Acta Derm Venereol 1999; 79: 281–284

CLINICAL REPORTS

Expression of the Psoriasis-associated Antigen, Pso p27, is Inhibited by Cyclosporin A

MORTEN DALAKER[1,2], TROND JACOBSEN[3], HILDE LYSVAND[3] and OLE-JAN IVERSEN[3]

Departments of [1]Dermatology, [2]Pathology and [3]Microbiology, University Hospital, NTNU, Trondheim, Norway

The psoriasis-associated antigen, pso p27, can be isolated from psoriasis scale and is present in complement-activating immune complexes in psoriatic scale, and in serum from patients with psoriasis. The antigen is produced by tryptase-positive cells in the skin lesions and is shown to be a major antigen in the immune reactions in psoriasis. The synthesis of this particular antigen is reduced with the remission of inflammation in the skin lesions. In this study we followed 3 patients with severe plaque psoriasis during treatment with cyclosporin A. In all patients we observed a decrease in the expression of the antigen pso p27 during the therapy. The effectiveness of the therapy varied among the patients, but there was a clear correlation between disease activity and expression of the antigen pso p27 as demonstrated by immunofluorescence in biopsies from selected skin lesions. This observation strengthens our hypothesis that the pso p27 antigen plays an important role in the inflammation in psoriasis. ***Key words: cyclosporin A; pathogenesis; psoriasis; pso p27.***

(Accepted December 17, 1998.)

Acta Derm Venereol 1999; 79: 281–284.

Morten Dalaker MD, Department of Dermatology, University Hospital, NTNU, N-7006 Trondheim, Norway.

The infiltration of inflammatory cells, which is an initial event in the development of skin lesions in psoriasis, strongly suggests that immune reaction plays a major role in the pathogenesis of this condition. This has also been verified through the clinical response of cyclosporin A in psoriasis.

Immunological analyses have shown that a protein present in psoriatic scale, pso p27, participates in the generation of complement-activating immune complexes, both in the psoriatic plaques (1 – 3) and in synovial fluid in patients with psoriasis arthritis (1). Recently, we presented evidence that the pso p27 antigen is expressed by mast cells in the skin lesions (4). The expression of the antigen was reduced or omitted when the lesions were in remission, even if the mast cells were still present. Indicating a direct correlation between the inflammation and the expression of pso p27 antigen. The N-terminal amino acid sequence of the psoriasis-associated antigen, pso p27, shows that pso p27 differs from other proteins (5). The biological function of the protein is still unknown.

In this study we followed 3 patients with extensive psoriasis during treatment with cyclosporin A (CsA), in order to investigate whether this treatment influenced the expression of pso p27 antigen.

PATIENTS AND METHODS

Patients and treatments

Three patients (2 men, 1 woman) volunteered to participate in this study. Each had a long history (11 – 17 years) of serious plaque psoriasis. One of the patients (patient 1) also suffered from moderate psoriasis-arthritis. Previously the patients had tried many kinds of ordinary topical treatments, phototherapy and systemic treatment with methotrexate, without significant response. All 3 patients received CsA 3 mg/kg/day in 2 doses/day. All other kinds of treatment except topical lubrications were stopped 4 weeks before entry into the study. We performed physical examinations with blood and urine tests according to the clinical guidelines for CsA therapy (6). The severity of the psoriasis was evaluated before starting the treatment and after 2, 6 and 12 weeks (patient 1), respectively, based on the Psoriasis Area and Severity Index (PASI) (7). One representative psoriasis plaque was selected as a reference lesion in each patient. These reference lesions were in the central part of the back of patients 1 and 2 and on the right upper arm of patient 3. The grade of scaling, erythema and thickness of the indicator lesions were determined on a scale from 1 – 7 for each parameter (1 absent, 2 trace, 3 mild, 4 mild-moderate, 5 moderate, 6 moderate-severe, 7 severe), with a total highest score of 21 and a lowest score of 3.

Specimens

Skin biopsies were taken from the indicator lesions as 4 mm punch biopsies. Before starting the CsA therapy we took a biopsy from the centre and the border of the plaque and from normal skin 5 cm from the border of the lesion. After 2 and 6 weeks we obtained biopsies from the centre of the indicator lesion and from normal skin. From patient 1 we also took a biopsy from the centre of the indicator lesion after 12 weeks and likewise from a new plaque on his left knee, which arose during treatment.

The specimens were shock frozen in liquid nitrogen and stored at – 70°C. Thin sections (4 – 5 μm) of the skin biopsies were analysed with respect to pso p27 antigen using monoclonal anti-pso p27 antibodies.

Monoclonal antibodies against pso p27

The production of monoclonal antibodies (Mabs) was performed essentially as described elsewhere (8). Purified pso p27 obtained from psoriatic scale was used as antigen (9).

Immunofluorescence analyses

Thin sections of the skin biopsies were incubated with anti-pso p27 Mabs (3A3D10 and 2C7D10) diluted 1 : 500 in PBS, followed by incubation with rabbit anti-mouse immunoglobulin Fluorescein Isothiocyanate Conjugate (DAKO). Finally, the thin sections were examined using a Leitz fluorescence microscope.

RESULTS

Clinical evaluation

The patients participating in this study responded to various extents to treatment with CsA. The PASI score indexes are presented in Table I . Patient 1 responded immediately to the treat-

© 1999 Scandinavian University Press. ISSN 0001-5555

Table I. *PASI score, plaque index and semiquantitative expression of pso p27 antigen during treatment with cyclosporin A*

Patient no./sex/age (years)	Week	PASI score	Plaque index	Fluorescence pso p27
1/M/45	0	37.7	20	++++
	2	6.2	5	+
	6	11.3	7	++
	12	3.1	3	+
	12	3.1	14	++++*
2/F/41	0	32	15	++++
	2	18	8	++
	6	6.5	3	+
3/M/34	0	15	19	++++
	2	12.1	14	+++
	6	10.4	10	++

* Now developed a new lesion with index 14 on his left knee.

ment and the PASI score dropped from 37.7 to 6.2 within 2 weeks. After 6 weeks, however, there was a tendency to relapse, as indicated by the PASI score index, due to an increasing thickness of the plaques sited on the trunk. An unexpected observation was made on this patient after 12 weeks of treatment. While the skin was almost normal, as demonstrated by a very low PASI score, a new psoriasis lesion appeared on his left knee. During the treatment period the patient recognized only a slight improvement in the arthritis.

The responses to treatment were different in these 3 patients. Patient 2 had only a moderate clinical effect after CsA treatment for 2 weeks, but had an excellent effect of the therapy after 6 weeks of treatment, as indicated by the PASI score and the plaque index (Table I). Patient 3, who had almost exclusively thick plaques of psoriasis on hands and fingers, had not the same good response to treatment as patients 1 and 2. However, in this patient also there was a significant improvement, as indicated in Table I, primarily registered as reduced thickness of the plaques.

In all 3 patients there were good correlations between the general clinical effect of the treatment and the plaque index of the indicator lesions (Table I). This indicates that the biopsies were obtained from representative plaques of psoriasis at various points of time. The effect of therapy was also verified through histological analyses of the skin biopsies as thinner stratum corneum, thinner epidermis and less inflammation.

The patients tolerated the therapy well and no serious side-effects were observed.

Analyses of psoriatic lesions and clinical uninvolved skin

The binding of monoclonal anti-pso p27 antibodies to psoriatic skin from patient 1 is shown in Table I and Fig. 1. Before starting therapy with CsA, biopsies made from the centre and the border of the indicator plaque from this patient (Figs. 1a and c) showed an extensive fluorescence for pso p27 antigen. The fluorescence was extensive in the stratum corneum and in the scale. Furthermore, we observed a high frequency of pso p27 positive cells in the dermal papillae and fewer positive cells in the reticular dermis. These cells were primarily tryptase positive cells (data not shown). No differences between the intensity of the fluorescence of pso p27 in the biopsies from the centre and the border of the indicator lesions could be demonstrated. In the biopsy from the uninvolved skin there was no visible fluorescence in the epidermis or the dermis (Fig. 1d). Before therapy, similar observations were made in corresponding biopsies from patients 2 and 3.

Analyses of selected skin lesions during treatment with CsA

The immunofluorescence analyses of pso p27 antigen in skin biopsies from the centre of a selected psoriasis lesion from patient 1 is presented in Figs. 1 a, e, f and g. The biopsy taken at day 0 demonstrated extensive fluorescence in the stratum corneum and in the scale. There was a high frequency of pso p27 positive cells in the dermal papillae and fewer positive cells in the reticular dermis (Fig. 1a). After 2 weeks of CsA treatment there was still strong fluorescence in the stratum corneum and in the scale of the biopsy. The number of positive cells in the dermis was decreased. The epidermis had become thinner and we observed a stronger autofluorescence in the biopsy by the same magnification (Fig. 1e). After 6 weeks a slight increase in the number of positive cells in the dermis compared with the biopsy performed after 2 weeks could be observed (Fig. 1f). The fluorescence in the stratum corneum and in the scale was even more intense. After 12 weeks when the skin was almost normalized, there was no positive fluorescence in the biopsy from the indicator lesion, except a weak fluorescence in the stratum corneum (Fig. 1g). However, in the biopsy from the new lesion on the knee which developed during therapy with CsA, we could observe a strong fluorescence both in the stratum corneum as well as in the dermis (Fig. 1h) The degree of fluorescence in this late biopsy was equal with the fluorescence in the biopsy from the indicator lesion before starting treatment with CsA. The density of mast cells was not significantly reduced during treatment and the expression of tryptase was not influenced by CyA (data not shown).

Consequently, there was a clear correlation between the response to therapy with CsA and the intensity of the fluorescence of the antibodies against pso p27. These observations are equal with the observations of patients 2 and 3. A certain amount of fluorescence was observed both in the stratum corneum and the dermis in the biopsy from patient 2 after 2 weeks, but after 6 weeks there was weak fluorescence in the stratum corneum only. Patient 3, who responded weakest to therapy among these 3 patients, after 6 weeks still had several positive cells in the dermis together with an intense fluorescence in the stratum corneum and the scale. However, the intensity of the fluorescence in these cells was much weaker than the fluorescence observed after 2 weeks of therapy.

The biopsies taken from the uninvolved skin before starting and during the CsA treatment, did not demonstrate any pso p27 positive reaction in the epidermis or the dermis. There was no change in this observation during the whole period of CsA therapy.

DISCUSSION

The initiation of psoriasis may be due to either microbial infection or physical damage to the skin or to systemic or local antigenic, autoantigenic, or superantigenic substances. Even psychological stress can be an initiating event (10). Without regard to the initiating event, psoriasis has the distinctive fea-

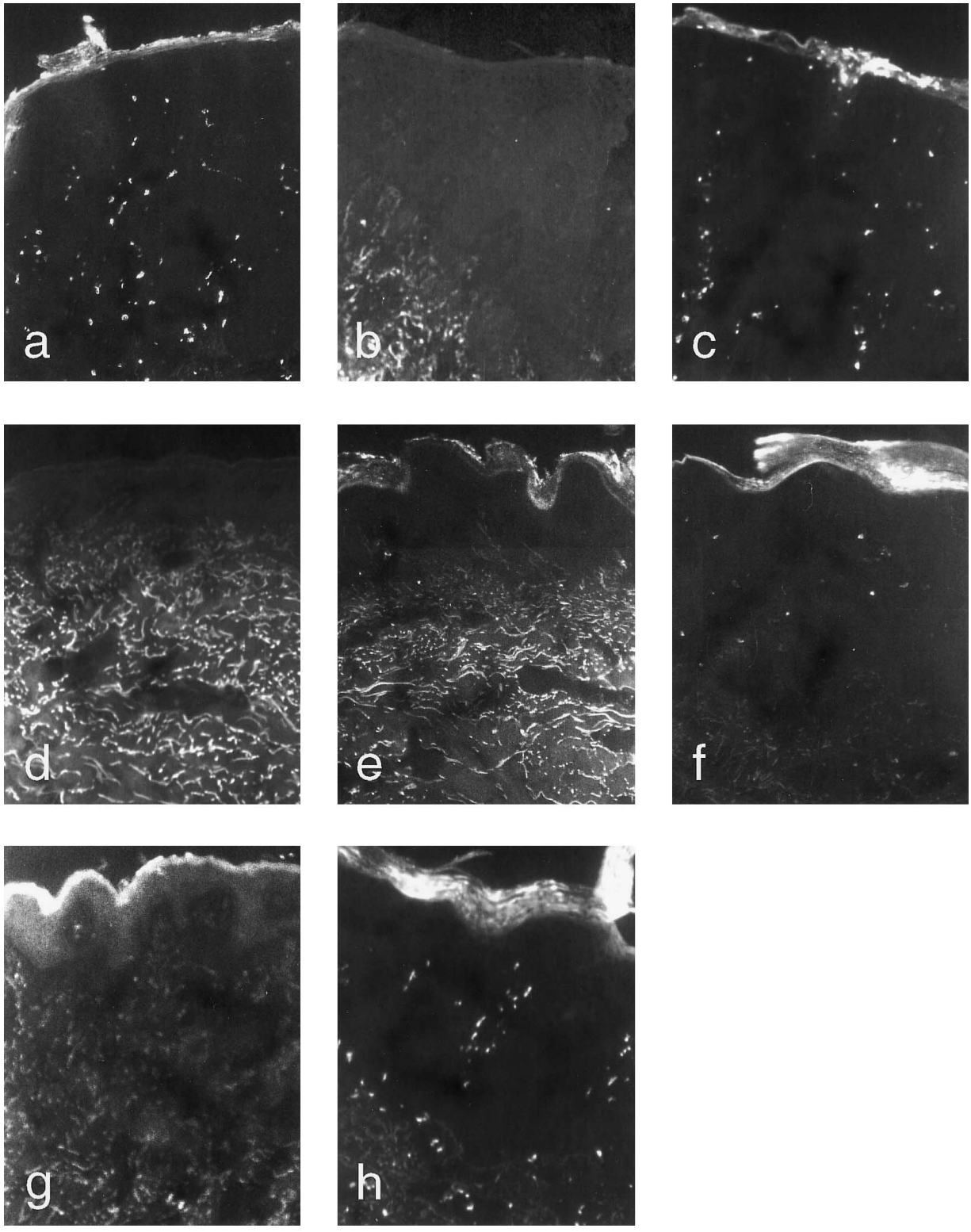


Fig. 1. Immunofluorescence of pso p27 in skin from patient 1. The biopsies are before therapy from (*a*) the centre of the indicator lesion, (*b*) control with an irrelevant antibody, (*c*) from the border of the lesion, and (*d*) from uninvolved skin and (*e*) after 2 weeks, (*f*) after 6 weeks and (*g*) after 12 weeks with CsA therapy. (*h*) A new plaque developed on the patient's left knee during CsA therapy.

tures of erythematous, scaly, infiltrating plaques of the skin. A main feature of psoriasis is an inflammatory reaction in the dermis with deposits of immunocomplexes and inflammatory cells.

There are generally antigens involved in immune reactions. It has never been possible to isolate an infectious agent as the cause of psoriasis, thus it is reasonable to assume that psoriasis is an autoimmune disorder. We have earlier focused on a pro-

tein antigen, pso p27, which is produced in mast cells in the skin lesions and which play an obvious role in the inflammatory reaction in psoriasis (3, 4).

The aim of this study was to investigate whether immunosuppressive treatment with CsA influenced the expression of the pso p27 antigen. The patients included in this study responded to various extents to treatment with CsA. We did not make any dose-adjustment during therapy, which could explain the various responses to therapy among these patients. Despite individual variation in response to therapy there was a clear correlation between disease activity expressed by PASI score and the plaque index of the indicator lesion. This means that the biopsies were representative for the general effect of the treatment. Furthermore, there was a good correlation between plaque index and to what extent the antigen was expressed, as demonstrated by the immunofluorescence.

Before CsA therapy there was a strong fluorescence both in the scale and the dermis. After 2 weeks there was a decrease in the intensity of the fluorescence in the mast cells of the dermis in biopsies from all 3 patients. However, the fluorescence was still strong in the stratum corneum. This could indicate that the production of the antigen is inhibited as an initial event of the CsA therapy, either as a direct effect of CsA on the antigen producing cells or indirectly through a suppression of the inflammatory process. In biopsies obtained from the clinically cured psoriasis plaque after 6 weeks with CsA therapy, there were no signs of the antigen. This indicates that the expression of the antigen is related to the activity of the disease and that the expression of the antigen is suppressed when the lesion goes into remission. This is in accordance with the results from earlier studies (4, 11).

The effectiveness of CsA in psoriatic treatment has been well established through many studies (12), especially in patients who have failed to response to phototherapy or methotrexate therapy. The immunosuppressive action of CsA in psoriasis has not yet been fully elucidated, but CsA binds to an intracellular receptor termed cyclophilin. The CsA-cyclophilin complex inhibits the phosphatase activity of the enzym calcineurin, which interferes with the nuclear factor of activated T-cells (NT-AT), which is a essential transcription factor for the transcription of the interleukin 2 (Il-2) gene (13). T-cell-derived lymphokines appear to play an important role in the pathophysiology of psoriasis. CsA inhibits the production of various numbers of cytokines, which have an effect on lymphocytes, antigen-presenting cells, keratinocytes and neutrophils (13). The effectiveness of CsA in psoriasis, strengthen the hypothesis of psoriasis as an immunological inflammatory skin disease.

We are led to speculate that the expression of pso p27 is induced by factors released by the inflammatory process and that the pso p27 antigen participates in the maintenance of the inflammatory process in the dermis through generation of immune complexes and activation of T-lymphocytes. In this way pso p27 plays a key role in the stationary psoriatic plaques. In accordance with this hypothesis, CsA decreases the expression of the antigen pso p27 by suppressing the inflammatory process either indirectly through inflammatory mediators or directly by inhibiting the production of the antigen.

Even if we do not know the exact action of this particular antigen, this study strengthens our earlier suggestion that the antigen plays an important role in the immunological reaction in psoriasis.

REFERENCES

1. Iversen O-J, Rødahl E. The major internal protein, p27, of a retrovirus-like particle participates in immune complex formation in psoriasis. Arch Virol 1985; 86: 37 – 45.
2. Aasbakk K, Bergh K, Iversen O-J. The psoriasis-associated antigen, pso p27, participates in the formation of complement activating immune complexes in psoriatic scale. APMIS 1990; 98: 143 – 149.
3. Iversen O-J, Bergh K, Lysvand H. Use of scale antibodies for the detection of antigens in psoriatic lesions. Acta Derm Venereol 1993; 73: 31 – 34.
4. Iversen O-J, Lysvand H, Jacobsen T, Bergh K, Lie BA. The psoriasis-associated antigen, pso p27, is expressed by tryptase positive cells in psoriatic lesions. Arch Dermatol Res 1995; 287: 503 – 505.
5. Iversen O-J, Lysvand H, Bergh K, Eriksen J, Elsayed S. The N-terminal amino acid sequence of the psoriasis-associated antigen, pso p27. Arch Dermatol Res 1995; 287: 761 – 763.
6. Witkamp L, Meinardi MMHM, Bossuyt PMM, Van de Kerkhof PCM, Arnold WP, De Hoop D, et al. A multicentre evaluation of the guidelines for the use of cyclosporin A in severe psoriasis. J Eur Acad Dermatol Venereol 1996; 7: 49 – 58.
7. Frediksson T, Pettersson U. Severe psoriasis – oral therapy with a new retinoid. Dermatologica 1978; 157: 238 – 244.
8. Bergh K, Iversen O-J, Aasbakk K. Monoclonal antibodies against the major zymosan-induced chemotactic factor in rabbit serum. J Immunol Methods 1988; 108: 179 – 187.
9. Jacobsen T, Lie BA, Lysvand H, Wiig M, Pettersen HB, Iversen OJ. Detection of psoriasis-associated antigen pso p27 in sarcoidosis bronchoalveolar lavage fluid using monoclonal antibodies. Clin Immunol Immunopathol 1996; 81: 82 – 87.
10. Farber EM, Nickoloff B J, Recht B, Fräki JE. Stress, symmetry and psoriasis: possible role of neuropeptides. J Am Acad Dermatol 1986; 14: 305 – 311.
11. Iversen O-J, Bergh K, Lysvand H, Jørstad S, Johnsson M, Kvernes S. Cascade apheresis in patients with psoriasis and psoriasis arthritis: effects on the expression of the psoriasis associated antigen pso p27 in skin lesions. Blood Purif 1993; 11: 204 – 205.
12. Schupack J, Abel E, Bauer E, Brown M, Drake L, Freinekel R, et al. Cyclosporine as maintenance therapy in patients with severe psoriasis. J Am Acad Dermatol 1997; 36: 423 – 432.
13. Wong RL, Winslow ChM, Cooper KD. The mechanisms of action of cyclosporin A in the treatment of psoriasis. Immunol Today 1993; Vol 14: 69 – 74.