

Long pentraxin 3: A marker of inflammation in untreated psoriatic patients

VALENTINA BEVELACQUA^{1,2*}, MASSIMO LIBRA^{2*}, MARIA C. MAZZARINO², PIETRO GANGEMI³, GIUSEPPINA NICOTRA⁴, SALVATORE CURATOLO¹, DANIELA MASSIMINO¹, ANTONIO PLUMARI¹, PAOLA MERITO², GUIDO VALENTE⁵, FRANCA STIVALA², STEFANO LA GRECA¹ and GRAZIA MALAPONTE²

¹Dermatology Unit, AORNAS, Garibaldi Hospital; ²Department of Biomedical Sciences, University of Catania; ³Division of Pathology, Vittorio Emanuele Hospital, Catania; ⁴Molecular Pathology Laboratory, Department of Medical Sciences, Amedeo Avogadro, University of Eastern Piedmont; ⁵Department of Clinical and Experimental Medicine, Amedeo Avogadro, University of Eastern Piedmont, Novara, Italy

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Abstract. Psoriasis is a common cutaneous disorder characterized by abnormal epidermal differentiation, proliferation and inflammation mediated by dermal infiltrates, such as T cells, neutrophils, dendritic cells and macrophages. There are renewed interest in the role of components of the innate immune system. Cytokines such as tumor necrosis factor- α (TNF- α) and interleukin (IL)-6, and -1 β involved in pathogenic phenomena in psoriasis are known as inducers of the acute phase response. Among the large group of acute phase reactants, C-reactive protein (CRP) and fibrinogen are of special interest in psoriasis. The PTX-3, a long pentraxin sharing similarities with the classical short proteins. Thus, considering the numerous biological roles of inflammatory cytokines and their relationship with inflammatory markers, such as CRP and fibrinogen we have investigated the role of PTX3 in psoriasis. To this aim PTX3, TNF- α , IL-6 and IL-1 β in plasma and in monocytic cultures by enzyme linked immunosorbent assay (ELISA) in 44 patients including severe and mild psoriasis were measured. An increased production of PTX3, both in supernatant of purified monocytes and in plasma from patients with severe psoriasis, was found. The significant correlation, between cellular production and plasma levels of PTX3 in psoriasis was found as a sign of cellular activation by monocytes/macrophages that first infiltrate the psoriatic lesion. In severe psoriasis, a significant correlation between psoriasis area and severity

index (PASI) score and TNF- α and IL-6 levels in both supernatant of monocytes and plasma was found. In contrast, no correlation was found for IL-1 β . By immunohistochemistry and immunofluorescence, a strong PTX3 staining in fibroblasts, endothelial cells and monocytes/macrophages in severe psoriatic lesional skin was detected. Finally, a positive correlation between PTX3 and disease activity of psoriasis was observed as PASI score was elevated. These findings suggest that PTX3 could be used as a further marker of disease activity of psoriasis.

Introduction

Psoriasis is a common cutaneous disorder characterized by abnormal epidermal differentiation, proliferation and inflammation mediated by dermal infiltrates, such as T cells, neutrophils, dendritic cells and macrophages (1). In recent years, important information has been provided by studies addressing the immunopathogenic mechanisms of the disease (2,3). Multiple factors contribute to the initiation of psoriasis. They include specific genetic factors such as alleles from the major histocompatibility complex and various trigger factors, such as stress and infections (4-9). Psoriasis is considered as a mediated autoimmune disease caused by dysregulation of innate and adaptive immunity (10,11). There is renewed interest in the role of components of the innate immune system. However, it may be that the overlap between the innate and acquired arms of the immune system can better explain immunopathogenesis in psoriasis (12). Inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β and IL-6, expressed in psoriatic patients, of the innate immune system initiate a cascade that activates inflammation locally in the skin and in the circulation (13,14).

Some of the cytokines involved in pathogenic phenomena in psoriasis are known as inducers of the acute phase response. Among the large group of acute phase reactants, C-reactive protein (CRP) and fibrinogen may be of special interest in psoriasis, given their relationship with inflammatory cytokines involved in the development of skin inflammation

Correspondence to: Dr Grazia Malaponte, Department of Biomedical Sciences, University of Catania, Via Androne 83, 95124 Catania, Italy
E-mail: g.malaponte@unict.it

*Contributed equally

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Table I. Patient characteristics with psoriasis vulgaris.

	Healthy controls (C)	mild Psoriasis (mP)	P-value mP versus C	severe Psoriasis (sP)	P-value mP versus sP
Subjects	25	18	-	26	-
Age (years) ^a	40±12.74	36.22±8.71	NS	45.85±8.05	NS
Male/female ^b	14/11	10/8	NS	16/10	NS
Psoriasis diseases	-	-	-	-	-
Arthritis psoriasis	-	5		10	-

^aP-value was calculated by Wilcoxon test; ^bP-value was calculated by Chi-square test. Age is expressed as mean ± standard deviation; NS, not significant.

(15). Several distinct larger proteins have been identified including 'the long pentraxins' (16). The PTX-3, a long pentraxin sharing similarities with the classical short proteins [CRP and serum amyloid P component (SAP)] but differs in the presence of an unrelated long N-terminal domain coupled to the C-terminal pentraxin domain, for gene organization, chromosomal localization (chromosome 3q25) and cellular source (17). PTX-3 is produced at sites of inflammation by several types of cells, primarily by dendritic cells, macrophages, fibroblasts, activated endothelia, and by other tissues; its production is induced by inflammatory mediators such as lipopolysaccharide (LPS), IL-1 β and TNF- α , but not IL-6 (18,19). In contrast, the classical short pentraxins (CRP and SAP) are produced only by hepatocytes in response to inflammatory cytokines, in particular IL-6 and IL-1 (20,21). In fact, various inflammatory diseases impair CRP and fibrinogen production (22-26).

In humans, PTX3 levels are detectable during severe infections, autoimmune, and degenerative conditions, as rheumatoid arthritis (RA), systemic scleroderma (SSc) and small vessel vasculitis (27-29). PTX3 plays an important role in innate resistance against selected pathogens, in female fertility, in the regulation of inflammatory reactions, and possibly in autoimmunity (18).

Thus, considering the numerous biological roles of inflammatory cytokines and their relationship with inflammatory markers (CRP, fibrinogen) (29) and given similarities and differences between PTX3 and CRP, it is important to assess the usefulness of PTX3 as a novel diagnostic tool, which may better reflect the involvement of tissue in inflammatory processes.

The aim of this study was to analyze PTX3 levels in both plasma and supernatant of purified monocytes of patients with mild and severe psoriasis. PTX3 was also correlated with worsening of the disease, CRP, fibrinogen and proinflammatory cytokines (TNF- α , IL-6, IL-1 β).

Materials and methods

Subjects. A total of 44 patients with psoriasis vulgaris (27 males, 17 females), mean age 42.2±9.4 (range: 23-58) years, were enrolled in this study. The patients were divided in two groups: 26 patients with severe psoriasis (age range: 36-58)

and 18 with mild psoriasis (age range: 23-46 years). The demographic and clinical characteristics are presented in Table I. Clinical evaluation of disease severity, expressed as the psoriasis area and severity index (PASI), was performed before the introduction of antipsoriatic treatment. The average PASI score in psoriatic patients was (range 3-33.4). None of the patients had received any local or systemic treatment before the collection of the blood samples. The control group consisted of 25 age- and sex-matched healthy individuals. Neither patients nor controls had a history of clinical or routine laboratory findings consistent with, impaired hepatic or renal function, nor parasitic or any other infection. All subjects signed an informed consent form.

Cell isolation, culture and stimulation of monocytes. Blood with anticoagulant, ethylenediamine tetraacetic acid (EDTA) was collected into pyrogen-free tubes from patients with psoriasis vulgaris and healthy subjects. Peripheral blood mononuclear cells were isolated as described by Böyum (30). Monocytes were prepared by centrifugation at 500 g for 30 min at room temperature on an isosmotic solution of 46% Percoll (Pharmacia, Uppsala, Sweden). The purity of monocytes was analysed on a FACScan flow cytometer (Becton Dickinson, Milan, Italy) with monoclonal antibodies CD14 and CD11c/CD18 (Becton Dickinson). The estimated purity was ~95%, and >90% of monocytes were positive for non-specific acid esterase activity. Cell viability was estimated, by the trypan blue exclusion test, to be ~95%. Monocytes were incubated for 20 h at 37°C in 5% CO₂ 95% air atmosphere, in hydrophobic Petriperm dishes (Heraeus, Hanau, Germany). They were cultured without stimulus for evaluation of the spontaneous production of PTX3 and proinflammatory cytokines. The supernatant was collected after a 20 h incubation and put through a filter with 0.2 nm pores (Sigma Chemical Co., St. Louis, USA) and stored at -80°C prior to analysis of PTX3 and cytokines. All reagents used were LPS-free, as determined by the Limulus amoebocyte lysate (LAL) assay (Kabi vitrum, Munich, Germany).

Preparation of blood samples. Blood was collected into pyrogen-free tubes with and without anticoagulant, EDTA and centrifuged within 30 min after collection to obtain plasma and serum. Subsequently, the plasma and serum

were stored at -80°C until further evaluation. The plasma was used for the evaluation of fibrinogen, PTX-3 and IL-6, IL-1, TNF- α cytokines, the serum for CRP.

Markers of inflammation: acute phase protein. Serum CRP was evaluated by nephelometry (N High sensitivity CRP; Dade Behring). To evaluate the plasma levels of fibrinogen we used a turbidimetric assay (DiaMed, Morat, Switzerland).

Immunohistochemistry. Formalin-fixed paraffin-embedded tissue sections were cut from cutaneous biopsies. Sections were mounted on glass slides, deparaffinized in xylene, rehydrated in graded alcohol and finally washed in water. Prior to incubation with antibodies, the sections were subjected to a heat-induced antigen-demasking reaction. Immunohistochemistry was performed by a standard procedure employing polyclonal rabbit antibodies against human PTX3, as primary antibody (diluted 1:250, catalogue number P0496 purchased from Sigma Chemical); as second step, the sections were incubated with a peroxidase-conjugated polymer carrying an antibody against rabbit immunoglobulins (ChemMate Dako EnVision kit; Dako Cytomation). After washing out the excess of antibodies, a reaction product was developed by using the ChemMate diaminobenzidine as chromogen (Dako Cytomation). Negative control was made either by omitting the primary antibody or by using in the first step an isotype matched polyclonal antibody specific for HBcAg (Dako; diluted 1:200) (not shown). Sections were then counterstained with hemalum (Merck, Germany). The cells were judged as positive when the reaction product was well detectable at low magnification. Representative areas were imaged using a microscope equipped with a digital camera.

Immunofluorescence. Biopsy sections were prepared as described for immunohistochemistry (see above). Incubation with the primary antibody anti-PTX3 (1:200 in phosphate buffer saline supplemented with 0.1% Triton X-100 and 4% foetal calf serum, for 16 h in a humid chamber at 4°C) was followed by a 1-h incubation with secondary antibodies conjugated with FITC (diluted 1:200 as above, for 1 h at room temperature in a humid chamber). Sections were also stained for actin, which marks the cell boarder, using a monoclonal antibody anti-human actin (diluted 1:200 as above; Sigma) followed by a TRITC-conjugated goat anti-mouse IgG, diluted 1:200 (Sigma). Excess of unbound antibody was removed by two washes with phosphate buffer saline. As negative control the cells were incubated with the secondary antibody alone. Stained sections were mounted with Slow-FAD (light anti-FADE kit, Molecular Probes) and observed under an immunofluorescence microscope (Zeiss fluorescence microscope equipped with a digital camera). Each biopsy was tested at least 3 times.

PTX-3 protein. PTX3 was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) (13). In brief, 96-well ELISA plates (Nunc, Roskilde, Denmark) were coated with 100 ml of anti-PTX3 monoclonal antibody (mAb) MNB4 in coating buffer (15 mM carbonate buffer pH 9.6) and incubated overnight at 4°C . After incubation, the plates were extensively

washed 3 times with washing buffer (phosphate-buffered saline containing 0.05% Tween-20), and 300 ml of 5% dry milk in washing buffer were added to block nonspecific binding sites. The plates were incubated for 2 h at room temperature and then washed 3 times with washing buffer. Purified human recombinant PTX3 standards (50 μl) (75 pg/ml to 10 ng/ml), or unknown plasma samples diluted in RPMI-1640 medium (Seromed, Berlin, Germany) and 2% bovine serum albumin (Sigma Chemicals), were added in triplicate to each well and incubated for 2 h at 37°C . The plates were washed 5 times with washing buffer and 100 μl of biotinylated rabbit IgG anti-PTX3 diluted 1:2000 in washing buffer were added. The plates were incubated for 1 h at 37°C , and then washed 5 times with 300 ml of washing buffer. Streptavidin-horseradish peroxidase (100 μl /well) (Amdex, Copenhagen, Denmark) diluted 1:4000 was subsequently added and the plates incubated for 1 h at room temperature. After incubation, the plates were washed 5 times and 100 ml of the chromogen substrate ABTS (Kirkegaard and Perry Laboratories, Gaithersburg, MD) were added. Plates were read after 15 min at 405 nm in an automatic ELISA reader.

Pro-inflammatory cytokine assay. Levels of TNF- α , IL-6, and IL-1 β were determined in duplicate samples with a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA), in accordance with the manufacturer's instructions. The lower limit of detection was 8 pg/ml for TNF- α , 3.5 pg/ml for IL-6 and 4.5 pg/ml for IL-1 β .

Statistical methods. Statistical analysis was performed using SPSS (version 10, SPSS Inc., Chicago, USA). Values are expressed as mean \pm standard deviation (SD). The distribution of males and females in the SP and MP groups was compared by Chi-square analysis. Differences among the groups were assessed by Student's t-test for two samples for normally distributed variables and Wilcoxon test for non-normally distributed variables. Spearman coefficients were calculated to determine the correlation among PTX3 and fibrinogen, CRP, PASI and cytokines. Significance tests were two-sided, and values $p < 0.05$ were considered significant.

Results

The results of our study show, on the one hand, inflammatory parameters between psoriatic patients and healthy controls, and, on the other, the changes between mild and severe psoriasis.

Immunohistochemistry and immunofluorescence analysis of PTX3. In order to assess the presence of PTX3 protein and to define the cell types involved in PTX3 production, PTX3 expression was analysed in normal skin and in psoriatic lesion skin by immunohistochemistry analysis (Fig. 1a-d). Additionally, immunofluorescence analysis of PTX3 was performed in psoriatic lesion skin (Fig. 2). Fig. 1a shows the absence of PTX3 in vessel walls of normal skin, while Fig. 1b and c describe a strong PTX3 staining in severe psoriatic lesional skin. In particular, in psoriatic lesional skin, intense staining in dermal fibroblasts, endothelial cells and infiltrating of monocytes/macrophages was present; the nuclei were

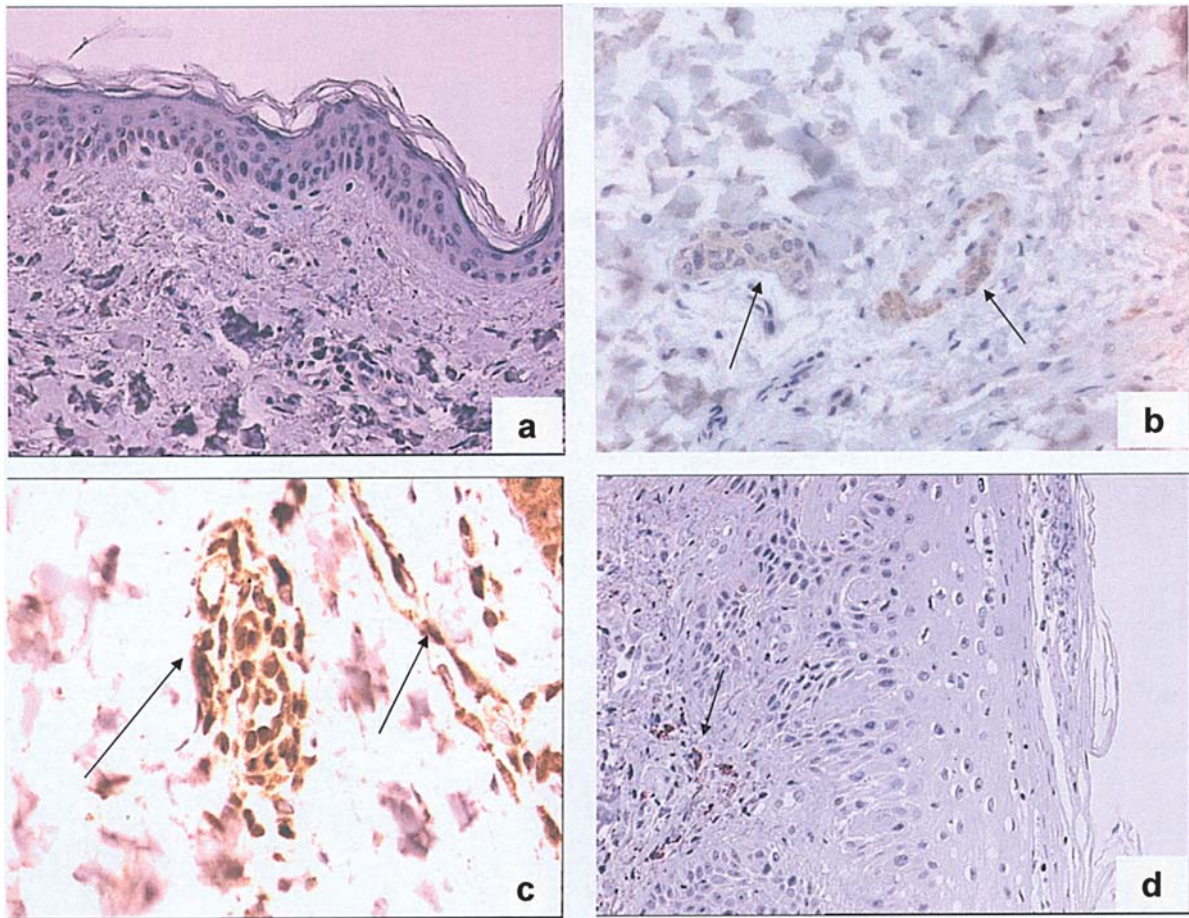


Figure 1. Expression of PTX3 by endothelial cells, macrophages and fibroblast. Section of human skin biopsy from normal skin shows the absence of PTX3 expression (a). Section of human skin biopsy from severe psoriasis shows the presence of expression of PTX3 in the endothelial cells (b) and macrophages (c). Section of human skin biopsy from mild psoriasis shows the presence of expression of PTX3 in macrophages (d).

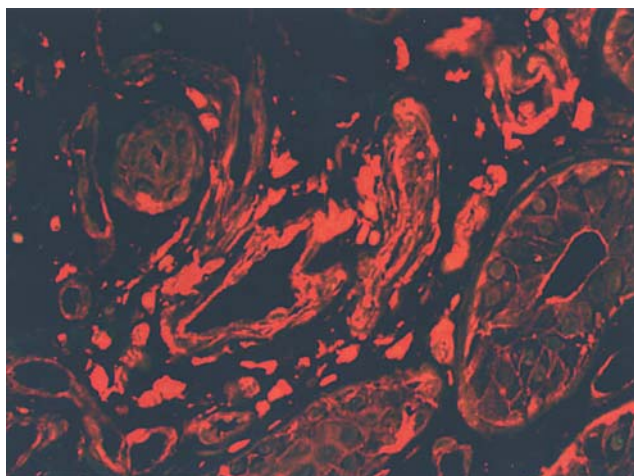


Figure 2. Immunofluorescence analysis of PTX3 in psoriatic lesion skin. Positivity was observed in endothelial cells and ‘macrophage-like cells’ in the dermal region.

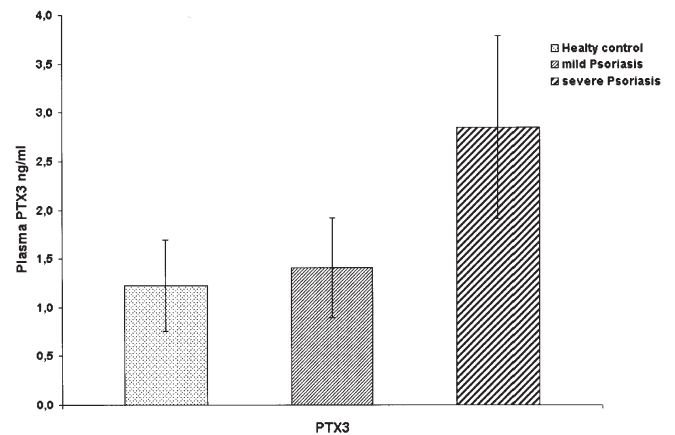


Figure 3. PTX3 plasma levels in mild and psoriatic patients.

constantly negative (Fig. 1c). These results were confirmed by immunofluorescence analysis of psoriatic lesional skin (Fig. 2). In mild psoriasis, a weak presence of PTX3 in vessel walls was evidenced by immunohistochemistry analysis and a few PTX3 positive cells (monocytes/macrophages) were detected in the dermis demonstrating a poor leucocyte infiltrate (Fig. 1d).

PTX3 levels in plasma and in monocytic cultures. Fig. 3 shows PTX3 plasma levels in psoriatic patients and in healthy controls. Patients with severe psoriasis showed PTX3 plasma levels significantly higher than those of healthy controls ($p < 0.0001$). Similarly, patients with severe psoriasis showed PTX3 plasma levels higher than those with mild psoriasis

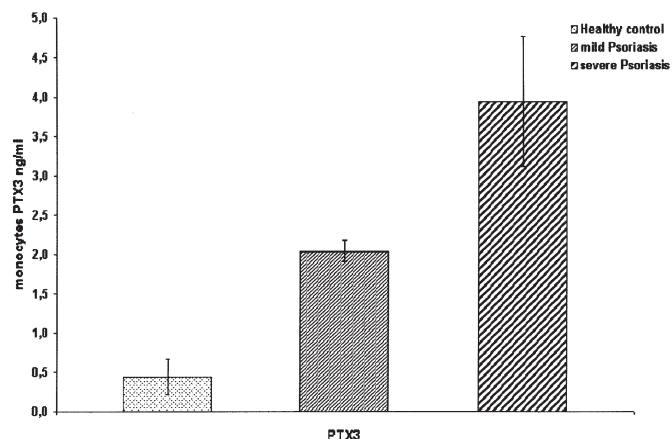


Figure 4. PTX3 levels in supernatant of monocytes cultures from mild and psoriatic patients.

($p < 0.0001$). While, no statistical significance were observed between PTX3 plasma levels of mild psoriasis patients and healthy controls (Fig. 3).

Fig. 4 shows the release of PTX3 in supernatant of purified monocytes from two groups of psoriatic patients (severe and mild psoriasis) and healthy controls. Monocytes from patients with severe psoriasis produced higher PTX3 levels when compared with those of mild psoriasis and healthy controls ($p = 0.006$, $p < 0.0001$, respectively). In contrast to PTX3 plasma levels, significant differences in PTX3 production were found in mild psoriatic patients compared to healthy controls ($p < 0.0001$) (Fig. 4).

CRP and fibrinogen levels. In addition to PTX3, CRP and fibrinogen levels were also studied as inflammatory markers. The mean serum level of CRP and plasma fibrinogen in severe, and mild psoriasis and the control groups are shown in Table II. Among group of patients with severe psoriasis, only 18 of 26 (69.23%) showed increased serum levels of CRP. The difference of CRP serum levels between severe and mild psoriasis was statistically significant ($p < 0.0001$). No significant differences were observed between CRP serum levels in mild psoriasis patients and healthy controls. In the group of patients with severe psoriasis, only 15 of 26 (34.09%) showed increased plasma levels of fibrinogen. Of note, 10 of these 15 patients were also affected by severe arthritis psoriasis. The difference of fibrinogen plasma levels between severe and mild psoriasis was statistically

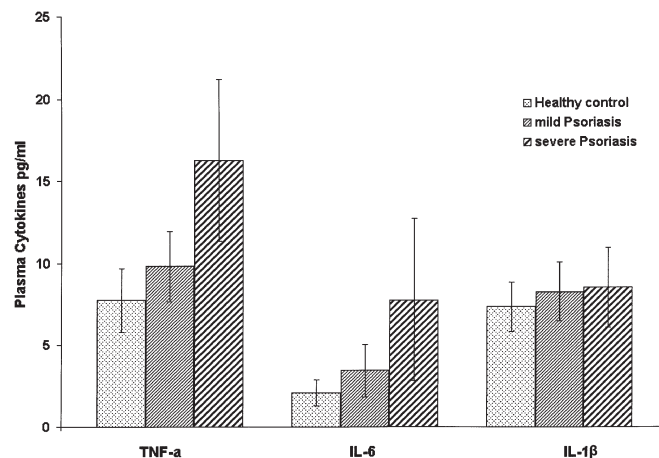


Figure 5. TNF- α , IL-6 and IL-1 β levels in mild and psoriatic patients. TNF, tumor necrosis factor; IL, interleukin.

significant ($p < 0.0001$). No significant differences were observed between CRP serum levels in mild psoriasis patients and healthy controls (Table II).

IL-1 β , TNF- α and IL-6 in plasma and in monocytic cultures.

The mean value of TNF- α plasma levels was higher in mild psoriatic patients than healthy controls (9.55 ± 2.18 pg/ml; 7.7 ± 1.96 pg/ml, respectively; $p > 0.05$) but lower than those with severe psoriasis (16.3 ± 1.96 pg/ml, $p < 0.0001$). The mean value of IL-6 plasma levels was higher in severe than in mild psoriasis patients and in healthy controls (7.8 ± 4.99 pg/ml; 3.4 ± 1.61 pg/ml, $p = 0.004$; 2.1 ± 0.79 pg/ml, $p < 0.001$, respectively). While, no differences were observed between mild psoriasis patients and healthy controls. IL-1 β plasma levels were very low in both psoriatic groups and healthy controls (7.4 ± 1.51 pg/ml; 8.3 ± 1.8 pg/ml; 8.5 ± 2.42 pg/ml, respectively) (Fig. 5).

In addition to plasma levels, we measured the concentration of TNF- α , IL-6 and IL-1 β in the supernatant of purified monocytes from the same psoriatic patients. In severe psoriasis, cellular release of TNF- α (150 ± 36.3 pg/ml) and IL-6 (184.73 ± 64.11 pg/ml) was statistically higher than in mild psoriatic group (63 ± 14.5 pg/ml and 30.5 ± 11.08 , $p < 0.0001$, respectively) and healthy controls (37 ± 6.45 pg/ml and 24.6 ± 9.28 pg/ml, $p < 0.0001$, respectively). In mild psoriasis, only TNF- α levels were higher than healthy controls ($p < 0.001$); IL-6 levels were similar than those from healthy controls. In

Table II. Inflammatory mediators in healthy controls and psoriatic patients.

	Healthy controls (C)	P-value mP versus C	mild Psoriasis (mP)	P-value mP versus sP	severe Psoriasis (sP)	P-value sP versus C
CRP mg/l	<3	NS	<3	<0.0001	7 ± 4.93	<0.0001
Fibrinogen mg/dl	232.36 ± 47.04	NS	257.39 ± 46.60	<0.0001	401.62 ± 74.44	<0.0001
PTX3 ng/ml	1.22 ± 0.47	NS	1.4 ± 0.5	<0.0001	2.84 ± 0.94	<0.0001

CRP, C-reactive protein; PTX3, pentraxin-3; P-value was calculated by Wilcoxon test; NS, not significant.

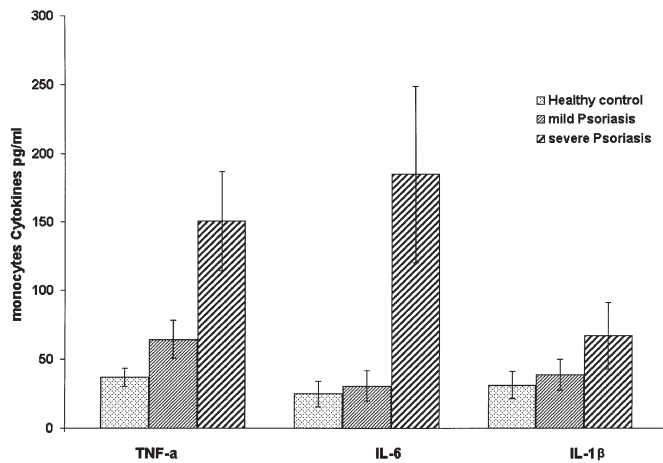


Figure 6. TNF- α , IL-6 and IL-1 β levels of supernatants of monocyte cultures from mild and psoriatic patients. TNF, tumor necrosis factor; IL, interleukin.

severe psoriasis, the production of IL-1 β was higher when compared with that of mild psoriatic group and healthy controls (67 ± 24.43 pg/ml; 38.5 ± 16.92 pg/ml; 31 ± 9.91 pg/ml, $p=0.002$, $p<0.0001$, respectively). In mild psoriasis, IL-1 β level was similar to that of healthy controls (Fig. 6).

Correlation between the PTX3 and mediators of inflammation levels. Plasma levels of PTX3 were correlated by Spearman test with CRP, fibrinogen, and cytokines (TNF- α , IL-6, IL-1 β) (Table III). In addition, we analyzed the correlation between PTX3 plasma levels and PASI score. No correlation was found between PTX3 and CRP in two psoriatic groups of patients; while, a positive correlation was found between PTX3 and fibrinogen levels only in patients with severe psoriasis. A positive correlation was also observed between PTX3 plasma levels and TNF- α in both mild and severe psoriasis. In contrast to TNF- α , no correlations were present between PTX3 versus IL-1 β and IL-6 in the two groups of patients. Notably, a significant correlation was found between PTX3 and PASI score in severe psoriasis, but not in mild psoriasis (Table III). A similar trend was observed when Spearman correlation test was performed between PTX3 and cytokines levels in supernatant of purified monocytes from patients with severe psoriasis (data not shown); the only positive correlation was observed between PTX3 levels and IL-1 β ($r=0.49$, $p=0.01$). Among group of mild psoriatic patients, the positive correlation was observed between PTX3 and both PASI score ($r=0.59$, $p=0.01$) and TNF- α ($r=0.52$, $p=0.02$). Correlation between PTX3 plasma levels and PTX3 levels in supernatant of monocytes from severe psoriatic patients were analyzed ($r=0.55$, $p=0.003$).

Discussion

Psoriasis is a chronic inflammatory disease of the skin, characterized by increased proliferation and differentiation of keratinocytes, leucocytes infiltration and activation of the cutaneous vasculature. It is characterized clinically by a relapsing and remitting course, many environmental factors

Table III. Correlation between PTX3 plasma levels and inflammatory markers in psoriatic patients.

PTX3 versus	mild Psoriasis r-value	severe Psoriasis r-value
CRP	NS	NS
Fibrinogen	NS	0.47 ($p=0.01$)
TNF- α	0.50 ($p=0.03$)	0.54 ($p=0.003$)
IL-1 β	NS	NS
IL-6	NS	NS
PASI score	NS	0.63 ($p=0.0006$)

PTX3, pentraxin-3; CRP, C reactive protein; TNF- α , tumor necrosis factor- α ; IL, interleukin; r-value was calculated by Spearman rank correlation test; NS, not significant.

are known to trigger relapses, including infection (7,8), cutaneous trauma (9) and stress (6). Recent progress in the understanding of psoriasis has shown that the hyperplasia in psoriasis reflects an exaggerated response as innate and adaptive immunity (12). PTX3 belongs to the pentraxin family and participates in the acute phase response to injury, infection and trauma (31-33), factors that could, as previously indicated, trigger psoriatic disease. PTX3 is an important element of innate immunity, since it contributes to the regulation of inflammatory response. In human, CRP together with SAP are acute-phase proteins belonging to classical short pentraxins and, similar to PTX3, are activators of innate immunity and modulator of adaptive immunity (34). Short pentraxins are produced by the hepatocytes in response to inflammatory cytokines (20). IL-1 β acts in synergy with IL-6 to enhance CRP and SAP gene induction (21,22). PTX3 is a member of the pentraxin family, structurally related to, yet distinct from, classical pentraxins (19). In contrast to short pentraxins, PTX3 is produced by dendritic cells, monocytes/macrophages, fibroblasts, endothelial cells and a variety of tissue cells upon exposure to inflammatory mediators such as LPS, IL-1 β and TNF- α , but not IL-6 (18,19). The importance of PTX3 in innate immune response and in inflammation has attracted our attention. Given the similarities and differences between PTX3 and CRP, we have studied PTX3 and its relationship with CRP, fibrinogen and the inflammatory cytokines in psoriasis.

This is the first study that investigated the role of PTX3 in psoriasis. In lesional skin, monocytic cultures and plasma from patients with psoriasis, a remarkable expression, production and release of PTX3 was found. In particular, we observed a strong PTX3 staining in fibroblasts, endothelial cells and monocytes/macrophages in severe psoriatic lesional skin. In sections of skin from patients with mild psoriasis only some endothelial cells and macrophages were stained for PTX3. Specific vascular changes have been described in psoriasis skin (35). Epidermal hyperplasia, fibroblast, endothelial cell activation and leucocyte infiltration are features of psoriasis and are associated with a chronic inflammatory reaction. In contrast, in section of normal skin, quiescent endothelial cells

of vessels did not express PTX3. In severe psoriasis, immunohistochemistry results were confirmed by immunofluorescence analysis.

In addition, to immunohistochemical and immunofluorescence findings, we found also an increased production of PTX3 both in supernatant of purified monocytes and in plasma from patients with severe psoriasis. The significant correlation, found between cellular production and plasma levels of PTX3 in psoriasis, is a sign of cellular activation of monocytes/macrophages, the first cells to infiltrate the psoriatic lesion (36,37). In mild psoriasis group, PTX3 production was also evident, even if lower than in severe psoriasis. We speculate that mild psoriatic patients with high production of PTX3 may evolve into severe psoriasis. This may be due to the activation of monocytes and was confirmed by initial leucocytes infiltration evidenced by immunostaining. Moreover, we found a positive correlation between PTX3 and disease activity of psoriasis indicated as PASI score. These findings suggest that PTX3 could be used as a further marker of disease activity of psoriasis. PTX3 levels were very low in serum and tissues of normal subjects but they rapidly increased in response to inflammatory stimulation in various diseases, such infections, autoimmune, and degenerative disorders (38,39). Elevated levels of PTX3 have also been found in synovial fluid of RA patients, systemic scleroderma (SSc), and small vessel vasculitis (27-29). In addition, among patients with active vasculitis, PTX3 expression in endothelial cells from biopsies of affected skin was demonstrated (29). Association of vasculitis and psoriasis has been reported in the literature (40,41).

Immunostaining and production for PTX3 in our psoriatic samples is caused by both elevated inflammatory cellular response and activation of cells infiltrating lesional skin. Of note, this activation is induced by an increased proinflammatory cytokine production and release (42). TNF- α , IL-6 and IL-1 β are known to be the inflammatory cytokines involved in most inflammations. It was demonstrated that the production of PTX3 is induced by inflammatory mediators such as LPS, IL-1 β and TNF- α (18). These cytokines have been previously indicated as important modulators in the psoriasis process (42). Similarly to other authors, in severe psoriasis we found a significant correlation between PASI score and TNF- α and IL-6 levels in both supernatant of monocytes and plasma (14,43-46). Differently, no correlation was found for IL-1 β .

Our results are different from those of Okubo and Michiyuki (42) and Mizutani *et al* (47). The authors did not find any correlation between monocyte production of TNF- α , IL-6 and IL-1 β and their plasma levels. These discrepancies may be due to the different geographic area of patients studied. In addition, patients unrolled in the present study had not been treated by topical or systemic therapy. In contrast, Okubo and Michiyuki (42) considered psoriatic patients treated with topical corticosteroids. Notably, in the present study some psoriatic patients were also complicated by arthritis. While, patients recruited by Mizutani and Michiyuki were affected by severe psoriasis but without arthritis (47).

Psoriasis arthritis is a heterogeneous disease with several articular patterns (48). Elevated levels of proinflammatory cytokines were observed in psoriasis arthritis (49-51). Our

results emphasize the increased reactivity of monocytes in psoriatic patients both as antigen presenting cells and as active cells producing cytokines along with PTX3. Human monocytes produce PTX3 in response to inflammatory stimuli (18). In severe psoriasis we found a significant correlation between PTX3 and TNF- α both in monocytes and in plasma levels. Similar correlation was found in monocytes between PTX3 and IL-1 β , not in plasma levels. In contrast, we did not find any correlation between PTX3 and IL-6 both in monocytes and in plasma levels. It has been demonstrated that the NF κ B proximal site in human promoter is essential for induction by TNF- α and IL-1 β . No induction of the human promoter was observed with IL-6 (52).

In the present study, in addition to PTX3, we analyzed CRP and fibrinogen levels. Several authors suggested that CRP is a sensitive, but non-specific indicator of inflammation of various origins (22-24). Previous studies reported the existence of a significant correlation between PASI score and levels of CRP (15). Rocha-Pereira *et al* (53) also pointed out that, among inflammatory markers, only CRP could be a marker for worsening of psoriasis. Although, CRP serum levels were higher in psoriatic patients than controls, in the present study no correlation was observed between CRP levels and PASI score. These findings may be due to the fact that the distribution of CRP serum levels was different among the patients.

Although in some patients affected by severe psoriasis, the levels of PTX3 partially overlap with those of CRP, no correlation was found between PTX3 and CRP levels. Similarly, non-significant correlation has been observed in other clinical studies (28,54). In agreement with previous studies, fibrinogen levels were increased in patients affected by psoriasis (53,55), although we did not find any correlation with PASI score. In patients with severe psoriasis we observed a moderate correlation between plasma levels of PTX3 and fibrinogen. This correlation was previously found in active vasculitis (29).

In conclusion, in the present study we observed that a network of monocytes, proinflammatory cytokines, acute phase proteins collaborate to create an inflammatory environment in the skin that stimulates the proliferation of resident keratinocytes and endothelial cells, producing a pattern of tissue growth that is recognized as psoriasis. In particular, PTX3 may fulfil in lesional skin of psoriasis the same function that CRP exert in the circulation (19). Therefore, PTX3, from a clinical point of view, could be a new marker for monitoring the progression of psoriasis. Studies are in progress to evaluate the expression and production of PTX3 in psoriatic patients with biological therapy and to better clarify the role of PTX3 in psoriasis.

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