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Plasma and scales levels of interleukin 18 in comparison with other possible clinical and laboratory biomarkers of psoriasis activity

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Abstract
The aim of the study was to assess plasma and scales levels of interleukin (IL) 18 collected from psoriatic patients with different disease activity. IL-18 concentrations were measured using an enzyme immunoassay in the plasma and scales of 34 patients with chronic plaque type psoriasis. IL-18 levels were analysed with respect to plasma-transforming growth factor β_1 (TGF-β_1), the disease duration and the duration of the present relapse, and psoriasis area and severity index (PASI). Plasma IL-18 concentration varied from 90 to 1300 pg ml^{-1} and means (368.2 $\pm$ 42.4 pg ml^{-1}) were significantly elevated in comparison with healthy controls (205.9 $\pm$ 31.8 pg ml^{-1}). The presence of IL-18 was also demonstrated in scales from skin lesions. Treatment caused a significant decrease of plasma IL-18 concentration to 250.2 $\pm$ 13.8 pg ml^{-1}. There was a significant correlation between plasma IL-18 levels and PASI values ($r = 0.554$). There was no correlation between IL-18 concentration in scales and PASI, between IL-18 concentrations in plasma and scales, and between plasma IL-18 and the disease duration or duration of present relapse. Plasma TGF-β_1 concentration demonstrated a significant correlation with PASI ($r = 0.353$), but not with IL-18 levels in plasma ($r = 0.063$) and scales (0.141). The sum of plasma levels of IL-18 and TGF-β_1 divided by the optimal coefficient demonstrated a statistically significant correlation with the highest r-value. The findings confirm an association between plasma IL-18 concentration and psoriasis severity. Moreover, it was shown that combined measurement of IL-18 and TGF-β_1 in plasma can be considered as a possible biomarker of psoriasis activity.

Keywords: *Interleukin (IL) 18, transforming growth factor (TGF) β_1, psoriasis, psoriasis area and severity index (PASI)*

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Introduction

Psoriasis is a skin disease characterized by hyperproliferation of keratinocytes associated with formation of inflammatory infiltrates consisting mostly of type-1 cytokine-producing T-cells. The cause of hyperproliferation in psoriasis is still unknown, but it can be a result of the persistent autocrine stimulation by cytokines released from T-cells in the inflammatory infiltrates (Krueger 2002). Epithelial cell proliferation can be inhibited by transforming growth factor (TGF)-β_1 (Wataya-Kaneda et al. 1996, Furue et al. 1997, Wang et al. 1997, Leivo et al. 1998). As

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demonstrated previously, plasma levels of TGF-β_1 are strongly associated with psoriasis severity and treatment efficacy (Flisiak et al. 2002, 2003). TGF-β_1 stimulates tissue inhibitors of metalloproteinases affecting the activity of extracellular matrix metalloproteinases responsible for collagen degradation (Flisiak et al. 2005). A crucial role among hyperproliferative factors play interferon gamma (IFN-γ) produced by T- and natural killer cells as a result of stimulatory activity of interleukin 18 (IL-18) (Gillespie & Horwood 1998, Ohta et al. 2001). The source of IL-18 is monocytes and macrophages, but in the skin this cytokine is first produced in keratinocytes (Companjen et al. 2000). Immunohistochemical analysis showed that the expression of IL-18 was increased in psoriatic lesional skin relative to that in normal skin (Ohta et al. 2001). Psoriasis activity can be related to an imbalance between stimulation and inhibition of keratinocytes proliferation. Therefore, analysis of IL-18 as a hyperproliferative factor in comparison with TGF-β_1 as an inhibitor of keratinocytes proliferation, as well as possible other biomarkers, can provide important information. Elevated levels of circulating IL-18 in psoriatic patients have recently been demonstrated in some studies, but they did not compare its levels with concentration in scales and with other possible biomarkers of the disease.

To assess the effect of psoriasis on circulating and skin IL-18, we analysed its pretreatment concentrations in plasma and scales collected from patients with different activity of the disease. Also analysed was the effect of treatment on IL-18 plasma concentrations. Moreover, to find out the best biomarker, the association between psoriasis area and severity index (PASI), IL-18 and TGF-β_1 was also evaluated.

Materials and methods

Patients

The study was carried out in the plasma and scales collected from 34 patients (11 females and 23 males) with chronic plaque-type psoriasis, aged between 17 and 72 years (mean 42.5 ± 3.0). Patients with other forms of psoriasis as well as persons with a history of any other inflammatory chronic disease were not included in the study. Patients were initially treated with topical application of salicyl and/or sulphur ointment for desquamation and then with dithranol ointment. Blood samples were collected before treatment and 14 days after treatment completion. Scales were collected from the skin lesions before the beginning of treatment. Pretreatment plasma levels of IL-18 were analysed with respect to PASI and TGF-β_1 plasma concentration. Additionally, this analysis included comparison with the duration of the disease (from the first relapse to sample collection) and its present relapse (from the appearance of papular lesions to sample collection). Comparison between pre- and post-treatment plasma IL-18 concentrations was also performed. To establish normal values of IL-18, plasma samples were collected from 18 healthy controls. The mean age of the controls (35.9 ± 1.2 years) did not differ significantly ($p = 0.116$) from the patients group (42.5 ± 3.0 years). The study was approved by the Bioethical Committee of the Medical University of Bialystok.

PASI was calculated by one investigator (I. F.) in all patients according to rules proposed by Fredriksson and Pettersson (1978). The head, trunk, upper and lower limbs were assessed separately for erythema, infiltration and desquamation. The degree of severity of each symptom in each body part was scored from 0 to 4. The area

covered by lesions of a particular body part was assigned a score from 0 to 6. The score for each of the four body parts was obtained by multiplying the sum of the severity scores of the three symptoms by the area score, then multiplied by the constant weighted value assigned to a particular body part as follows: head, 0.1; trunk, 0.3; upper limbs, 0.2; and lower limbs, 0.4. The sum of the scores of body parts gives the PASI.

IL-18 measurement

Venous blood was collected on ice using vacutainer tubes with EDTA and centrifuged at 1000*g* within 30 min of collection. Plasma samples were additionally centrifuged at 10 000*g* for 10 min at 2–8°C for complete removal of platelets. Scales were collected on ice from plaque lesions demonstrating the most intensive inflammation and desquamation, mashed immediately with a homogenizer in buffer (50 mM Tris-HCl, 75 nM NaCl, 1 mM phenylmethyl sulphonyl fluoride) and centrifuged at 1000*g* within 30 min of collection. Plasma and scales samples were diluted 1:5 and stored at −20°C. Thawed samples were assayed in duplicate with the quantitative sandwich ELISA technique using two monoclonal antibodies against two different epitopes of human IL-18 precoated onto microtitre wells (Medical & Biological Laboratories Co., Ltd, Nagoya, Japan). Optical density was read with a microtitre plate photometer Stat Fax® 2100 (Alab, Warszawa, Poland) at 450 nm. The concentration of IL-18 in the sample was determined by interpolation from a standard curve prepared with standard samples supplied by the manufacturer. Plasma IL-18 concentration was expressed as pg ml^{-1}, whereas concentration in scales was calculated in pg mg^{-1} protein, which was measured by the method of Lowry et al. (1951).

TGF-β1 measurement

Plasma samples were assayed by the quantitative sandwich enzyme immunoassay technique using recombinant human TGF-β-soluble receptor type II (TβRII) as a solid phase precoated onto a microplate (Quantikine®, R&D Systems, Inc., Minneapolis, MN, USA), as described (Flisiak et al. 2002, 2003).

Statistical methods

Values were expressed as the mean and standard error (±SE). The statistical comparison of group means was calculated by a two-tailed Student's *t*-test. For correlation analysis, the Pearson product moment correlation was used and linear regression performed. $p < 0.05$ was considered as being statistically significant.

Results

Individual values of plasma IL-18 concentration varied from 90 to 1300 pg ml^{-1}. Mean IL-18 plasma concentration (368.2 ± 42.4 pg ml^{-1}, $n = 34$) was significantly ($p = 0.013$) elevated in comparison with healthy controls (205.9 ± 31.8 pg ml^{-1}, $n = 18$). The presence of IL-18 was also demonstrated in scales from the skin lesions of psoriatic patients (1459 ± 298 pg mg^{-1}). Treatment caused a significant decrease of plasma IL-18 concentration to 250.2 ± 13.8 pg ml^{-1} (Figure 2). PASI score varied from 3 to 34. As shown in Figure 1, there was a significant correlation between plasma

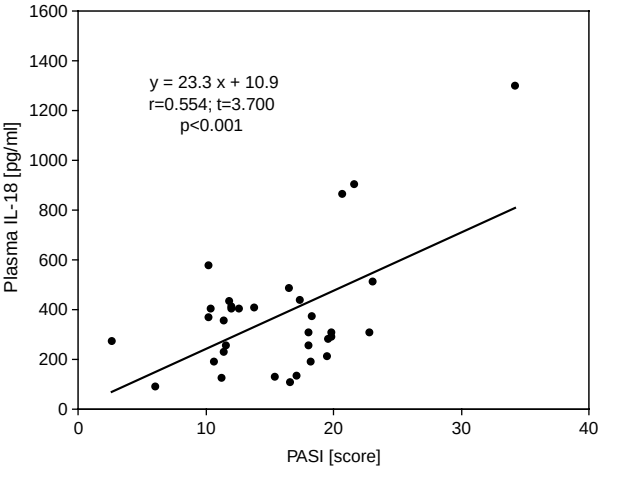

Figure 1. Correlation between plasma IL-18 concentrations and psoriasis area and severity index (PASI) in 34 patients with psoriasis.

IL-18 levels and PASI values ($r = 0.554$). However, there was no correlation between IL-18 concentration in scales and PASI (Table I) and no association was shown between plasma and scales IL-18 ($r = 0.109$). As shown in Table I, there was also no correlation between plasma IL-18 and the disease duration or duration of the present relapse. Plasma TGF-β_1 concentration demonstrated a significant correlation with PASI ($r = 0.353$), but not with IL-18 levels in plasma ($r = 0.063$) and scales ($r = 0.141$).

To find the index with the highest degree of correlation with PASI, the sum of plasma IL-18 and TGF-β_1 values was calculated ($I+T$). Since TGF-β_1 plasma concentrations were much higher, its values were divided by an optimal coefficient of 68 that resulted with the highest r-value. As shown in Figure 3, correlation between this cumulative index and PASI was statistically significant ($r = 0.631$) and distinct from that demonstrated by separate calculation for plasma IL-18 or TGF-β_1.

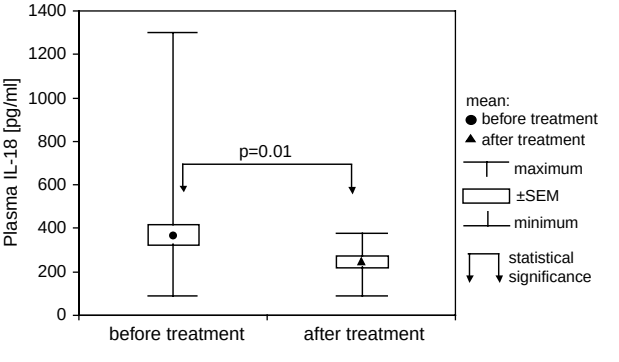

Figure 2. Comparison of IL-18 plasma concentrations in psoriasis patients before and after treatment. Values are means (±SE) and ranges of 34 patients.

Table I. Plasma TGF-β_1, psoriasis area and severity index (PASI), duration of the disease and its present relapse in psoriatic patients, and correlation with plasma or scales IL-18 concentrations.

		r	
Parameter measured in psoriatic patients ($n=34$)	Mean $\pm$ SE	Plasma IL-18	Scales IL-18
Plasma TGF-β_1 (ng ml^{-1})	16.0 $\pm$ 1.7	0.063	0.141
PASI (score)	15.6 $\pm$ 1.0	0.554*	−0.121
Disease duration (years)	17.9 $\pm$ 2.0	0.287	−0.029
Duration of present relapse (months)	2.7 $\pm$ 0.3	−0.221	−0.288

Values are the mean $\pm$ SE of 34 patients.
*Statistically significant ($p < 0.05$) correlation.

Discussion

IL-18 as a member of the interleukin 1 family with pro-inflammatory and tumour-suppressive properties is a key mediator of peripheral inflammation and host defence responses. The immunomodulatory function of IL-18 is indicated by its ability to enhance the production of interferon-gamma. High levels of IL-18 are detected in human diseases associated with immunoactivation including infections and chronic inflammation (Muhl & Pfeilschifter 2004). The presence of IL-18 and its mRNA was determined using polymerase chain reaction in human keratinocyte cultures (Naik et al. 1999, Mee et al. 2000). According to Wittmann et al. (2005), keratinocytes functionally respond to IL-18 with up-regulation of MHC II and production of the chemokine CXCL10/IP-10 supporting an important role of IL-18 in inflammatory skin diseases in the epidermal compartment. In active and progressive lesions of early phases of psoriasis, Companjen et al. (2004) demonstrated an elevated expression of total IL-18 protein and it was more prominent than in established psoriatic lesions. In contrast, down-regulation of IL-18 expression is observed in human keratinocytes in response to corticotropin-releasing hormone, which is main coordinator of stress reaction involved in the pathogenesis of psoriasis (Park et al. 2005).

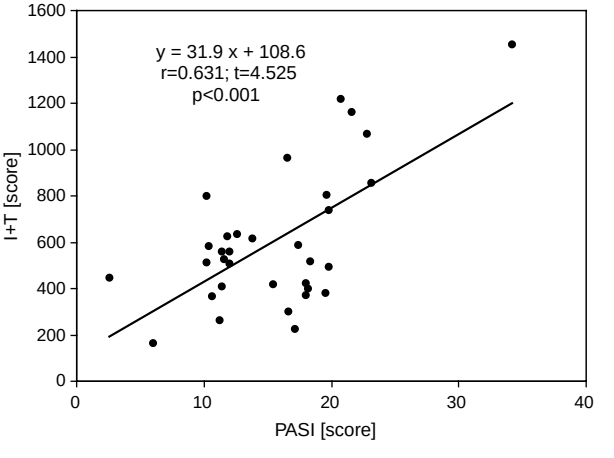

Figure 3. Correlation between $I+T$ index and psoriasis area and severity index (PASI) in 34 patients with psoriasis.

The study confirmed a high concentration of plasma IL-18 and its correlation with PASI. Similar results were demonstrated by Pietrzak et al. (2003) and Gangemi et al. (2003). However, these studies were carried out in much smaller groups of 12 and 16 patients, respectively, and did not include scales or any other skin measurements. To confirm production of IL-18 in keratinocytes, the present paper demonstrated its presence in scales. Of course, it could be more representative to perform analysis in skin samples, but biopsy procedures for research purpose is ethically doubtful, and therefore it was decided to perform measurements in scales. Scales measurement was previously applied by Ohta et al. (2001), who demonstrated expression of IL-18 in extracts of psoriatic scales using immunohistochemical assay, RT-PCR, Western blotting and ELISA. These findings indicate that human keratinocytes are a source of biologically functional IL-18 and participate in the local IFN-gamma-dependent inflammatory processes related to Th1 response in psoriatic lesions. Therefore, therapies targeting IL-18 are currently considered in the treatment of psoriasis (Piskin et al. 2004, Numerof et al. 2005).

The present study did not demonstrate an association between plasma and scales concentrations of IL-18 as well as between its scales levels and PASI. This observation can be explained by similar production of IL-18 in psoriatic lesions irrespective of involved skin area and the disease severity.

As demonstrated previously, measurement of TGF-β_1 in plasma can be considered as a possible biomarker of psoriasis activity during its management (Flisiak et al. 2002, 2003). These results were confirmed in the present study by a statistically significant correlation between PASI and plasma TGF-β_1 concentrations. To obtain the best possible biomarker of psoriasis activity, the authors tried to combine measurement of plasma levels of these two bioactive substances representing contrary effects on keratinocytes proliferation. A calculated $I+T$ index demonstrated a statistically significant correlation with PASI that resulted with higher r-value than observed with respect to individual calculations performed for IL-18 and TGF-β_1.

These findings confirm an association between plasma IL-18 concentration and psoriasis severity. Moreover, it was shown that combined measurement of IL-18 and TGF-β_1 in plasma can be considered as a possible biomarker of psoriasis activity.

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