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IL-6 and FAS (CD95) serum level in patients suffering from psoriasis and in relation to the lymphocyte subpopulations

Interleukin 6 (IL-6), originally identified as a B-cell differentiation factor, is now known to be a multifunctional cytokine that regulates the immune response, hematopoiesis, the acute phase response, and inflammation. Disregulation of this cytokine production is implicated in the pathology of various disease (4). Interleukin-6 (IL- 6) is engaged as a key mediator in immune and inflammatory responses in psoriasis (10). Moreover, being a mitogenic factor for keratinocytes Il-6 is also possibly involved in epidermal hyperproliferation (1, 6). So its concentration can reflect the processes occurring in epidermis and skin (6, 11, 15). IL-6 considerably affects the function of T lymphocytes, being a secondary signal for the induction of IL-2 expression by CD4 T lymphocytes after the mitogen stimulation (1, 14). Along with IL-2 and IFN-γ, IL-6 influences the development of cytotoxic T lymphocytes from immature cells (1, 4, 14).

Apoptosis is a physiological form of cell death that is responsible for the deletion of unwanted cells (16). The cells of immune system in particular macrophages and dendritic cells clear away the apoptotic cells. Epidermal keratinocytes are supposed to be regulated by cell proliferation and cell death balance leading to structural homeostasis. Psoriatic skin shows marked thickening of the epidermis, suggesting the imbalance of homeostasis, which might be related to abnormal apoptotic process (16). In psoriatic lesions the decrease of apoptosis index in comparison to the normal epidermis and its increase during disease regression induced by PUVA-therapy and dithranol treatment were observed (7). Lots of data indicate that apoptosis of activated T lymphocytes may be mediated by CD95 (FAS/ APO-1) antigen (12). This antigen is also called the death factor (12). Takahashi et al. (16) investigated the expression of various apoptosis-related molecules in the psoriatic hyperproliferative epidermis. Real time quantitative RT-PCR analyses revealed that FAS mRNAs in the psoriatic lesional epidermis were increased by 4.2 fold, compared with the uninvolved epidermis values. No significant difference in the expression of mRNAs of FAS ligand was detected between the involved and uninvolved epidermis. There was no publication dealt with CD95 blood level in psoriatic patients as far as we know.

The aim of the study was to analize the concentration of IL-6 and FAS (CD95) antigen in relation to T cell's subpopulations in the peripheral blood of psoriatic patients.

MATERIAL AND METHODS

Twenty patients with guttate psoriasis triggered by infection and 17 healthy controls were included in the study. The mean age of the patients and controls were 33.8 ± 12.0 years and 32.2 ± 11.0 years respectively. The PASI score ranged from 22 to 48, mean 29.09 ± 8.04. In all the patients infection of the upper respiratory tract, throat or ears was diagnosed 3–8 weeks before the following recurrence of psoriatic lesions.

The patients were examined before the beginning of the study. None of them was treated with topical or systemic steroids before the examination, nor any form of dithranol and retinoids therapy was used. Bacteriological examinations were not performed because all the patients had previously been treated with antibiotics and antiflogistics by their family physicians.

1. Analysis of the subpopulation of peripheral blood lymphocytes. Flow cytometry was used to analyze the above subpopulation. Peripheral blood samples were collected in 10 ml vacutainer tubes with EDTA. The cell surface antigens in each case were determined on fresh cells at the time of sample submission. Mononuclear cells were isolated by density centrifugation on Lymphoprep (Nycomed, Norway) and washed twice in phosphate buffered saline (PBS) containing 1% bovine serum albumin. Double color immunofluorescence studies were performed using combinations of phycoerythrin (PE) and fluorescein isothyocyanate (FITC) conjugated monoclonal antibodies. Monoclonal antibodies were obtained from Ortho Diagnostic Systems (Germany), Becton Dickinson (Germany), or Dako (Denmark). Commercial monoclonal antibodies were used according to the instructions given to the producers. The analysis of the following cells was done: CD3+, CD19+, CD45+/CD14 +, CD45RA+ , CD45RO+, CD4+/CD8+, CD4+, CD8+, CD25+, CD3+CD19+, CD3+CD16+CD56+, CD56+. 10^6 cells were incubated with monoclonal antibodies for 30 min at 4° C and washed twice with PBS afterwards. The analysis of T cell subpopulations were performed using the flow cytometry method (12). All samples were measured with the cytoron flow cytometer (Ortho Diagnostic Systems). 10,000 cells were analysed per test. In order to quantify the levels of fluorescence, the mean fluorescence intensity and fluorescence signal strength of the studied antigens were calculated. The mean fluorescence intensity and fluorescence signal strength of histogram were measured from the upper limit of the negative control.

2. Measurement of IL-6 and FAS antigen concentration. The presence and concentration of IL-6 and FAS antigens was examined in serum using the immunoenzymatic ELISA method. Measurements were done in duplicates according to the instructions included in commercial kits for human IL-6 (h IL-6- ELISA kit 600 R&D System < 0.094 pg/mL) and FAS-APO A (CD95 ELISA kit R&D System). The results were put to statistical analysis using the Whitney-Mann test. P values smaller than 0.05 were considered as significant.

RESULTS

The results of our study in relation to the lymphocyte subpopulations in psoriatic patients, using flow cytometry, were analogous to the previously published (11). Comparing patient's with the control values (Pt versus C) we observed statistically significant decrease of the percentage of CD3 lymphocytes (58.04±14.30 vs 68.98±10.66 , $p < 0.02$) , a highly significant decrease of the percentage (39.25±9.40 vs 50.82±6.74, $p < 0.0001$) and expression of CD4 lymphocytes (99.44±10.69 vs , 107.41±3.74, $p < 0.01$) a decrease of CD25 cells expression (67.68±7.80 vs 75.72±8.97, $p < 0.02$) and an increase of the percentage of CD45RO cells in the psoriatic patients (3.72±4.05 vs 1.04±1.04, $p<0.006$). Plasma levels of IL-6 estimated in acute psoriatic group were significantly higher than in the controls ($p < 0.05$) (Fig.1), while FAS concentration was 390.07+172.16 and 462.65+154.7 pg/ml in psoriatic patients and in the controls, respectively, although the differences was not statistically significant,

$p > 0.05$ (Fig. 2). The correlations between the examined parameters were also analyzed. There was correlation established between IL-6 and CD95 with $p = 0.06$ and $R = 0.41$. IL-6 concentration correlated positively with percentage of CD45RO+ cells ($p = 0.05$, $R = 0.28$) and negatively with percentage of CD3+ cells ($p = 0.02$, $R = -0.34$), CD4+ cells ($p = 0.02$, $R = -0.34$), and CD45 RA + cells ($p = 0.04$, $R = -0.68$).

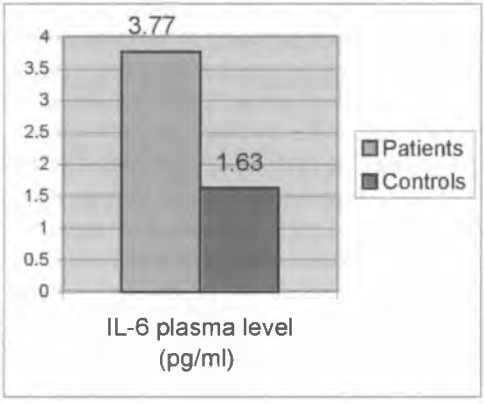

Fig. 1. The concentration of IL-6 in the blood of psoriatic patients and healthy individuals

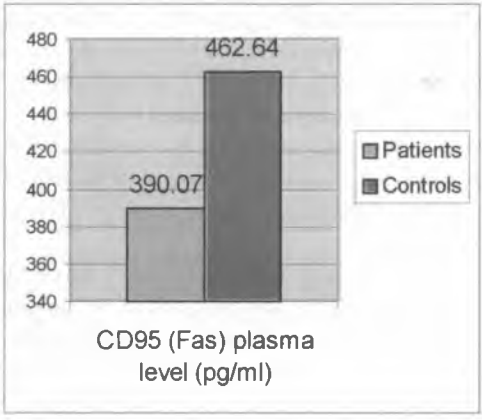

Fig. 2. The concentration of CD95 (FAS) in the blood of psoriatic patients and healthy individuals

DISCUSSION

To explain the molecular pathology of psoriasis it is necessary to consider not only the regulation of epidermal growth and differentiation, but also the potential role of cytokines in these pathogenic events and their partake in immune inflammation (5). The IL-6 overexpression observed in hyperplastic psoriatic tissue may explain the phenomena characteristic of psoriasis: keratinocyte proliferation, immune activation and tissue inflammation (1, 10). Thus, IL-6 could help activate the cellular elements in the local inflammatory infiltrate (T cells, macrophages, and polymorphonuclear leukocytes) leading to an exacerbation of the local lesion (5).

The increased IL-6 levels in the peripheral blood were previously observed in psoriatic patients (1, 12, 16). The fact that this cytokine has not been detected in all but only in some patients with severe psoriasis, especially with arthropatic psoriasis (1, 3, 10) and *pustulosis palmaris et plantaris*

(8), should be underlined. IL-6 increased concentration in the psoriatic lesions and/or in the blood serum is reported by the majority of authors (1, 3, 6, 9 ,11, 15).

One of the parameters responsible for epidermis homeostasis is loss of keratinocytes adherence leading either to keratinocyte differentiation or to apoptosis. Keratinocytes can be induced to undergo apoptosis by loss of cells interactions following cross-linking of FAS (CD95) or UV treatment (2). Apoptosis of keratinocytes may be inhibited *in vitro* by several growth factors like epidermal growth factor (EGF), keratinocyte growth factor (KGF) or nerve growth factor – NGF – (1, 2, 13). It is possible that the abnormally low rate of apoptosis can be (at least) partially responsible for epidermal hyperproliferation (2, 13). Some data suggest that psoriatic keratinocytes were refractory to apoptosis (2, 13). The results of our study showing the lowering of CD95 (FAS) serum level in patients with psoriasis may suggest the decrease of apoptosis rate in this disease.

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SUMMARY

In the presented study the subpopulations of blood lymphocytes as well as serum concentrations of IL-6 and FAS in 20 patients with psoriasis triggered by infection and 17 healthy controls were analyzed. The flow cytometry technique and ELISA method were used. We detected statistically significant higher serum level of interleukin-6 (IL-6) in patients with active disease and tendency to the lowering of FAS level, although not statistically significant. Analysis of the correlation between the parameters showed that it was close to statistical significance for IL-6 and CD 95, $p = 0.06$ and $R = 0.41$. IL-6 level correlated positively with percentage of CD45RO+ cells ($p = 0.05$, $R = 0.28$) and negatively with percentage of CD3+ cells ($p = 0.02$, $R = -0.34$), CD4+ cells ($p = 0.02$, $R = -0.34$), CD 45RA+ cells ($p = 0.04$, $R = -0.68$).

Stężenie intereukiny-6 (IL-6) i antygenu FAS (CD95) w surowicy krwi chorych na łuszczycę w odniesieniu do subpopulacji limfocytów

Badano subpopulację limfocytów krwi obwodowej za pomocą cytometru przepływowego u 20 osób z łuszczycą poinfekcyjną w porównaniu z 17 zdrowymi ochotnikami z grupy kontrolnej oraz stężenie interleukiny 6 (IL-6) i antygenu CD 95 (FAS) w surowicy krwi przy pomocy metody ELISA. Stwierdzono istotne statystycznie podwyższenie surowiczego stężenia IL-6 u pacjentów w aktywnym okresie choroby oraz tendencję do obniżenie stężenia CD95. Badając korelacje pomiędzy poszczególnymi parametrami stwierdzono korelację bliską istotności ($p = 0,06$, $R = 0,41$) pomiędzy IL-6 a CD95, chociaż nieistotną statystycznie, dodatnie korelacje pomiędzy IL-6 a ekspresją - [Mean Fluorescence Intensity (MFI)] komórek NK ($p = 0,003$, $R = 0,43$, odsetkiem komórek CRO ($p = 0,05$, $R = 0,28$), natomiast ujemną pomiędzy IL-6 a odsetkiem komórek CD3+ ($p = 0,02$, $R = -0,34$), CD4+ ($p = 0,02$, $R = -0,34$), odsetkiem komórek CRA ($p = 0,04$, $R = -0,68$).