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Somatostatin Immunoreactive Cells in Lesional Psoriatic Human Skin during Peptide T Treatment

OLLE JOHANSSON[1], MARITA HILLIGES[1], TOOMAS TALME[2], JAN A. MARCUSSON[2] and LENNART WETTERBERG[3]

[1]Experimental Dermatology Unit, Department of Neuroscience, Karolinska Institute, [2]Department of Dermatology, Huddinge Hospital, and [3]Department of Psychiatry, S:t Göran's Hospital, Stockholm, Sweden

Peptide T has been shown to be an effective treatment in psoriasis. The mechanism through which peptide T works in psoriasis is at present unknown. Furthermore, a clearance of psoriasis has also been registered using the inhibitory peptide somatostatin. These observations all focus on the fact that peptide T, somatostatin, and/or other peptides, might provide a clue to understanding the etiology and pathogenesis of psoriasis. Therefore, the effect of peptide T administration on somatostatin containing cutaneous cell populations was investigated. Ten psoriatic patients were treated with peptide T (D-Ala-peptide T amide; 2 mg/day i.v.) for 28 days. Serial biopsies were obtained from the psoriatic lesions before, once weekly during and 4 weeks after discontinuation of the peptide T treatment. An indirect immunofluorescence procedure was performed using a polyclonal antiserum against somatostatin. Clinically, most of the patients responded successfully to the treatment. Immunohistochemical investigations of the serial biopsies revealed the appearance of extensive changes in the number of dermal somatostatin immunoreactive dendritic cells. We believe that peptide T may stimulate the local synthesis and/or release of somatostatin, or proliferation and/or migration of certain dendritic cell populations in psoriatic lesions during healing. Since the benefits of peptide T treatment of psoriatic patients parallel earlier investigations using somatostatin infusions, it is likely that somatostatin given exogenously or synthesized/released endogenously plays a vital role in inducing the healing process. Because of the very few (so far reported) side-effects of peptide T treatment, as compared to somatostatin (or somatostatin analogue) injections/infusions, there is much hope that peptide T in the future can be used in the medical treatment of the large group of patients suffering from psoriasis. ***Key words: Peptides; Dermatology; Immunohistochemistry; Dendritic cells.***

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O. Johansson, Experimental Dermatology Unit, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Peptide T (Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr) is a ligand for the CD4/T4 receptor. It was designed as a part of the HIV envelope protein gp 120 (1). Synthetic peptide T and its analogues, D-Ala-peptide T amide and pentapeptide T_{4-8}, inhibit the binding of HIV envelope to brain membranes as well as the HIV infection of human T cells in vitro (1, 2). Therefore it was tested as a possible compound for treating HIV. As an unexpected finding, psoriasis cleared during peptide T treatment in one HIV seropositive patient (3). This improvement of the psoriasis was later also reproduced in seronegative patients (4, 5). The mechanism through which peptide T works in psoriasis is at present unknown. It has been suggested that peptide T might block the CD4 receptor, thus preventing the penetration of a putative psoriasis-causing retrovirus. Peptide T might also alter the function of CD4 positive cells found in the lesional skin of psoriasis (6).

A clearance of psoriasis (7–15), similar to the described improvement during and after peptide T treatment, has been registered after infusions of the inhibitory peptide somatostatin (16). Endogenous cutaneous somatostatin has, by immunohistochemistry, been located to a dermal dendritic cell population (17–20) and to a small number of dermal nerve fibers (21).

The purpose of the present study was to investigate if peptide T induces immunohistochemically detectable changes of the somatostatin content of the skin. Thus, we report dynamic changes of the dermal dendritic somatostatin-containing cell population during peptide T treatment of 10 HIV seronegative psoriatic patients. This could indicate one step of the mechanism through which peptide T improves the healing of psoriasis.

MATERIAL AND METHODS

Ten patients (2 females) were selected for this investigation. In order to be included they had to be healthy except for psoriasis. Furthermore, the disease had to be widespread, recalcitrant and resistant to conventional topical therapy. Two weeks prior to peptide T, during the treatment and after, during the observation period, only indifferent emollients were applied to the skin. The patient material, including clinical evaluation (patients no. 1–9), has been described earlier (22). For patient no. 10, see Table I.

Table I. *Results of peptide T treatment on somatostatin immunoreactive dendritic cells*

Estimation of the amount of somatostatin immunoreactive cells made on day 7, 14, 21, and 28, including pre- (= day 0) and post-treatment (= day 56) biopsies. Peptide T was given at 2 mg/day during day 1–28. L = upper leg; C = chest; A = upper arm. 0 = no immunoreactive cells; 1 = single cells; 2 = small groups of cells; 3 = large groups of cells; 4 = total coverage of cells. The scale also included intermediate steps (e.g. 1.5).

Pat./ Sex	Age	Biop. site	Day 0	Day 7	Day 14	Day 21	Day 28	Day 56
1/F	52	L	3	4	0.5	0	0	1
2/M	59	C	3	0	0.5	0	0.5	1
3/M	37	A	1.5	3	3	4	0.5	3
4/F	55	A	0.5	2	1	0.5	0	0.5
5/M	36	L	2	1	3	1	3	3
6/M	40	A	0	0	0.5	0	2	0
7/M	43	L	0.5	0.5	3	3	0	0
8/M	67	L	0.5	1	1	2.5	3	0.5
9/M	45	L	1.5	2	2	2	0	1
10/M	73	L	2	4	1	1	1	1

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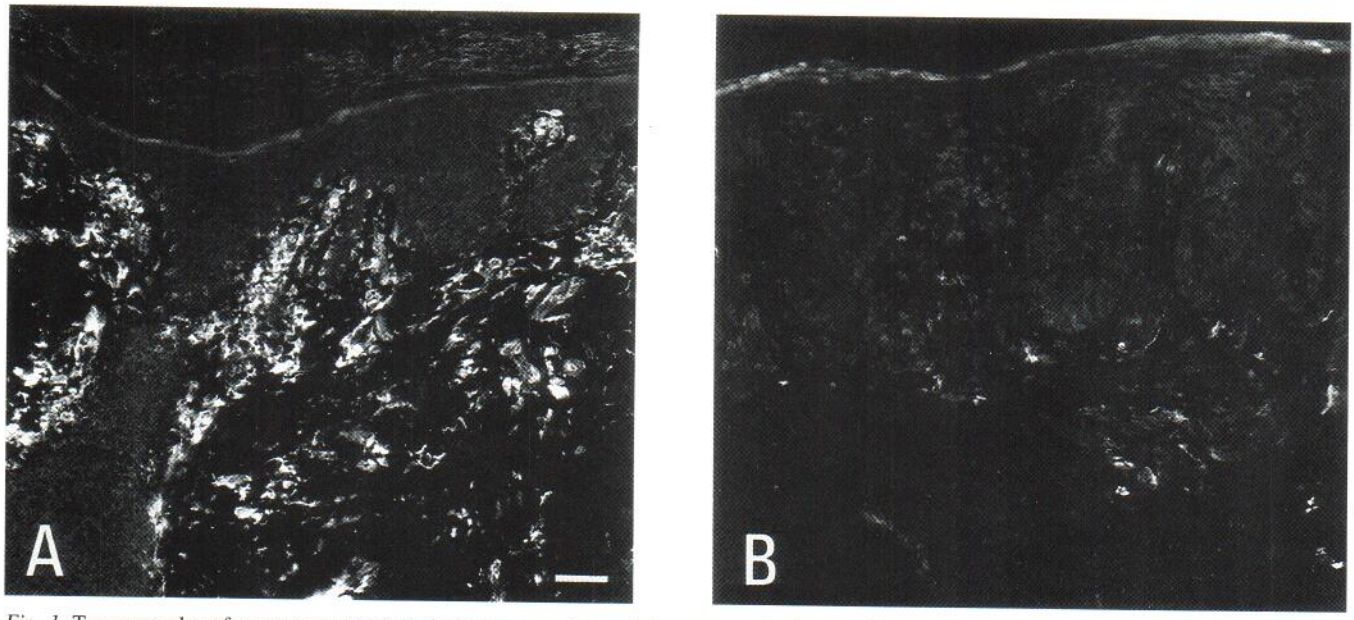

Fig. 1. Two examples of cutaneous somatostatin immunoreactive cellular patterns during peptide T treatment. (A) Immunoreactive cells in patient no. 8 four weeks post-treatment with peptide T. (B) Immunoreactive cells in patient no. 4 after four weeks of treatment with peptide T. *Bar* represents 50 μm.

All patients were treated i.v. with peptide T (D-Ala-peptide T amide), 2 mg/day (diluted in 500 ml saline) for 28 consecutive days.

One lesion was individually chosen in each patient as biopsy site (cf. Table I). Punch biopsies (4 or 6 mm) were taken from involved skin before, every week during and 4 weeks after treatment. Lidocaine without epinephrine was used as local anaesthesia, except for patient no. 10 where chlorethyl spray was utilized. The specimens were immersed for 3 h in 4% paraformaldehyde and 14% saturated picric acid in 0.1 M Sörensen's phosphate buffer (pH 7.4) at 4°C, and then rinsed in the same buffer containing 10% sucrose for at least 24 h. Cryostat sections (14 μm) thawed onto gelatin-coated slides were processed for indirect immunofluorescence (23). The primary antiserum was a rabbit polyclonal anti-somatostatin$_{15-28}$ diluted 1:400 in 0.01 M phosphate buffered saline (PBS), in which the sections were incubated overnight at 4°C in a humid atmosphere, followed by incubation for 30 min at 37°C in tetramethylrhodamine-isothiocyanate isomer R (TRITC)-conjugated goat anti-rabbit IgG antiserum diluted 1:80. Additional sections were incubated with the primary antiserum preadsorbed with the antigen, or with the secondary antiserum only. All antisera contained 0.3% Triton X-100. All rinses before and after the incubations were performed with PBS. The mounting media contained para-phenylenediamine to prevent fading of the fluorescence. The material was examined in a Nikon Microphot-FXA or Optiphot fluorescence microscope. Three to six sections were investigated/biopsy. Immunohistochemical ranking was performed as follows: 0 = no immunoreactive cells; 1 = single cells; 2 = small groups of cells; 3 = large groups of cells; 4 = total coverage of cells. The scale also included intermediate steps (e.g. 1.5). To this estimate, usual detailed qualitative assessments were added. The specimens were blind-coded during the whole laboratory handling.

RESULTS

Immunoreactivity towards somatostatin was seen in small dendritic cells located to the upper part of the dermis (cf. Fig. 1). They were single, scattered in the dermal papillae and in the connective tissue just below or in groups of different sizes with the same locations, however, the groups seemed preferably to be located to the stratum papillare. One biopsy (patient no. 6, at one week of treatment) showed stained cells in the deeper part of dermis only. The number of immunoreactive cells differed considerably from one patient to the other in the biopsies taken before treatment. Nevertheless, changes in the somatostatin positive dermal cell population were seen in all cases during the treatment period. In patients with a sparse occurrence of cells (patients no. 4 and 6–8) the population was increased in number, and sometimes also in fluorescence intensity, followed by a decrease in number. The rate and time axis differed. Some of the cases (patients no. 1, 2, 9 and 10) showed a relatively higher number of immunoreactive cells from the beginning. In these, decreases of the number were seen. Finally, 2 patients (nos. 3 and 5) displayed a fluctuating pattern, starting low and ending high in the relative cell density numbers.

Control sections incubated with the primary antiserum preabsorbed with the antigen or with only the secondary antiserum showed no immunofluorescence.

Some medical side-effects were seen during infusion, such as drop in blood pressure, headache, fatigue, lethargy, dizziness, flatulence, and feelings of well-being. However, the effects were short-termed and not general among the patients, of which 3 reported no side-effects at all. During the post-treatment period, no side-effects remained or developed.

DISCUSSION

In the present investigation, the biopsies from the psoriasis lesions, before, weekly during and 4 weeks after cessation of the peptide T infusions, were analysed for the presence of somatostatin-like immunoreactivity. We found that the treatment leads to major alterations in the number of immunoreactive dendritic cells. All patients, except one (no. 2), reached, during the peptide T infusion, levels in the cell numbers not seen in normal skin from healthy volunteers. Patients no. 1 and 2 had remarkably high numbers of positive cells even before treatment. Such alterations in immunoreactivity could reflect changes in intracellular synthesis and/or release of somatostatin from certain dermal dendritic cells. On the other hand, it could

indicate true differences in cell numbers and show increases and decreases, respectively, of the somatostatin-producing cell population(s). In that context, cell proliferation and/or migration could, of course, not be excluded. It is our experience that immunohistochemical methods best label compounds bound to, or contained within, cellular organelles. "Free" cytoplasmatic, or "free" extracellular, somatostatin is consequently not stained with certainty. Specimens with a low ranking in immunoreactivity could actually reflect a very active situation, with e.g. an increased release of somatostatin. Also the fact that the biopsies were taken from three different body regions must be taken into consideration.

Somatostatin is a potent inhibitor of human growth hormone secretion and has been used for treatment of psoriasis and psoriatic arthritis in several open studies. In those communications, a clearance rate between 30–80% was found (7–15). One placebo-controlled double-blind study has demonstrated that 7/9 patients receiving somatostatin cleared, compared to 1/11 in the placebo group (13). The mechanism of action is unknown, but a growth hormone inhibition, and subsequent decline of and release of epidermal growth factor and insulin growth factors, has been discussed (24, 25). Somatostatin has been reported to regulate epidermal growth factor levels locally in duodenal mucosal tissue (26), and a similar mechanism could be possible in the skin. Normal epidermal proliferation has been proposed to be regulated by a growth inhibitory epidermal pentapeptide (27). If psoriatic skin lacks or has reduced levels of this regulatory substance, peptide T treatment could induce a replacement inhibitor. Maybe somatostatin could fulfil this task, as somatostatin is known to be a powerful inhibitor in many systems.

Regarding the exact nature of the somatostatin immunoreactive dendritic cells we can only at present be speculative. The location of single and smaller groups of cells seems preferably to be perivascular. Recently, factor XIIIa$^+$ perivascular dendritic cells have been reported to be proliferating in psoriatic dermis (28). Also the presence of Langerhans' cells in the dermis and epidermis in psoriatic lesions is well established (29).

As noted clinically, very few side-effects were observed. This is, of course, of great importance since injections or infusions of somatostatin may result in gastro-intestinal problems such as diarrhoea, abdominal pain, disturbed liver function, gall bladder malfunction, hyperglycemia, decreased glucose tolerance, loss of appetite, slow bowel symptoms, and, in rare cases, even ileus, general bowel paralysis, gall bladder concrements, etc.

Based on our present data, we propose that peptide T may have a direct local effect on endogenous somatostatin synthesis and/or release; however, changes in cellular patterns due to proliferation and/or migration should not be ruled out. Furthermore, we also propose that this change in the somatostatin system is directly involved in the beneficial effects of the peptide T treatment. The implications of the fact that a small part of a viral protein envelope can exert a pharmacological effect on a certain peptidergic cellular system are at present unknown but could explain some symptoms and signs accompanying viral infections. In a recent study (30), 14 psoriatic patients were treated with intralesional infusions of peptide T for 2 weeks, after which these demonstrated a clearing effect upon the psoriatic lesions compared to infusion with saline only. The result indicates that the improvement following peptide T treatment is not explained by an unspecific placebo effect. The authors (30) agree that the molecular mechanism involved in the clinical effect of peptide T should be defined. It must, however, finally be pointed out that Reubi & Hunziker (31) have recently shown an absence of somatostatin receptors in psoriatic skin lesions. Thus, the last word is not said, and further experimental investigations trying to elucidate the role(s) of peptides in the field of dermatology are highly important and may form a rationale for future clinical treatment strategies.

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