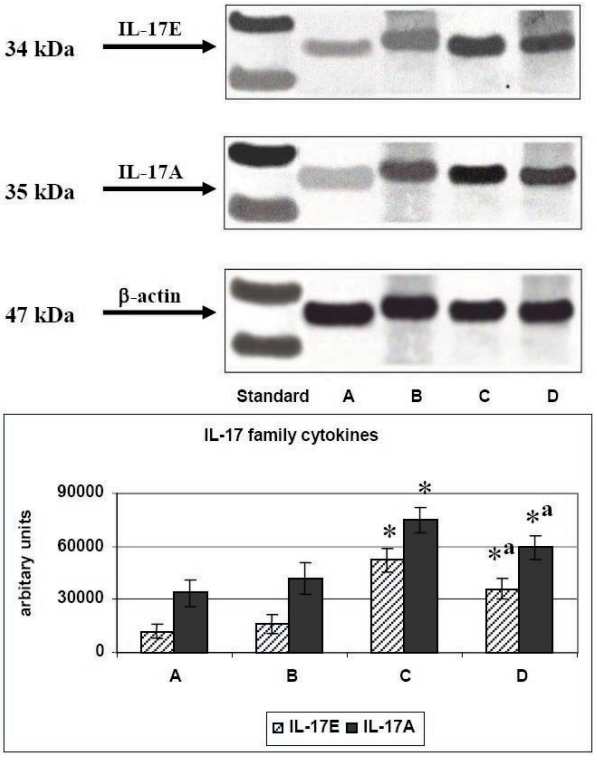
Results were elaborated statistically using the Mann-Whitney *U* test and the t-Student test. $P<0.05$ was considered statistically significant.

RESULTS

IL-17E and IL-17A expressions in PMN and PBMC of patients with oral inflammation assessed using the Western blot method

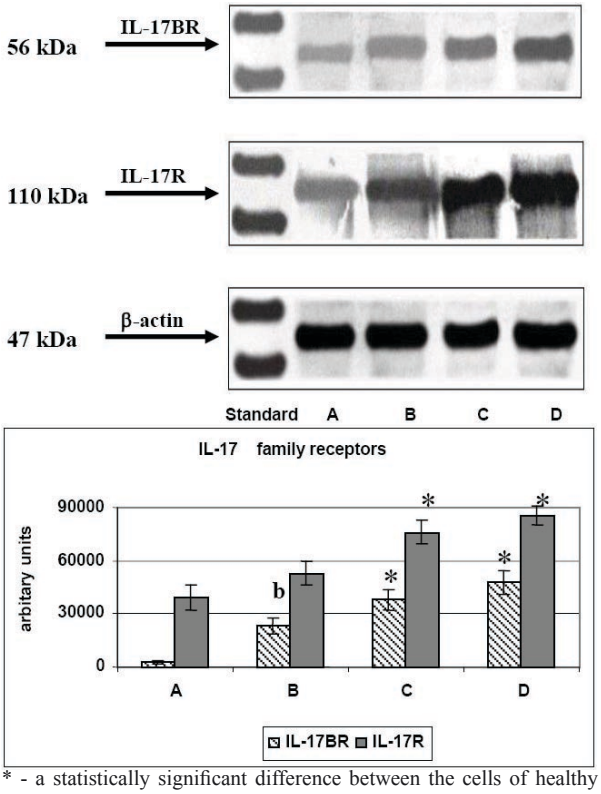
The expressions of IL-17E (34kDa) and IL-17A (35kDa) were found in cell lysates of PMN and PBMC in healthy subjects

Figure 1. **Expressions of IL-17E and IL-17A in PMN and PBMC in healthy subjects and patients with oral inflammation assessed using the Western blot method.**



* - a statistically significant difference between the cells of healthy subjects and patients ($p<0,05$)
a – a statistically significant difference between PMN and PBMC of patients ($p<0,05$)
A- PMN of healthy subject; C- PMN of patients; B- PBMC of healthy subject; D- PBMC of patients

Figure 2. **Expressions of IL-17BR and IL-17R in PMN and PBMC in healthy subjects and patients with oral inflammation assessed using the Western blot method.**



* - a statistically significant difference between the cells of healthy subjects and patients ($p<0,05$)
b - a statistically significant difference between PMN and PBMC of healthy subjects ($p<0,05$)
A- PMN of healthy subject; C- PMN of patients; B- PBMC of healthy subject; D- PBMC of patients

and patients with oral inflammation (*Fig. 1*).

In patients, the expressions of IL-17E and IL-17A in PMN and PBMC were significantly higher, as compared to healthy subjects. The levels of the two cytokines were found to be significantly higher in PMN than in PBMC of patients (*Fig. 1*).

IL-17BR and IL-17R expressions in PMN and PBMC of patients with oral inflammation assessed by the Western blot method

The expressions of IL-17BR (56 kDa) and IL-17R (110 kDa) were found in cell lysates of PMN and PBMC in healthy subjects and patients with oral inflammation (*Fig. 2*).

In the patient group, the expression of IL-17BR and IL-17R in PMN and PBMC were found to be significantly higher than in healthy subjects. PMN and PBMC of patients exerted expression of IL-17R and IL-17BR on the same level (*Fig. 2*).

IL-17E and IL-17A concentrations in serum and cell supernatants of healthy subjects and patients with oral inflammation assessed by the ELISA method

In patients, PMN and PBMC had a significantly greater ability to release IL-17E and IL-17A, as compared to the control group. Moreover, no differences were revealed in the concentrations of IL-17E and IL-17A between the culture supernatants of PMN and PBMC. Significantly higher serum levels of these proteins were observed in patients with oral inflammation, in comparison to the control subjects (*Tab. 2*).

No correlations were found of IL-17E and IL-17A levels between cells supernatants and serum (*Tab. 1*).

DISCUSSION

An interesting relationship between enhanced expressions IL-17E and IL-17A in neutrophils of patients with oral inflammation was observed in the present study. Lower expression of anti-inflammatory IL-17E accompanied higher expression of pro-inflammatory IL-17A in these cells. Although a similar relation

Table 2. **IL-17E and IL-17A concentrations in blood serum and cell supernatants of PMN and PBMC in healthy subjects and patients with oral inflammation using ELISA method.**

	IL-17E pg/ml			IL-17A pg/ml		
	PMN x ± SD	PBMC x ± SD	Serum x ± SD	PMN x ± SD	PBMC x ± SD	Serum x ± SD
Control group	**8.04** ± 1.64	**9.03** ± 1.47	**10.03** ± 1.29	**9.18** ± 2.97	**10.52** ± 3.26	**15.27** ± 4.32
Patients	**26.23*** ± 2.65	**23.30*** ± 4.52	**31.75*** ± 13.43	**36.16*** ± 9.03	**34.97*** ± 8.94	**76.78*** ± 5.98

* - a statistically significant difference between healthy subject and patients ($p<0,001$)

between these cytokine in PBMC was demonstrated, PMN exerted higher expression IL-17E and IL-17A than PBMC. The above observations indicate an essential role for neutrophils in the reactions controlled by IL-17A promoting the development and maintenance of the inflammatory process. Overexpression and secretion of IL-17A may lead to increased production of inflammatory cytokines, such as IL-1β, IL-6, IL-8, IL-12 or TNF-α by mononuclear cells, as well as neutrophils. High expression of receptor IL-17R, demonstrated in both kinds of cells, may suggest a high sensitivity of these cells to IL-17A in the patient group. Furthermore, NF-κB activation in stromal cells by IL-17A, by inducing the production of GM-CSF and chemokines, involving CXC, stimulates granulopoiesis and recruitment of neutrophils [9]. An increase in expression of adhesion molecules (ICAM-1, ICAM-3 and E-selectin), HBD-2, MIP-3, iNOS or COX-2 modulated by IL-17A may additionally enhance the recruitment and activation of many inflammatory cells [4,5,10,11].

The excessive IL-17A-dependent activity of PMN and PBMC in patients with oral inflammation may be in part counterbalanced by the increased expression and release of IL-17E by these cells, as compared to the control group demonstrated in these studies. The presence of substantial amounts of IL-17E in the patients' circulation may cause the increase in production of anti-inflammatory Th2 cytokine profile: IL-4, IL-5, IL-10 and IL-13 by mononuclear cells through enhanced IL-17BR receptor expression [12]. Moreover, enhanced IL-17E secretion may inhibit proliferation of human bone marrow progenitor cells of the granulocytic-macrophage series (CFU-GM), thus reducing leukocyte count [10]. On the other side, IL-17E can also increase recruitment of B cells and increase the production of specific antibodies [13,14].

CONCLUSIONS

Concluding, these preliminary examinations indicate a predominance of pro-inflammatory effects of IL-17A, controlled by PMN, as well as PBMC in patients with oral inflammation. Results obtained also suggest a more significant involvement of PMN in the IL-17E and IL-17A dependent reactions.

Further studies concerning the expression and release of IL-17A, IL-17E in relation to their natural regulators and their biological effects may contribute to a complete definition of the basics of cell immunoregulation with the involvement of these cytokines in patients with oral inflammation and indicate a new direction of immunotherapy associated with IL-17 family proteins modulation.

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