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Interleukin-18 serum concentration in patients with psoriasis triggered by infection

Psoriasis is a chronic, inflammatory skin disease, affecting approximately 2% of Caucasians. It is characterized by a hyperproliferative epidermal layer and a mononuclear cellular infiltrate composed predominately of T lymphocytes, dendritic cells (DCs) and macrophages (2). An early cellular event in the development of psoriatic lesions is the infiltration of target sites by activated T cells which, in turn, produce inflammatory mediators, such as INF-γ, induce epidermal hyperplasia, and may act with keratinocytes and dermal macrophages to sustain a cycle of inflammation that finally leads to the psoriatic phenotype (4, 12). The strongest association between a bacterial infection and psoriasis is that of GAS (group A streptococcal antigen), and the acute guttate form of the disease in which patients have positive streptococcal throat cultures and raised antistreptococcal titers (2). Patients with guttate or chronic plaque psoriasis both show increased PBMC responses to GAS antigens (2). Furthermore, streptococcal antigen-reactive T-cell lines were isolated from the lesional skin of 66% of psoriasis patients (guttate and chronic plaque), but they were less frequently found (41%) in a group of disease controls with other skin disorders such as eczema, lichen planus or pityriasis rosea (2). T-cell lines isolated from guttate skin lesions showed strong proliferative responses to GAS of different M serotypes; T-cell lines isolated from one of the GAS-reactive T-cell lines responded in a HLA-DR restricted manner and produced high levels of interferon-gamma (INF-γ) but no interleukin-4 (IL-4) or tumour necrosis factor-alpha (TNF-α) (2). Interferon-gamma is believed to be one of the important mediators in the cytokine cascade of psoriasis (1).

IL-18 is a potent proinflammatory cytokine able to induce IFN-γ, GM-CSF, TNF-γ and IL-1 in immunocompetent cells, to activate killing by lymphocytes, and to up-regulate the expression of certain chemokine receptors. IL-18 induces naive T-cells to develop into Th2 cells. Moreover, IL-18 induces IL-13 and/or IL-4 production by NK cells, mast

cells and basophils. Therefore, IL-18 should be seen as a unique cytokine that enhances innate immunity and both Th1- and Th2-driven immune responses (8).

IL-18 mRNA is expressed in a wide range of cells including Kupffer cells, macrophages, T cells, B cells, dendritic cells, osteoblasts, keratinocytes, astrocytes, and microglias. Thus, the pathophysiological role of IL-18 has been extensively tested in the organs that contain these cells (9).

However, the overproduction of IL-12 and IL-18 induces severe inflammatory disorders, suggesting that IL-18 is a potent proinflammatory cytokine that plays a pathophysiological role in several inflammatory conditions.

OBJECTIVE

The aim of the study was to check if the psoriasis has any influence on IL-18 serum level and subpopulation of lymphocytes in peripheral blood.

MATERIAL AND METHODS

Fourteen patients participating in the study were diagnosed with psoriasis triggered by infection on the basis of clinical criteria. The patient's sex and age distribution was as follows: 8 females, 6 males, mean age 30.5±4.3. The control group were sex- and age-matched. The PASI (psoriasis area and severity index) score ranged from 20 to 48, mean 28.3±7.2. The patterns of psoriasis were: pinpoint, guttate, and guttate and nummular. We included the patients with clinical evidence for infection 3-8 weeks prior to admission.

The patients were examined before the beginning of treatment. 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 since all the patients had previously received anti-infectious treatment.

ELISA

Plasma samples were collected using EDTA as an anticoagulant, and centrifuged for 10 min. at 1,000 x g. After centrifugation, the samples were stored at -80°C. The samples were thawed immediately before the determination of IL-18 level. The cytokine was measured with enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instruction.

The high sensitivity ELISA kit (MBL; Japan) was used (sensitivity <12.5 pg/ml). The assay uses two monoclonal antibodies against two different epitopes of human IL-18.

Standards and 100 µl of prepared samples were added to each well of an anti-human IL-18 monoclonal antibody precoated strip well plate (96-wells). They were incubated for 60 min. at room temperature. After washing, a peroxidase conjugated anti-human IL-18 monoclonal antibody was added to the wells and incubated for 60 min. After another washing, the peroxidase substrate was mixed with the chromogen and allowed to incubate for an additional period of 30 min. Subsequently, an acid solution was added to each well to terminate the enzyme reaction and to stabilise the developed colour. Next, the optical density (O.D.) of each well was measured at 450 nm using a microplate reader. The concentration of human IL-18 was calibrated from a dose response curve, based on the reference standards. The optical densities were measured on the el x 800 (Biocom) microplate reader. O.D. values derived from standards were plotted as a standard curve. The concentration of samples was calculated by salving for the equation of the standard curve.

FLOW CYTOMETRY

Percentages and mean fluorescent intensity (MFI) of the following cells were studied: CD3+ (T lymphocytes), CD19+ (B lymphocytes), CD4+8+ (double positive lymphocytes CD4+, CD8+), CD4+ (T helper lymphocytes), CD8+ (T suppressor lymphocytes), NK (natural killer cells), CD25+3+ (T lymphocytes with alpha chain of IL-2R), CD25+ (alpha chain of IL-2R on all lymphocytes), CD45RO+ (memory cells on all lymphocytes), CD45 RO+/CD4+ (memory cells T helper lymphocytes). Equally, the expression of the aforementioned antigens on the same cells was measured as Mean Fluorescent Intensity (MFI).

In each case, the cell surface antigens 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 colour immunofluorescence studies were performed using combinations of phycoerythrin (PE) and fluorescein isothyocyanate (FITC) conjugated monoclonal antibodies. The monoclonal antibodies were obtained from Ortho Diagnostic Systems (Germany), Becton Dickinson (Germany), and Dako (Denmark). The combinations of the same antibodies as described in our previous article published in "Annales UMCS" (7) were used.

RESULTS

The results presented in Table 1 do not demonstrate any big differences with respect to the percentage and mean fluorescence intensity in many subpopulations of lymphocytes. In psoriasis, we observed lower expression of MFI in CD3+ (T

lymphocytes) and CD19+ (B lymphocytes) cells, in comparison with the control group: in CD3+, MFI equalled 114.2±6.5 vs. 118.71±5.81 ($p<0.05$); also in CD19+, MFI was 69.54±22.4 the group of psoriatic patients vs. 90.6±23.50 in controls ($p<0.05$). The percentage of CD45RO+/CD4+ (memory cells on T helper lymphocytes) in psoriatic patients was significantly higher than in controls: 5.12±3.6 vs. 1.7±1.71 ($p<0.004$). Similarly, the percentage of CD45RO (memory cells on all lymphocytes) cells was significantly higher in patients than in the control group: 4.1±3.05 vs. 1.6±1.02 ($p<0.02$). The mean level of IL-18 in psoriatic patients was higher in patients suffering from psoriasis, reaching the value of 280.4±185.15 vs. 175.69±78.90 pg/ml, but this difference was not statistically significant ($p>0.40$).

DISCUSSION

Companjen et al. (3) demonstrated that human keratinocytes constitutively expressed IL-18 at the mRNA and at the protein level. The protein was mainly expressed intracellularly in the 24 kD unprocessed pro-form, but was also secreted. Consequently, human keratinocytes relative to monocytes, PBMC or leukocytes produce a considerably larger amount of pro-IL-18, which is also readily released. The authors supposed that high constitutive levels of IL-18 might contribute to the skewing towards a Th-1-like environment, which is apparent in many human inflammatory skin diseases (3).

Immunohistochemical and RT-PCR analysis showed that the expression of IL-18 was increased in psoriatic lesional skin relative to that in normal skin. Western blotting and an ELISA for IL-18 in combination demonstrated that the immunoreactive IL-18 in extracts of psoriatic scales contained the mature form of IL-18, but most of the IL-18 were pro-IL-18 (10).

Koizumi et al. (6) favour the interpretation that IL-18 plays a role in the maintenance of homeostasis of a Th-1 dominant state in normal skin.

Recent reports suggested that even in the absence of cell-to-cell contact, T cells could be activated through cytokines such as IL-12 and IL-18 (6). On the other hand, multiple tissue damage may be induced by high levels of IL-18 expression (6). TPA induces protein kinase C downregulation (6). The latter has been demonstrated in psoriatic epidermal cells (6). In patients with psoriasis, Th-1 induction may exacerbate the disease state and IL-18 may contribute to disease progression in these patients (5).

Puren et al. (11) described that in human unstimulated peripheral blood mononuclear cells (PBMC), IL-18 induces the synthesis of IL-1β, TNF-α, IL-6, IL-8, MIP-1-α, and MCP-1 as well as enhances the production of adhesion molecules such as ICAM-1. There may be excessive connections between these cells and the net of cytokines and their receptors in psoriatic skin and psoriatic blood, especially in relation to the increase the percentage of CD45RO4 memory cells on psoriatic lymphocytes. We observed the higher concentration of IL-18 in psoriatic patients but this increase was

not significant. Because IL-18 is involved in immunologically mediated tissue damage, the lack of significant increase of this cytokine level was unexpected, and we are not able to explain this phenomenon (4). IL-18 can play a potential immunoregulatory role in the human defence system, especially in inflammatory, infectious, and autoimmune diseases (13). The activity of IL-18 can be diminished by: specific blockage of IL-18, neutralising anti-IL-18 antibodies, soluble receptors to IL-18, non-agonistic antibodies that bind the IL-18R ligand binding or the IL-1Rrp. IL-18 blocking may enhance the risk of *Mycobacterium* or *Salmonella* infections because of decreased production of INF-γ (13).

Table 1. The comparison of the parameters between psoriasis triggered by infection and the control group

	Controls n=10	*Psoriasis postinfectiosa* N=14	p
Percentage of CD45+ lymphocytes	97.41±1.21	96.87±1.6	>0.5
CD45 MFI	123.78±6.86	129.5±8.6	>0.1
Percentage of CD3+ lymphocytes	66.15±13.02	62.07±15.3	>0.3
CD3+ MFI	118.71±5.81	114.2±6.5	<0.05
Percentage of CD19+ lymphocytes	3.95±1.55	4.14±1.96	>0.8
CD19+ MFI	90.6±23.50	69.54±22.4	<0.05
Percentage of CD4+8+ lymphocytes	0.88±0.70	1.68±2.45	>0.2
CD4+ 8+ MFI	88.31±10.73	88.98±12.86	>0.8
Percentage of CD4	48.71±9.89	40.22±13.07	>0.1
CD4 MFI	106.38±5.00	102.99±7.77	>0.2
Percentage of CD8+	25.62±7.41	23.44±12.6	>0.2
CD8+ MFI	108.84±7.71	104.00±22.64	>0.9
Percentage of NK	15.1±7.4	18.41±13.03	>0.5
NK MFI	91.8±15.8	90.25±23.8	>0.9
Percentage of CD25+3+	2.92±2.0	5.86±5.26	>0.09
CD25+3+ MFI	78.5±15.3	83.26±19.1	>0.4
Percentage of CD25+	0.88±0.61	1.48±1.6	>0.5
CD25+ MFI	80.4±15.06	82.8±18.9	>0.8
Percentage of CD45RO4	1.7±1.71	5.12±3.6	<0.004
CD45 RO4 MFI	83.4±33.2	77.2±17.45	>0.9
Percentage of CD45RO	1.6±1.02	4.1±3.05	<0.02
CD45 RO MFI	79.97±25.92	74.6±14.7	>0.8
Percentage of CD45RA4	22.54±12.10	18.57±11.0	>0.5
CD45 RA4 MFI	116.40±35.96	103.89±12.80	>0.5
Percentage of CD45RA	33.43±7.67	34.02±7.6	>0.6
CD45 RA MFI	124.41±8.47	119.7±12.08	>0.6
IL-18	175.69±78.90	280.4±185.15	>0.4

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SUMMARY

The blood serum concentrations of IL-18 and the subpopulation of blood lymphocytes in peripheral blood were investigated in 14 patients with psoriasis preceded by a bacterial infection. The IL-18 concentrations were measured with the ELISA method, and the lymphocyte subpopulation – by flow cytometry. The elevated IL-18 concentrations in blood serum were observed; however, the increase was statistically insignificant in the group of psoriatic patients in comparison with the control ($p>0.4$). The result of flow cytometry showed that a statistically significant increase of the percentage of CD45RO4 and CD45RO cells, and a statistically significant decrease of MFI expression of CD19+ and CD3+ cells ($p<0.004$; $p<0.02$; $p<0.05$; $p<0.05$) occurred in the patients.

Stężenie IL-18 w surowicy pacjentów z łuszczycą poinfekcyjną

U 14 chorych z łuszczycą poprzedzoną infekcją bakteryjną przeprowadzono badania stężenia IL-18 w surowicy krwi metodą ELISA i subpopulacji limfocytów krwi krążącej za pomocą cytometrii przepływowej. Zaobserwowano podwyższenie stężenia IL-18 w surowicy krwi, z tym że nieistotne statystycznie w grupie chorych z łuszczycą w porównaniu z kontrolą ($p>0.4$). W cytometrii przepływowej u chorych występowało istotne statystycznie podwyższenie odsetka komórek CD45RO4 i CD45RO oraz istotne statystycznie obniżenie ekspresji MFI komórek CD19 oraz CD 3 ($p<0.004$, $p<0.02$; $p <0.05$, $p <0.05$).