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Plasma transforming growth factor β_1 as a biomarker of psoriasis activity and treatment efficacy

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Transforming growth factor-β_1 (TGFβ_1) is thought to be an inhibitor of the keratinocyte hyperproliferation associated with psoriasis. The aim of this study was to evaluate plasma TGFβ_1 and TGFβ_2 concentrations in psoriatic patients as possible indicators of treatment efficacy. TGFβ concentrations were measured in the plasma of 26 patients with psoriasis using an enzyme immunoassay and analysed with respect to the psoriasis area and severity index (PASI) before and after treatment with salicylic acid and/or sulphur followed by dithranol ointment. Baseline plasma concentrations of both TGFβ_1 and TGFβ_2 (20.3 ± 2.2 ng ml^{-1} and 0.14 ± 0.02 ng ml^{-1}, respectively) did not differ significantly from control values (18.3 ± 1.6 ng ml^{-1} and 0.14 ± 0.03 ng ml^{-1}, respectively). However, a significant positive correlation ($r = 0.69$) between the baseline PASI and TGFβ_1, but not TGFβ_2, values was demonstrated. The pretreatment TGFβ_1 concentration in patients with a PASI ≥ 15 (26.6 ± 3.2 ng ml^{-1}) was significantly higher than control values. There were no significant elevation of pretreatment TGFβ_1 concentrations in patients with a PASI < 15, or with respect to TGFβ_2 in both groups. Treatment caused a significant decrease in TGFβ_1, but only in patients with a PASI ≥ 15. Patients with baseline TGFβ_1 concentrations exceeding the mean of the control group had a PASI value that was significantly higher than that of patients with a TGFβ_1 concentration below the mean of the controls. These results confirmed an association between plasma TGFβ_1 concentration and psoriasis severity, and demonstrated its normalization during treatment. Measurement of TGFβ_1 in plasma should be considered as a possible biomarker of psoriasis activity during its management.

Keywords: transforming growth factor-β_1, psoriasis, psoriasis activity, severity index.

Introduction

Psoriasis is characterized by hyperproliferation and altered differentiation of keratinocytes associated with an inflammatory infiltrate in the epidermis (Kadunce and Krueger 1995). Since persistent autocrine stimulation of the epidermal growth factor receptor by transforming growth factor-α leads to hyperplasia of psoriatic keratinocytes, the pathogenesis of this disease may be explained by an imbalance in the factors responsible for epidermal proliferation (Krueger *et al*. 1990, Kondo *et al*. 1992, Powell *et al*. 1999). Transforming growth factor-β_1 (TGFβ_1) is a potent inhibitor of epithelial cell proliferation, and its effects on growth and differentiation have been extensively characterized in cultured keratinocytes. Impaired down-regulation of TGFβ_1 may important for the pathogenesis of psoriasis (Wataya-Kaneda *et al*. 1996, Furue *et al*. 1997, Wang *et al*. 1997, Leivo *et al*. 1998).

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Moreover, the role of TGFβ$_1$ is supported by its predominant synthesis in the upper, differentiated layers of the epidermis, the stratum granulosum and stratum corneum. TGFβ$_2$ is also known to be present in the lower epidermal layer (Gold *et al*. 2000). As we have demonstrated recently (Flisiak *et al*. 2002), plasma levels of TGFβ$_1$ are strongly associated with psoriasis severity and therefore can be considered as a possible biomarker for the assessment of disease activity. Despite its established role in the pathogenesis of psoriasis, the use of plasma TGFβ levels in disease management has not been evaluated to date.

The aim of this study was to evaluate TGFβ$_1$ and TGFβ$_2$ concentrations in the plasma of psoriatic patients as possible indicators of treatment efficacy and prognosis.

Materials and methods

Patients

Concentrations of TGFβ$_1$ and TGFβ$_2$ were measured in plasma collected from 26 patients (eight females and 18 males) with chronic plaque-type psoriasis aged 15–72 years (mean ± SEM 39.3 ± 3.4 years). Patients with other forms of psoriasis as well as those with a history of other inflammatory chronic diseases were not included in the study. Patients were treated initially with a topical application of salicylic acid and/or sulphur ointment for desquamation, and then with dithranol ointment. TGFβ$_1$ and TGFβ$_2$ plasma levels were determined before treatment and after 14 days, and analysed with respect to the disease activity, which was measured using the psoriasis area and severity index.

Normal values of plasma TGFβ$_1$ and TGFβ$_2$ were determined in 13 healthy controls. The mean age of the controls (47 ± 2 years) did not differ significantly ($p = 0.11$) from that of the patients.

The study was approved by the Bioethical Committee of the Medical University of Bialystok, and informed consent was obtained from all patients.

Psoriasis area and severity index

The psoriasis area and severity index (PASI) was calculated according to rules proposed by Fredriksson and Pettersson (1978) and standardized by Thompson and Feutren (1997). The head, trunk, upper and lower limbs were assessed separately for erythema, infiltration and desquamation. The degree of severity of each symptom in each of the body parts was scored from 0 to 4. The area of each body part covered by lesions 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, and then multiplying the product by the constant weighted value assigned to particular body part (head, 0.1; trunk, 0.3; upper limbs, 0.2; lower limbs, 0.4). The sum of the scores for the four body parts gives the PASI.

TGFβ$_1$ and TGFβ$_2$ measurement

Venous blood was collected through a wide gauge needle on ice using Vacutainer tubes with ethylene diamine tetra-acetic acid as an anticoagulant and centrifuged at 1000 *g* within 30 min of collection. The plasma obtained was additionally centrifuged at 10 000 *g* for 10 min at 2–8°C for the complete removal of platelets, which are the main source of TGFβ in the blood. To activate latent TGFβ$_1$ to immunoreactive TGFβ$_1$, 0.1 ml of a mixture of 2.5 N acetic acid and 10 M urea was added to 0.1 ml of platelet-poor plasma and incubated for 10 min at room temperature. Acidified samples were neutralized by adding 0.1 ml of a mixture of 2.7 N NaOH and 1 M HEPES. TGFβ$_2$ was activated through acidification of samples with 1 N hydrochloric acid and subsequent neutralization with mixture of 1.2 N NaOH and 0.5 M HEPES

Activated samples were diluted and assayed in duplicate using the quantitative sandwich enzyme immunoassay technique with recombinant human TGFβ soluble receptor type II as a solid phase precoated onto a microplate (Quantikine®, R&D Systems Inc., Minneapolis, USA), as described previously (Flisiak *et al*. 2000). The optical density was read with a microtitre plate photometer Stat Fax® 2100 (Alab, Warsaw, Poland) at 450 nm and corrected by subtraction of readings at 540 nm. The concentrations of TGFβ$_1$ and TGFβ$_2$ were determined by interpolation from calibration curves and expressed as ng ml^{-1}. The cross reactivity between TGFβ$_1$ and TGFβ$_2$ is 0.48%; other cytokines do not cross-react in this assay.

Statistical methods

Values were expressed as the mean ± SEM. The range of normal values was calculated as the mean ± 2SD. Statistical comparisons of the group means were performed using the two-tailed Student's *t*-test. The Pearson product moment correlation was used for correlation analysis, and linear regression was performed. Values of $p < 0.05$ were considered to be significant.

Results

The PASI before treatment ranged from 2.6 to 34.2 (mean 15.6 ± 1.3; median 16.0). The second evaluation performed after the completion of treatment demonstrated a significant decrease in the PASI mean value to 0.8 ± 0.1. Baseline plasma concentrations of both TGFβ_1 and TGFβ_2 (20.3 ± 2.2 ng ml^{-1} and 0.14 ± 0.02 ng ml^{-1}, respectively) did not differ significantly from control values (18.3 ± 1.6 ng ml^{-1} and 0.14 ± 0.03 ng ml^{-1}, respectively). As shown in Table 1, the mean value of TGFβ_1 decreased after treatment, but the difference between both baseline and after-treatment measurements was not statistically significant ($p = 0.18$). Treatment did not cause any concentration changes with respect to TGFβ_2 (Table 1).

Analysis of individual patients demonstrated a significant positive correlation ($r = 0.69$) between baseline PASI and TGFβ_1 values (Figure 1). There was no such as association with respect to TGFβ_2 concentrations ($r = 0.02$). Evaluation carried out after the treatment showed no correlation between PASI and TGFβ_1 ($r = 0.29$) or TGFβ_2 ($r = 0.01$). We did not demonstrate any association between the concentrations of the two growth factors before ($r = 0.14$) or after ($r = 1.3$) treatment.

As demonstrated in Table 2, division of patients into two groups according to the PASI (< 15 and ≥ 15) demonstrated apparent differences in TGFβ_1 concentrations, whereas the mean TGFβ_2 values were almost the same in both groups. The baseline TGFβ_1 concentration (26.6 ± 3.2 ng ml^{-1}) in patients with a PASI ≥ 15 was significantly higher than control values ($p = 0.03$). There were no significant elevation of pretreatment TGFβ_1 concentrations in patients with a PASI < 15, or with respect to TGFβ_2 in both PASI groups (Table 2). The difference in baseline TGFβ_1 plasma levels between the two groups was statistically significant (Figure 2). Treatment caused a significant decrease in TGFβ_1 but only in patients with more severe disease (PASI ≥ 15).

Patients with a baseline TGFβ_1 concentration exceeding the mean of the control group (18.3 ng ml^{-1}) had a PASI value (19.9 ± 1.5) that was significantly ($p = 0.0001$) higher than that in patients with a TGFβ_1 value below the mean of the controls (11.2 ± 1.1). Four patients with a TGFβ_1 level exceeding the upper limit of normal had a mean PASI value of 23.3.

Table 1. Mean (±SEM) plasma TGFβ_1 and TGFβ_2 concentrations before and after local treatment of psoriatic patients compared with control values.

	Before treatment	After treatment	Controls
TGFβ_1 (ng ml^{-1})	20.3 ± 2.2	16.6 ± 1.5	18.3 ± 1.6
TGFβ_2 (ng ml^{-1})	0.14 ± 0.02	0.15 ± 0.02	0.14 ± 0.03

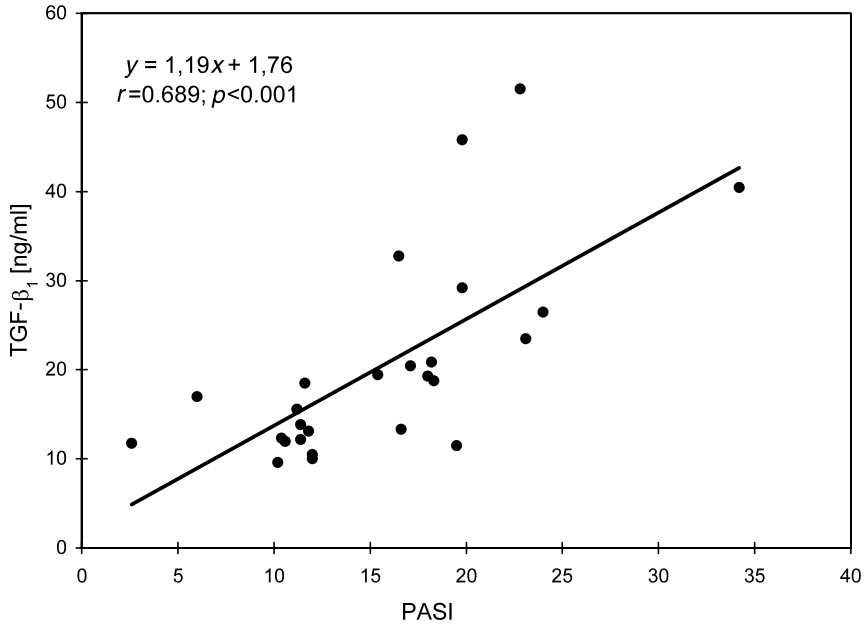

Figure 1. Correlation between plasma TGFβ$_1$ level and PASI.

Table 2. Mean (±SEM) plasma TGFβ$_1$ and TGFβ$_2$ concentrations before and after local treatment of psoriatic patients divided according to baseline PASI values.

	PASI < 15		PASI ≥ 15	
	Before	After	Before	After
TGFβ$_1$ (ng ml^{-1})	14.8±1.4	15.1±1.0	26.6±3.2*	16.4±2.7
TGFβ$_2$ (ng ml^{-1})	0.13±0.01	0.11±0.03	0.14±0.03	0.16±0.02

* Statistically significant ($p < 0.05$) compared with controls.

Discussion

Psoriasis is characterized by a marked hyperproliferation of keratinocytes in association with vascular expansion, fibroblast activation, leukocyte infiltration and alterations in cytokine production (Kapp 1993, Suomela *et al*. 2001). The intracellular localization of TGFβ$_1$ demonstrated in keratinocytes indicates the synthetic ability of these cells (Kane *et al*. 1990, Borkowski *et al*. 1996, Gold *et al*. 2000). Its role was first established with respect to cutaneous fibrosis and wound healing (Singer and Clark 1999). However, TGFβ has also been found to antagonize the effects of TGFα, which is thought to be responsible for the hyperplasia of psoriatic keratinocytes (Kondo *et al*. 1992, Powell *et al*. 1999). As demonstrated recently, TGFβ activity may be mediated at least in part by the Mad1 transcription factor (Werner *et al*. 2001). Thus this cytokine acts as a multi-functional regulator of both cell growth and differentiation and is very important for the pathogenesis of psoriasis.

Since the main source of blood TGFβ is platelets, they had to be removed before degranulation for reliable measurement of circulating TGFβ, which should

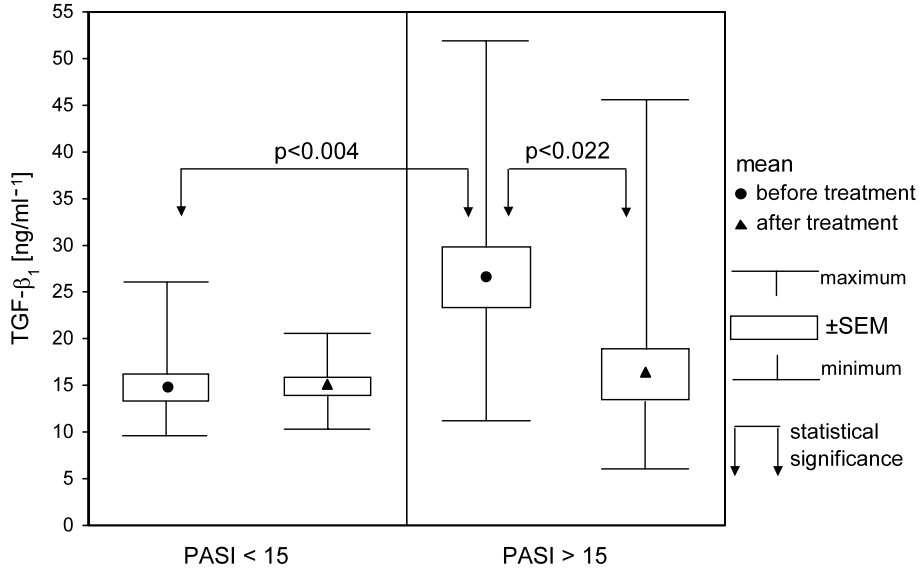

Figure 2. Comparison of means (±SEM) and ranges of TGFβ_1 plasma concentrations before and after treatment according to baseline PASI values.

be performed in plasma. According to Grainger *et al*. (2000), the protocol used in the present study minimizes platelet degranulation and improves extraction procedures.

The mean plasma concentration of TGFβ_1 in the patients in this study (20.3 ± 2.2 ng ml^{-1}) was slightly higher than that demonstrated previously in another group of psoriatic patients (15.7 ± 1.4 ng ml^{-1}; Flisiak *et al*. 2002), but the levels in both studies did not differ significantly from control values. TGFβ_1 levels in psoriatic patients were much lower than those measured previously in our laboratory in patients with active chronic liver disease (Flisiak *et al*. 2000). Concentrations of TGFβ_2 in both our psoriasis-related studies were similar to normal. This can be explained by the predominant expression of TGFβ_2 in the lower epidermal layer, which is not affected during psoriasis (Gold *et al*. 2000). Moreover, the values were not associated with disease activity. In our previous research there was also no association between disease activity and TGFβ concentration in scales from psoriatic lesions, so in this study we evaluated only plasma samples. We were able to demonstrate a strong correlation ($r = 0.69$) between the PASI, a generally accepted clinical method of psoriasis assessment, and concentrations of TGFβ_1 but not TGFβ_2. This correlation together with the simultaneous lack of significant difference compared with normal values indicates the necessity of grouping patients with respect to disease severity. The significant difference demonstrated between the pretreatment results in the two categories supported the possible application of TGFβ_1 measurement as a biomarker of psoriasis activity. On the other hand, plasma TGFβ_1 can be elevated in other inflammatory and fibrotic diseases, and so is not specific for psoriasis. However, once other reasons for TGFβ_1 elevation have been excluded, this can be another tool for psoriasis assessment. Its reduction resulting from successful treatment

indicates its usefulness in disease management. The prognostic value of TGFβ_1 measurement was supported by the significantly elevated pretreatment PASI in patients with a baseline concentration exceeding the mean value of the controls.

One possible explanation of the increased plasma TGFβ_1 concentration in psoriatic patients is as a result of inflammation (Blobe *et al*. 2000). On the other hand, it may be the result of the activation of endothelial cells and fibroblasts that is associated with vascular expansion (Singer and Clark 1999, Oyama *et al*. 2000). Since elevation of plasma TGFβ_1 can be observed in some other inflammatory and fibrotic diseases (pulmonary, hepatic, bowel), we excluded such patients from our study.

Our findings confirmed an association between plasma TGFβ_1 concentration and psoriasis severity. Moreover, we demonstrated its reduction during successful treatment. Therefore TGFβ_1 measured in plasma should be considered as a possible biomarker of psoriasis activity during its management.

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