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**SOMATOSTATIN, DENDRITIC CELLS AND
PEPTIDE T IN PSORIASIS**

A clinical, immunohistochemical and functional study

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Stockholm 2000

SUMMARY

Peptide T is an octapeptide designed to block the CD4 receptor. In a phase 1 study 9 psoriasis patients were treated with 2 mg peptide T i.v. once daily for 28 days and followed for a further 3 months. Five patients improved their PASI score >50%. The clinical results were confirmed by assessments of the histopathological score and epidermal thickness on biopsies. Peptide T treatment caused an increase in CD1-positive dendritic cells and a decrease in infiltrating lymphocytes. Immunohistochemical analysis revealed major changes in a population of somatostatin-immunoreactive dendritic cells during the treatment.

Somatostatin is a neuropeptide that inhibits the release of several hormones. It also affects fundamental lymphocyte functions, such as proliferation and antibody production, and somatostatin has been used in several studies of psoriasis. We found more somatostatin-immunoreactive cells in psoriasis lesions than in normal skin. These cells did not co-express CD1a, CD35, CD45RB, CD45RO, CD68, factor XIIIa or S-100, and they probably represent a specific population of dendritic cells. A subgroup co-expressed HLA-DR, which suggests that these cells can present antigens to T-cells.

Changes in somatostatin- and factor XIIIa-immunoreactive dendritic cells in psoriatic skin were then studied in biopsies taken during treatment with clobetasol propionate or calcipotriol. We found a significant reduction in the number of somatostatin- and factor XIIIa-positive cells after both treatments. The rate of reduction in the somatostatin-positive cells differed between the groups and closely paralleled healing.

Most somatostatin-positive cells in psoriasis are found in the dermis. In view of the known effects of somatostatin on the immune system, these cells may regulate the function of surrounding lymphocytes. Somatostatin exerts its effects by binding to five distinct receptors (SSTR1-5). We used RT-PCR to map the mRNA expression of these receptors in normal blood T-lymphocytes and in eight leukemic T-cell lines. Normal T-cells, but not the leukemic T-cells, expressed mRNA for SSTR1 and SSTR 5. SSTR2, 3 and 4 were present in all the T-cells examined. SSTR5 was selectively expressed in activated normal T-cells. Normal T-lymphocytes and all the T-cell lines were negative with respect to somatostatin mRNA expression. Thus, although T-cells can respond to somatostatin, they do not seem to produce somatostatin themselves.

A prerequisite for the localization of T-lymphocytes in specific tissue sites, such as the skin, is that they can adhere specifically to endothelial cells and extracellular matrix (ECM) components. Somatostatin and somatostatin analogs specific for SSTR2 and/or SSTR3 enhanced the adhesion of T-cells to fibronectin, and to a certain extent also to collagen type IV and laminin. Thus, somatostatin regulates T-cell adhesion to ECM components via distinct receptor subtypes. Our findings indicate that somatostatin may be a major regulator of a fundamental lymphocyte function, the capacity of adhesion to ECM components. They also suggest that SSTR subtypes may be useful targets for therapy in disorders involving T-cells.

To Lotta, Henrik and Kristian

MAIN REFERENCES

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I. Peptide T - a new treatment for psoriasis? A study of nine patients. Jan A Marcusson, Toomas Talme, Lennart Wetterberg, Olle Johansson. *Acta Derm Venereol* 1991; 71(6): 479-483
- II. Histopathological and immunohistochemical changes in psoriatic skin during peptide T treatment. Toomas Talme, Barbro Lundh Rozell, Karl-Gösta Sundqvist, Lennart Wetterberg, Jan A Marcusson. *Arch Dermatol Res* 1995; 287(6): 553-557
- III. Somatostatin immunoreactive cells in lesional psoriatic human skin during peptide T treatment. Olle Johansson, Marita Hilliges, Toomas Talme, Jan A Marcusson, Lennart Wetterberg. *Acta Derm Venereol* 1994; 74(2): 106-109
- IV. Somatostatin- and factor XIIIa-immunoreactive cells in psoriasis during clobetasol propionate and calcipotriol treatment. Toomas Talme, Marianne Schultzberg, Karl-Gösta Sundqvist, Jan A Marcusson. *Acta Derm Venereol* 1999; 79(1): 44-48
- V. Colocalization of somatostatin- and HLA-DR-like immunoreactivity in dendritic cells of psoriatic skin. Toomas Talme, Marianne Schultzberg, Karl-Gösta Sundqvist, Jan A Marcusson. *Acta Derm Venereol* 1997; 77(5): 338-342
- VI: Somatostatin receptor (SSTR) expression and function in normal and leukemic T-cells. Evidence for selective effects on adhesion to extra-cellular matrix components via SSTR2 and/or 3. Toomas Talme, Jyrki Ivanoff, Maria Hägglund, Anna Ivanoff, RJ Joost van Neerven, Karl-Gösta Sundqvist. Submitted for publication

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CONTENTS

| | |
|---|----|
| Abbreviations | 7 |
| Introduction | 8 |
| Psoriasis | 8 |
| T-cells in psoriasis | 9 |
| Somatostatin | 10 |
| Somatostatin and psoriasis | 12 |
| Dendritic cells and psoriasis | 13 |
| Peptide T | 15 |
| Aims of the study | 16 |
| Material and methods | 17 |
| Subjects and treatments | 17 |
| Clinical evaluation | 17 |
| Histopathological procedures and evaluation | 18 |
| Immunohistochemical procedures and evaluation | 18 |
| Cell preparations and culture conditions | 21 |
| Analysis of somatostatin mRNA expression | 21 |
| Cell attachment assays | 22 |
| Statistical analysis | 23 |
| Results | 24 |
| Peptide T treatment of psoriasis | 24 |
| Histopathological and immunohistochemical changes in psoriasis lesions during peptide T treatment | 26 |
| Somatostatin immunoreactive cells | 26 |
| Somatostatin-immunoreactive cells in psoriasis lesions during peptide T treatment | 28 |
| Somatostatin- and factor XIIIa-immunoreactive cells in psoriasis | |

| | |
|--|----|
| lesions during clobetasol propionate and calcipotriol treatment | 29 |
| Immunohistochemical characterization of the somatostatin-immunoreactive cells in psoriasis | 31 |
| Lack of somatostatin mRNA expression in T-cells | 32 |
| mRNA expression of SSTR subtypes in human leukemic and normal T-cells | 32 |
| Somatostatin and T-cell adhesion to ECM proteins | 33 |
| Adhesion experiments with somatostatin analogs having various receptor affinities | 34 |
| Discussion | 35 |
| Peptide T | 35 |
| Somatostatin-immunoreactive cells | 37 |
| Somatostatin receptor expression and function in T-cells | 39 |
| Conclusions | 42 |
| Acknowledgements | 44 |
| References | 46 |
| Papers I - VI | 58 |

ABBREVIATIONS

| | |
|--------|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| BSA | Bovine Serum Albumin |
| CIV | Collagen Type IV |
| CD | Cluster of Differentiation |
| ECM | Extracellular Matrix |
| FCS | Fetal Calf Serum |
| FITC | Fluorescein Isothiocyanate |
| FN | Fibronectin |
| FXIIIa | Subunit A of Clotting Factor XIII |
| GTP | Guanosine-triphosphate |
| HLA | Human Leukocyte Antigen |
| IFN | Interferon |
| IL | Interleukin |
| Ig | Immunoglobulin |
| LAM | Laminin |
| MHC | Major Histocompatibility Complex |
| mRNA | Messenger RNA |
| PASI | Psoriasis Area and Severity Index |
| PUVA | Psoralen and Ultraviolet light type A |
| PCR | Polymerase Chain Reaction |
| PBS | Phosphate-Buffered Saline (pH 7.4) |
| RT-PCR | Reverse Transcription - Polymerase Chain Reaction |
| SSTR | Somatostatin Receptor |
| TPA | 12-myristate 13-acetate |
| TRITC | Tetramethylrhodamine-Isothiocyanate Isomer R |
| VIP | Vasoactive Intestinal Peptide |

INTRODUCTION

Psoriasis

Psoriasis is a common inflammatory skin disease which affects about 2% of the Scandinavian population (Hellgren 1967). It can start at any age, and the age of onset shows two peaks: an early one at 16-22 years, and a later one at 57-60 years (Henseler & Christophers 1985). Patients with a family history of psoriasis tend to have an earlier age of onset (Henseler & Christophers 1985).

Clinical findings. Psoriasis is classified as a papulosquamous disease and appears in several clinical forms. The commonest variant is the *plaque form*, with well demarcated erythematous infiltrated lesions covered with silvery scales. The plaques tend to be symmetrical in distribution, the elbows, knees, scalp and nails being the most frequently affected locations. The *guttate form*, characterized by a rapid onset of teardrop-shaped lesions, often follows streptococcal pharyngitis. Guttate psoriasis tends to occur more often in younger persons. The *pustular form* is characterized by erythema, scaling and varying numbers of sterile pustules, which may be localized or occasionally generalized. Rarely psoriasis presents a generalized *erythroderma* when the skin becomes diffusely red and profoundly scaling. Psoriasis is associated with *arthritis*. The most widely accepted classification divides psoriasis arthritis into five subgroups: oligoarticular asymmetrical arthritis (the commonest form), symmetric polyarthritis, arthritis mainly involving the distal interphalangeal joints, the severe form of arthritis mutilans and the spinal form, including ankylosing spondylitis (Moll and Wright 1973).

Histopathology. The histological picture in a fully developed psoriasis plaque is characterized by elongated and club-shaped rete ridges resulting in acanthosis, elongation and edema of the papillae, thinning of the suprapapillary portions of the epidermis and absence of a granular layer. In the epidermis there is an increase in the number of keratinocyte mitoses, which is not limited to the basal layer as in normal skin, but is extended up to two rows of the cells above. The loss of keratinocyte differentiation is revealed by retention of nuclei within the stratum corneum (parakeratosis). The capillaries are dilated and tortuous. There is an accumulation of inflammatory cells consisting mainly of mononuclear cells, but with an admixture of neutrophil granulocytes, especially during exacerbation's. Increased numbers of lymphocytes are also found in the epidermis. Upwards migrating neutrophils may accumulate with formation of Kogoj spongiform pustules in the stratum spinosum and Munro microabscesses in the stratum corneum.

Genetics. Psoriasis is believed to be a multigene disease of which the expression is in part dependent upon external factors (Trupe 1995). The association of psoriasis to HLA-B13 was reported already 30 years ago (Russell et al 1972). Genetic associations have also been identified with the MHC alleles HLA-Cw6 (Tiilikainen et al 1980, Marcusson et al 1981), especially among patients with early onset (Enerback et al 1997), and to a HLA-DR7 (Marcusson et al 1981). The molecular genetic techniques of today try to identify disease gene loci by linkage studies. Because of the known association of HLA with psoriasis, much genetic research has focused on the sixth chromosome, where these genes are known to be located. Linkage for markers in that area have been shown by several research groups (Burden et al 1996, Trembath et al 1997, Enlund et al 1999). Other suggested disease loci have been reported in chromosome 17 (Tomfohrde et al 1994, Enlund et al 1999), chromosome 1 (Bowcock et al 1998, Capon et al 1999), chromosome 4 (Matthews et al 1996), and recently chromosome 3 (Enlund et al 1999). A complete genetic model for psoriasis at present, however, is not available.

The rest of this introduction will deal with T-cells, somatostatin, dendritic cells and peptide T in psoriasis, the main subjects in this thesis.

T-cells in psoriasis

The focus of research has gradually shifted towards activated immunocytes as being primarily involved in the pathogenesis of psoriasis. The T-cells form a major constituent of the inflammatory infiltrate in the papillary dermis, and they are also present in the epidermis. Immunohistochemical studies in the initial stages of psoriasis show that T-lymphocytes infiltrate skin lesions early in the evolution of plaques and before other cellular alterations (Baker et al 1984). Reports of the appearance (Gardembas-Paine et al 1990) and disappearance (Eedy et al 1990) of psoriasis after bone marrow transplantation indicate that the immune system plays a crucial role in the pathogenesis of psoriasis. Dermal endothelium is activated and expresses cellular adhesion molecules required for the recruitment of lymphocytes into inflamed tissue, particularly ICAM-1 and E-selectin (Groves et al 1991). The immunological events in psoriasis closely resemble a T-helper 1 type immune response, primarily accompanied by production of IL-2 and IFN- γ (Barker et al 1991). ICAM-1 (intercellular adhesion molecule 1) is expressed on keratinocytes in psoriasis lesions, unlike in normal skin (Lisby et al 1989). Epidermal hyperproliferation can therefore be facilitated by an interaction between infiltrating T-lymphocytes and keratinocytes (Valdimarsson et al 1986, Stoof et al 1992). Activated T-lymphocytes may also induce keratinocyte production of autocrine growth

factors, and the keratinocytes can in turn secrete costimulatory factors to maintain the T-cell activation (Bos and De Rie 1999).

The T-lymphocytes in psoriasis lesions are mainly activated memory T-cells, consisting of CD4⁺ (helper) and CD8⁺ (cytotoxic/suppressor) subsets. The CD8⁺ T-cells form the majority in the epidermis and the CD4⁺ cells in the dermis. It is still uncertain which of the two subsets is primarily involved in psoriasis. Some studies show an initial influx of CD4⁺ T-cells (Baker et al 1984), others report an early infiltration of CD8⁺ T-cells (Hammar et al 1984). In support of CD4⁺ T-cells, it has been shown that intralesional injection of CD4⁺ T-cells from peripheral blood of patients with psoriasis can induce conversion of normal skin to psoriasiform eruptions in severe combined immunodeficiency (SCID) mice engrafted with healthy skin from psoriasis patients (Schon et al 1997, Nickoloff et al 1999). There are also reports of psoriasis improving after blocking the CD4 receptor by systemic administration of chimeric monoclonal antibodies to CD4 (Morel et al 1992). Others have suggested that intraepidermal CD8⁺ T-cells mediate the disease and that successful treatment of psoriasis correlates most closely with a reduction in the CD8⁺ T-cells (Gottlieb et al 1995).

Several factors are known to trigger the immunological process leading to psoriasis. As early as 1878, Koebner noted that injury to the skin could start this process (Koebner 1878). Other factors reported to initiate psoriasis include drugs such as anti-malarials, lithium, and β -blockers (Abel et al 1986), the withdrawal of systemically-administered corticosteroids (Baker and Ryan 1968), gluten intolerance (Michaelsson et al 2000), psychological stress (Farber and Nall 1974), alcohol abuse (Poikolainen et al 1990) and infection with HIV (Garbe et al 1994). The role of streptococcal infection in provoking acute guttate psoriasis has long been recognized (Norlind 1950). Since there is a sequence homology between streptococcal M protein and keratins it has been hypothesized that psoriasis is induced by streptococcal superantigen-mediated T-cell activation and is maintained by memory T-cells cross-reacting with epidermal keratin, which then leads to epidermal hyperproliferation (Valdimarsson et al 1996). Furthermore, it has been shown that streptococcal superantigen activation of T-cells selectively induces expression of the skin homing receptor, CLA-1 (Leung et al 1995).

Psoriasis responds to treatment modalities aimed at directly ablating the function of T-lymphocytes. Cyclosporin (Mueller et al 1979) and tacrolimus (Abu-Elmagd et al 1991) inhibit activated T-cell cytokine production, and are used as therapy for severe psoriasis. Clinical trials have been made with the toxin DAB389IL2 that depletes activated T-cells expressing the IL-2 receptor

(Gottlieb et al 1995), the recombinant CD2-binding protein LFA3TIP (Krueger 1999), and monoclonal antibodies directed against CD4 (Morel et al 1992) and CD11a (Papp et al 1999).

Somatostatin

Somatostatin-producing cells occur in high densities throughout the central and peripheral nervous systems, in the endocrine pancreas, and in the gut. They are also found in the thyroid, adrenals, kidneys, prostate, placenta and the skin (Patel 1992, Patel et al 1999, Reichlin 1983). Somatostatin functions as a neurotransmitter in the brain and inhibits the release of growth hormone and thyroid-stimulating hormone (TSH) (Brazeau et al 1973, Epelbaum et al 1994). In the gastrointestinal tract, somatostatin inhibits the release of virtually all hormones including insulin, glucagon, and gastrin, and it has a generalized inhibitory effect on gut exocrine secretion (Reichlin 1983). It also blocks the release of growth factors (IGF1, EGF, PDGF), and of cytokines (IL-6, IFN- γ) (Blum et al 1992, Hayry et al 1993, Elliott et al 1998). Somatostatin affects several fundamental lymphocyte functions. It inhibits lymphocyte proliferation induced by mitogens and antibody production (Payan et al 1984, Blum et al 1993). Adhesive interaction with ECM components is of pivotal importance to migration and proliferation of the immune system and somatostatin has recently been reported to augment binding of peripheral blood T-cells to FN (Levite et al 1998).

Somatostatin exists in two bioactive forms, as a 14 amino acid peptide (Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)), and as a cogener of somatostatin-14 extended at the N-terminus called somatostatin-28 (Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)) (Patel et al 1999). Like other protein hormones, somatostatin is synthesized from a large preprosomatostatin 92 amino acid precursor molecule (Patel 1999). The human gene of preprosomatostatin maps to the long arm of chromosome 3. Secretion of somatostatin is affected by a broad array of secretagogues, ranging from ions and nutrients, to neuropeptides, neurotransmitters, classical hormones, growth factors, and cytokines (Patel 1992, Patel 1999, Reichlin 1983).

Somatostatin mediates its actions by a family of seven transmembrane-domain G-protein-coupled receptors which consists of five distinct subtypes (SSTR 1-5) that are encoded by separate genes segregated on different chromosomes (Raynor et al 1989). These receptors have recently been cloned and their function has been characterized, using selective ligands for the individual receptor subtypes (Rens-Domiano et al 1992, Yasuda et al 1992, Raynor et al

1992, Raynor et al 1993, Patel 1997, Hocart et al 1998). Various receptor subtypes may mediate different actions of somatostatin. For example it has been reported that SSTR2 and SSTR5 are the subtypes responsible for somatostatin mediated inhibition of growth hormone from the pituitary, while SSTR5 mainly inhibits insulin secretion from the pancreas (Tallent et al 1996, Fagan et al 1998). The natural somatostatin peptides, somatostatin-14 and -28, bind to all five receptor subtypes. Short synthetic peptides have been developed, which bind well to the SSTR subtypes 2, 3 and 5 (Hocart et al 1998, Patel and Srikant 1994).

The synthetic somatostatin analog octreotide, which has a longer duration of action, is now used in clinical practice to suppress the symptoms of neuroendocrine tumors, acromegaly, and prevent complications after pancreatic surgery (eds, Lancet 1992). Somatostatin has been shown to suppress the inflammatory reaction (Karalis et al 1994), and somatostatin has been used in several clinical studies to treat inflammatory disorders, such as psoriasis (Camisa 1994) and rheumatoid arthritis (Fioravanti et al 1995). New potent and more selective somatostatin analogs effectively inhibit proliferation and induce apoptosis in various cancer and leukemia cell lines (Keri et al 1996, Gianetti et al 2000). Consequently some somatostatin analogs have also been evaluated in clinical trials on malignancies known to express receptors for somatostatin, including breast cancer (O'Byrne et al 1999), hepatocellular cancer (Raderer et al 2000), and lymphoma (Sulkowski et al 1999).

Somatostatin and psoriasis

Several neuropeptides have been identified in human skin by immunohistochemical staining and radioimmunoassay, such as calcitonin gene-related peptide (CGRP), neuropeptide Y, somatostatin, substance P and vasoactive intestinal peptide (VIP) (Wallengren et al 1987, Pincelli et al 1993). Neurogenic inflammation is caused by the release of neuropeptides from free nerve endings. Neuropeptides have biological actions on cells involved in the psoriasis process, such as keratinocytes, endothelial cells, neutrophils, lymphocytes, macrophages, and mast cells (Raydchaudhuri and Farber 1998). The concentrations of neuropeptides in the skin range from about 0.1 to 5 pmol/g of tissue (Eedy et al 1991). Somatostatin immunoreactive nerve fibers and cells with dendritic morphology are found in human skin (Johansson and Nordlind 1984, Johansson and Vaalasti 1987). Somatostatin immunoreactivity has also been reported in Merkel cells (Fantini and Johansson 1995, Wollina 1992).

Somatostatin has been used in several open-label trials as infusional therapy for psoriasis. In 1981, Weber et al reported that 6 of 8 psoriatic patients were markedly improved by intravenous infusions of somatostatin given twice daily for 14 days (Weber et al 1981). A subsequent study by Weber et al showed that 22 of 26 patients improved with these infusions (Weber et al 1982). When Guilhou et al repeated these experiments, 6 of 20 patients had an almost complete remission and 2 a partial clearing (Guilhou et al 1982). Venier et al gave a continuous intravenous infusion for 96 h to 20 patients with severe forms of psoriasis, including 6 with severe arthropathy, and obtained total clearing in 11 patients, persistence of some psoriasis in 9 patients, and marked improvement in the 6 arthropathic patients (Venier et al 1988). In a study of somatostatin treatment in 18 patients with psoriatic arthritis, Matucci-Cerinic et al reported of a marked improvement in 8 patients and less marked in 4 (Matucci-Cerinic et al 1988). Only two double-blind placebo-controlled trials have been done. They had conflicting results. In a study of 21 patients, Matt et al found a significant clinical improvement in 70% of patients assessed after 15 days of somatostatin treatment (Matt et al 1989), while Gorsulowsky et al found no improvement in 20 patients treated (Gorsulowsky et al 1987). Camisa et al performed a double-blind, placebo-controlled trial with 12 weeks octreotide treatment on 24 patients with plaque psoriasis. No significant improvement in body surface involvement was seen, although physician global assessment improved in the high dose group (Camisa 1994).

Although the test protocols in the different trials are not comparable, the compiled data suggest that somatostatin probably improves psoriasis and psoriasis arthritis, but the therapy has several drawbacks. Its duration of action with a half-life in the circulation of about 3 min necessitates a continuous intravenous infusion for sustained action. The effect of somatostatin is unselective with rebound hypersecretion of inhibited hormones, hyperglycemia and gastrointestinal side-effects.

Dendritic cells and psoriasis

Dendritic cells in human skin were first described by Paul Langerhans, who showed their presence in the epidermis in 1868 by staining with gold chloride (Langerhans 1868). These cells now bear his name. The Langerhans cells have characteristic inclusions of tennis racket-like granules in the cytoplasm, called Birbeck granules (Birbeck et al 1961). Langerhans cells represent about 2 - 4% of all epidermal cells. They are continuously repopulated by a mobile pool of precursor cells derived from the bone marrow (Stingl et al 1980). Human Langerhans cells express MHC class II antigens, CD1a and to a lesser extent CD4, on the cell surface, and they are able to interact with neighboring

keratinocytes and T-lymphocytes (Stingl et al 1978, Groh et al 1986). Langerhans cells function as specialized epidermal antigen presenters, since they induce allogeneic and antigen-specific T-cell activation in the epidermal lymphocyte reaction, and they are needed for epidermal cell-induced generation of cytotoxic T-lymphocytes (Stingl et al 1980, Pehamberger et al 1983, Braathen & Thorsby 1980). The number of Langerhans cells in psoriasis has been reported to be reduced (Haftek et al 1983, Lisi 1973, Bieber & Braun-Falco 1989) and increased (Baker et al 1984, Baker et al 1985). Studies of the earliest phases of development of psoriatic lesions indicate that these cells become involved in the pathological process at a very early stage (Placek et al 1988, Chowaniec & Jablonska 1979). An electron microscopic study of Koebner-induced psoriasis showed close contact between Langerhans cells and lymphocytes (Heng et al 1991). This suggests that Langerhans cells are also involved in the psoriasis process.

In psoriasis increased numbers of other cell populations with dendritic morphology are found especially in the dermis, located beneath the hyperproliferating keratinocytes and surrounded by T-cells. HLA-DR⁺ dermal dendritic cells, isolated from plaque psoriasis, are demonstrated to be effective stimulators of T-cell proliferation. They also mediate a T-cell response with production of high level of IL-2 and IFN- γ (Nestle et al 1994). Subunit A of clotting factor XIII (FXIIIa) is expressed in a population of dermal dendritic cells mainly distributed in the upper dermis and around superficial blood vessels (Cerio et al 1989). FXIIIa-positive cells seem to be bone marrow-derived (Hle-I⁺) and coexpress monocyte, macrophage and antigen-presenting cell markers (HLA-DR, LFA-I, HLA-DQ, OKM5, Mo1, LeuM3), but not markers for Langerhans cells (CD1) or T-lymphocytes (CD2) (Cerio et al 1989). The number of FXIIIa-positive cells is increased in atopic eczema, psoriasis and mycosis fungoides (Cerio et al 1989, Fivenson & Nickoloff 1995). FXIIIa-positive dermal dendritic cells have reported to be one of the three most proliferating cell types in psoriatic skin, the other two being T-cells and endothelial cells (Morganroth et al 1991). Other members of the dendritic cell family reported in increased numbers in psoriatic lesions are epithelium-lining cells that express CD45 (leukocyte common antigen) and HLA-DR, but not CD1 or FXIIIa (van den Oord and de Wolf-Peters 1994), and spindle-shaped cells expressing the macrophage marker CD68 (Boehncke et al 1995). Exactly how these different subsets of dendritic cells interact with each other, and with adjacent cells, such as T-lymphocytes, endothelial cells, fibroblasts and keratinocytes in psoriasis is still unclear.

Peptide T

Peptide T is a synthetic octapeptide (Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr) which has a sequence homologous with the envelope glycoprotein 120 (gp 120) of human immunodeficiency virus (HIV) (Pert et al 1986). Gp 120 is essential to attachment of the virus to the CD4 receptor on T-helper cells (Fauci 1988), but this receptor is also present on other cells, such as Langerhans cells and cells of the nervous system. Peptide T was designed to block the CD4 receptor in order to prevent binding and penetration of the HIV virus into the CD4-positive cell (Pert et al 1986). Gp 120-like neurotoxic activity of cerebrospinal fluid from HIV-infected patients can be blocked by peptide T (Buzy et al 1992).

In 1987, Wetterberg et al. reported an improvement in coexisting psoriasis in one patient, who was treated with peptide T for AIDS (Wetterberg et al 1987). Peptide T was then tested on 3 HIV-negative psoriasis patients. In 2 of them, all psoriasis lesions immediately improved, while in the third case, severe deterioration preceded clearance two months after cessation of this therapy (Marcusson & Wetterberg 1989, Marcusson et al 1989). These preliminary results suggested that peptide T may have an effect on psoriasis and we therefore decided to perform an open-label pilot study of peptide T in the treatment of 9 additional patients with psoriasis (I).

AIMS OF THE STUDY

- to evaluate the clinical effects of peptide T treatment on psoriasis in a phase 1 study (I)
- to study histopathological changes in psoriatic lesions during peptide T treatment, with special emphasis on Langerhans cells, lymphocytes and somatostatin-immunoreactive cells (II, III)
- to compare the number and distribution of somatostatin-immunoreactive cells in psoriasis and healthy skin (IV, V)
- to study changes in the somatostatin- and factor XIIIa-positive cell populations in psoriasis during treatment with calcipotriol or clobetasol propionate (IV)
- to characterize the somatostatin-immunoreactive dendritic cells in psoriatic skin (V).
- to determine the expression of five somatostatin receptor subtypes in T-lymphocytes and T-cell lines (VI)
- to describe the effects of somatostatin receptor subtype specific signaling on T-cell adhesion by using somatostatin analogs specific for various somatostatin receptors (VI)

MATERIAL AND METHODS

Subjects and treatments (I, II, III, IV, V, VI)

(I). Nine HIV-negative patients with chronic recalcitrant plaque psoriasis were treated once daily with 2 mg peptide T in 500 ml saline intravenously (22-34 µg/kg bodyweight) for 28 consecutive days. Topical and systemic therapies for psoriasis were discontinued 2 weeks before the study, except in patient no. 6, who was unsuccessfully treated with PUVA and etretinate until one week before. Only innocuous emollients were permitted during the study. All patients were hospitalized for the first week and then treated as out-patients. Patient no. 6 was an in-patient during the 4-week long treatment because of severe arthritis.

(II, III). Ten psoriasis patients were treated with peptide T for 4 weeks (9 patients in paper I and one previously treated patient). Punch biopsies were taken from one lesion before, once weekly during the treatment, and 4 weeks after treatment for histological and immunohistochemical examinations.

(IV). Twenty patients with chronic plaque psoriasis participated in the study. All previous therapy was stopped two weeks before admission to the study. Ten patients were treated with a topical corticosteroid, 0.05% clobetasol propionate ointment (Glaxo Wellcome Operations), applied twice daily during the first week, once daily during the second, every other day during the third and two days during the fourth week; 10 patients were treated with a topical vitamin D analog, calcipotriol 50 µg/g ointment (Lövens Kemiske Fabrik) twice daily during 4 weeks. Punch biopsies were taken from a selected lesion before and after 1, 2 and 4 weeks of treatment. Biopsies of uninvolved skin from 6 of the psoriasis patients and from 10 healthy volunteers served as controls.

(V). Patients with chronic plaque psoriasis (5 patients in the double-labeling study, 8 patients in the elution-restaining study), who had not been treated for at least 4 weeks, were included in this study. Punch biopsies were taken from lesional psoriatic skin and uninvolved skin in the elbow region.

(VI). Lymphocytes were obtained from 10 healthy blood donors.

Clinical evaluations (I, V)

(I). Clinical activity of psoriasis was assessed by using the Psoriasis Area and Severity Index (PASI), which rates the degree of scaling, erythema and thickness (induration) of plaques, as well as the percentage of body surface involved (Fredriksson and Pettersson 1978). In addition, three chosen target

lesions were rated concerning to erythema, desquamation and induration, and scored as 0 = no visible lesion, 1 = mild, 2 = moderate, 3 = severe (target lesion score). Clinical examinations were performed on day zero, once weekly during the first 8 weeks and every other week for the remaining 8 weeks over a total observation period of 16 weeks.

(IV). The clinical activity of the target lesion was scored for desquamation, erythema and induration, as described above.

Histopathological procedures and evaluations (II, V)

Histological score

(II). Biopsies from psoriasis lesions during peptide T treatment were fixed in 4% neutral buffered formaldehyde, dehydrated through a series of graded alcohol's, embedded in paraffin, and 4 μ m thick sections were cut and stained with hematoxylin-eosin. All specimens were coded and scored on a scale from 0 (normal skin) to a maximum of 19, based on the cardinal microscopic features of psoriasis (Trozak 1991, Farber et al 1991). The following criteria were evaluated and scored: elongated and/or club-shaped rete ridges, elongation and edema of the dermal papillae, perivascular mononuclear infiltrates, absence of a granular layer, parakeratosis, suprapapillary plate thinning, mitoses above the basal layer, Munro microabcesses, and spongiform pustules. The number of lymphocytes infiltrating the dermis was estimated and scored as follows: (+) a few lymphocytes, + small, ++ medium-sized and +++ large lymphocytic infiltrates.

Epidermal thickness

(II, V). The healing process of psoriasis was followed by measurements of the thickness of the living epidermis from the granular layer to the tips of the rete ridges (epidermal thickness), using an ocular micrometer. We calculated the mean of 20 consecutive rete ridges in two sections per biopsy.

Immunohistochemical procedures and evaluations (II, III, IV, V)

Immunoperoxidase staining

(II). Biopsies from psoriasis lesions during peptide T treatment were immediately frozen and sections (5 μ m thick) cut in a cryostat. The sections were fixed in acetone at 4°C for 10 min and incubated overnight with mouse monoclonal antibodies against CD1, CD3, CD4, CD8, CD25 and HLA-DR (for details and working dilution, see *Table 1*). The reactivity of antibodies to the biopsy sections was assessed using a biotin-avidin detection system (Vectastain ABC, Vector Laboratories) (Hsu et al 1981). All rinses before and after the

Table 1. Primary antibodies used in the immunohistochemical experiments (II, III, IV, V).

| Antigen (study) | Dilution | Species | Cellular distribution | Source |
|----------------------------|----------------|------------------------|---|------------------|
| Somatostatin (III,IV,V) | 1:200 - 400 | Rabbit (polyclonal) | Nerve fibers, dendritic cells | Peninsula |
| Factor XIIIa (IV,V) | 1:800 | Rabbit (polyclonal) | Dendritic cells | Calbiochem |
| S-100 (V) | 1:200 | Rabbit (polyclonal) | Melanocytes, Schwann cells, Langerhans cells | Dako |
| HLA-DR (II,V) | 1:50 | Mouse (monoclonal) | Class II MHC | Becton Dickinson |
| CD1a (II,V) | 1:25-50 | Mouse (monoclonal) | Langerhans cells | Becton Dickinson |
| CD3 (II) | 1:50 | Mouse (monoclonal) | T-lymphocytes | Becton Dickinson |
| CD4 (II) | 1:25 | Mouse (monoclonal) | T-helper cells | Becton Dickinson |
| CD8 (II) | 1:25 | Mouse (monoclonal) | T-suppressor cells | Becton Dickinson |
| CD25 (II) | 1:50 | Mouse (monoclonal) | IL-2 receptors | Becton Dickinson |
| CD35 (V) | 1:10 | Mouse (monoclonal) | Follicular dendritic cells | Dako |
| CD45RB (V) | 1:25 | Mouse (monoclonal) | B-cells, T-cell subsets monocytes, macrophages | Dako |
| CD45RO (V) | 1:50 | Mouse (monoclonal) | Activ.T-cells, monocytes,macro- phages,granulocytes | Dako |
| CD68 (V) | 1:25 | Mouse (monoclonal) | Macrophages, monocytes | Dako |

incubations were performed with PBS. The primary antisera were omitted in control sections. Quantitation of the labeled cells per linear mm in the epidermis, papillary and upper reticular dermis were made using a light microscope (Leitz Dialux 22) at 400x magnification. We estimated the percentage of keratinocytes that expressed HLA-DR.

Immunofluorescence staining

(III, IV, V). We used an indirect immunofluorescence staining technique according to Coons (Coons 1958). The skin biopsies were immersed for 3 h in 4% paraformaldehyde containing 14% saturated picric acid in 0.1 M Sørensen phosphate buffer (pH 7.4) at 4°C, and then rinsed in the same buffer containing 10% sucrose for at least 24 h. Sections (14 µm (II), 12 µm (IV, V)) were cut with a cryostat. The sections were incubated with the primary antibodies (for details and working dilution, see *Table 1*) in a humid atmosphere at 4°C overnight. The sections were then incubated for 60 min at room temperature or for 30 min at 37°C with a secondary antibody labeled with a FITC or TRITC (Dako). All rinses before and after the incubations were performed with PBS. The antisera contained 0.3% Triton X-100. Para-phenylenediamine was added to the mounting medium to prevent fading of the fluorescence, Para-phenylenediamine was added to the mounting medium (Johnson and Nogueira Araujo 1981). The specificity of the somatostatin antibody was tested by preabsorption with somatostatin-14 and in addition omission of the primary antisera served as control. The material was examined in a fluorescence microscope (Nikon Microphot-FXA (II); Zeiss Axioplan with a MC 100 Camera (IV, V)). All readings and measurements with the microscope were performed on coded sections.

(III). Sections from psoriasis lesions taken during the peptide T study were incubated with somatostatin antibodies. The number of somatostatin immunoreactive cells was ranked as follows: 0 = no immunoreactive cells; 1 = single cells; 2 = small groups of cells; 3 = large groups of cells; 4 = total coverage of cells. Three to six sections were examined per biopsy.

(IV). Sections from psoriasis lesions treated with clobetasol propionate or calcipotriol were incubated with antibodies raised against somatostatin or FXIIIa. Sections from uninvolved skin from psoriasis patients and from healthy volunteers served as controls. The labeled cells were counted per linear mm in the epidermis, the papillary dermis and the upper 0.25 mm of the reticular dermis in two sections per biopsy at 400x magnification.

(V). In the double-labeling experiments, sections from untreated psoriasis lesions were incubated with a mixture of somatostatin antiserum and antibodies

against one of the following antigens: HLA-DR, CD1a, CD35, CD45RB, CD45RO or CD68. The material was then examined with attention to colocalization of immunoreactivity against somatostatin and the other antisera. The somatostatin positive- and double-labeled cells were counted.

(V). In the elution-restaining experiments, sections from untreated psoriasis lesions were incubated with somatostatin antiserum, followed by incubation with TRITC-labeled secondary antibodies. After photographing of the somatostatin-positive cells, the antibody complex was removed by immersion of the sections for 1 min in a solution containing 1.6 ml 3% KMnO₄, 2 ml 5% H₂SO₄ and 50 ml distilled water (Tramu et al 1978). The complete elution of the antibodies was controlled in all sections by reincubation with secondary antibodies. Sections with no immunofluorescent cells were incubated over-night with antibodies against FXIIIa or S-100, followed by incubation with TRITC-labeled antibodies. Micrographs of the same fields were taken and compared with those of somatostatin-positive cells.

Cell preparations and culture conditions (VI)

Mononuclear cells were isolated from ten healthy blood donors by centrifuging heparinized venous blood on a Ficoll gradient (Lymphoprep, Nycomed). Recovered cells in the interface were washed in PBS and the remaining erythrocytes were lysed with a buffer containing 0.15M NH₄Cl, 0.01M KHCO₃ and 0.1M EDTA. The remaining mononuclear cells were then treated with carbonyl iron and phagocytic cells removed by a magnet. The CD2⁺ blood T-cells for preparation of RNA were purified using anti-CD2-coated Dynabeads (Dynal). Recovered cells were resuspended in RPMI 1640 (Life Technologies Inc.) supplemented with 2 mM L-glutamine, 0.16 % sodium bicarbonate, 100 IU/ml benzylpenicillin, 100 μ g/ml streptomycin and 10 % FCS. The cells were cultured in Nunclon bottles in a humidified CO₂ incubator at 37C \pm and the cell attachment assays were done the following day. We also performed experiments on the human leukemic T-cell lines P 30, CCRF-HSB2, CCRF-CEM, DND-CCRF, Jurkat and Molt-4 (American Type Culture Collection), on the S zary cell line HUT-78 (American Type Culture Collection), and on the normal human T-cell clone AF 24 (ALK Research Laboratories). The cell lines were cultured in complete RPMI, supplemented with 10% FCS, as described above.

Analysis of somatostatin receptor mRNA expression (VI)

Isolation of cellular mRNA

Somatostatin and SSTR mRNA were isolated from non-activated and TPA-activated CD2-positive human blood T-cells, and from the T-cell lines

mentioned above (Chomczynski and Sacchi 1987). Cells from decidua and trophoblasts (T220) were used as a positive control (Watkins et al 1980). 10×10^6 cells were collected by centrifugation, washed in 1x10ml phosphate-buffered saline (PBS) and 2x10ml DEPC-treated PBS before being dissolved in 1 ml of solution D2 (4M guanidine isothiocyanate, 25mM sodium citrate pH 7.0, 0.5% N-lauroyl sarcosine and 0.1- β -mercaptoethanol). RNA was isolated from each of the cell lines, using 500 μ l of a cell suspension (= 5×10^6 cells). The following steps were carried out on ice in a RNase-free area: 100 μ l sodium acetate, 500 μ l TE-saturated phenol (mix), and 200 μ l chloroform/isoamyl alcohol (24:1) were added. The mixture was placed on ice for 15 min. After centrifugation (25 min, 14,000 rpm at 4 $^{\circ}$ C), the upper phase, containing RNA, was carefully removed and transferred to new tubes. An equal volume of isopropanol (500 μ l) was added, and the tubes were kept at -20 $^{\circ}$ C overnight. Centrifugation (20 min, 14,000 rpm at 4 $^{\circ}$ C) was carried out to pellet RNA. The supernatant was removed and the pellets resuspended in 400 μ l of solution D2. 800 μ l of 100% ethanol was added, and the tubes were kept at -20 $^{\circ}$ C overnight. RNA pellets were collected by centrifugation (20 min, 14,000 rpm at 4 $^{\circ}$ C) and washed 2x1 ml in 70% ethanol. After the final wash, the tubes were allowed to air-dry for 45 min. The pellets were then resuspended in 30 μ l of DEPC-treated water + 0.5 μ l RNasin. RNA solutions were stored at -70 $^{\circ}$ C).

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR from 0.5 μ l of purified RNA was done in the presence of RNasin. Temperature cycling for the reaction was: step 1, 42 $^{\circ}$ C for 15 min; step 2, 99 $^{\circ}$ C for 5 min.

Polymerase chain reaction (PCR)

The primers for somatostatin and SSTR1-5 used for PCR are described in Paper VI. The temperature cycling for all reactions was: step 1, 94 $^{\circ}$ C for 1 min; step 2, 55 $^{\circ}$ C for 1 min and step 3, 72 $^{\circ}$ C for 2 min. Steps 1 through 3 were repeated 35 times, followed by 72 $^{\circ}$ C for 10 min (Takeba et al 1997). Amplified products were run on 1.5% agarose gels with 0.5 μ g/ml ethidium bromide. Bands were visualized on a UV-table and photographed using a CCD-camera.

Cell attachment assays (VI)

In the cell attachment assays, plastic Petri dishes (60 mm, Becton Dickinson) were coated with one of the ECM components FN, CIV and LAM, or with BSA 10 μ g/ml in a buffer containing 0.02M NaH₂PO₄ and 0.15M NaCl (pH 7.4) at 22 $^{\circ}$ C overnight. After coating with these proteins, the dishes were washed in the coating buffer. A total of 2×10^6 /ml of T-cells were washed in RPMI supplemented with 10 % FCS, resuspended in complete medium and used in the

adhesion experiments. The T-cells were preincubated with somatostatin-14 10^{-4} - 10^{-8} M (in some experiments 10^{-4} - 10^{-14} M), with one of the somatostatin analogs listed in *Table 2* or with a negative control; in a humidified CO₂ incubator for 2 h at 37°C. Somatostatin was also present during the adhesion assay. Unbound cells were removed after 1 h by gentle aspiration. Bound cells were fixed in cold glutaraldehyde 2.5% in PBS for 10 min and the adherent cells were counted using an inverted microscope (IMT-2, Olympus). Cells from five microscope fields were counted and the mean of the adherent cells was determined.

Table 2. Binding selectivity of somatostatin analogs to the somatostatin receptor subtypes (Patel 1994)

| Peptide | Receptor selectivity | Source |
|-----------------|----------------------|------------------|
| Somatostatin 14 | SSTR 1 2 3 4 5 | Sigma, Peninsula |
| Somatostatin 28 | SSTR 1 2 3 4 5 | Peninsula |
| Octreotide | SSTR 2 3 5 | Novartis |
| RC 160 | SSTR 2 5 (3 4) | Peninsula |
| NC 8-12 | SSTR 2 3 | Affinity |

Statistical analysis (I, II, IV, V, VI)

Results were analyzed using one-way or two-way analysis of variance (ANOVA) with repeated measures of one factor. In the event of significant interaction between each treatment group and time, simple effects were examined, *i.e.*, effects of one or more factors when other factors were fixed. The p-values were then corrected by the Bonferroni procedure. The distribution of some of the variables was skewed and the data were therefore log-transformed to meet the requirements for an adequate ANOVA. When the compound symmetry assumptions were not valid in ANOVA, the F-test was adjusted according to Greenhouse & Geisser (Kirk 1968). In case of only two measures the results were analyzed by using Wilcoxon signed rank test within group and with Mann-Whitney U test between two independent groups.

RESULTS

Peptide T treatment of psoriasis (I, II)

Clinical course

(I). Nine psoriasis patients were treated daily with 2 mg peptide T i.v. for 4 weeks and they were evaluated for a further 3 months, when no additional treatment was given. Five patients (nos. 1, 3, 5, 7, 8) improved in their PASI more than 50% during the study (referred to as responders), while 4 patients (nos. 2, 4, 6, 9) did not improve (referred to as nonresponders) (*Fig. 1*). Two patterns of healing were noted: first, a continuous improvement beginning with the peptide T infusions (patients nos. 1, 3, 5, 8); secondly, an improvement preceded by deterioration (patient no. 7). No positive effect on the arthritis of patient no. 6 was observed, and he was excluded from the study after day 60 because of deterioration. All patients reported less desquamation and a reduced need for emollients during the treatment.

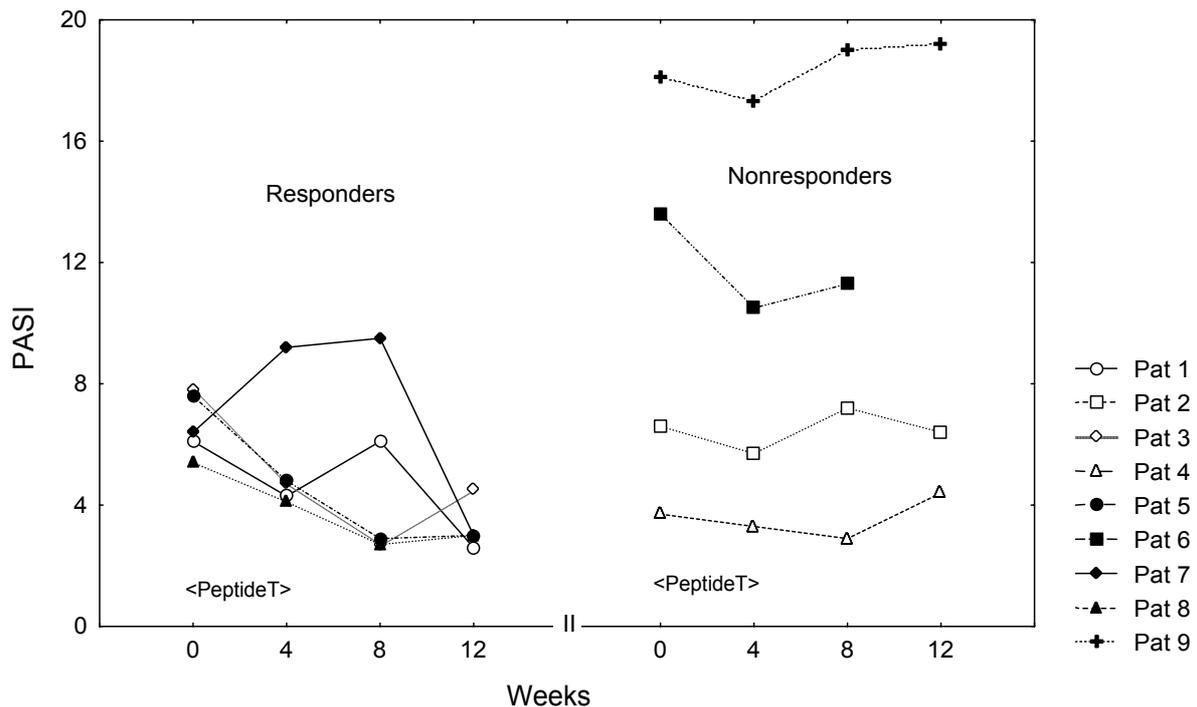


Fig. 1. PASI before treatment, after 4 weeks of treatment with peptide T, and during the follow-up. Five patients improved in their PASI >50% during the study (responders), while 4 patients did not respond to the treatment (nonresponders). Pat 6, who also had a psoriasis arthritis, was excluded after 8 weeks because of deterioration.

Histological score and epidermal thickness during peptide T treatment

(II). We also evaluated the peptide T treatment by assessing the histological score and epidermal thickness on blind coded specimen. All 5 patients who improved clinically also showed a reduction in the histological score (*Fig 2*) and the epidermal thickness. Among the 4 patients who did not respond, 2 patients showed initial improvement especially in the histologic score directly after treatment, but this change disappeared after cessation of peptide T (*Fig 2*).

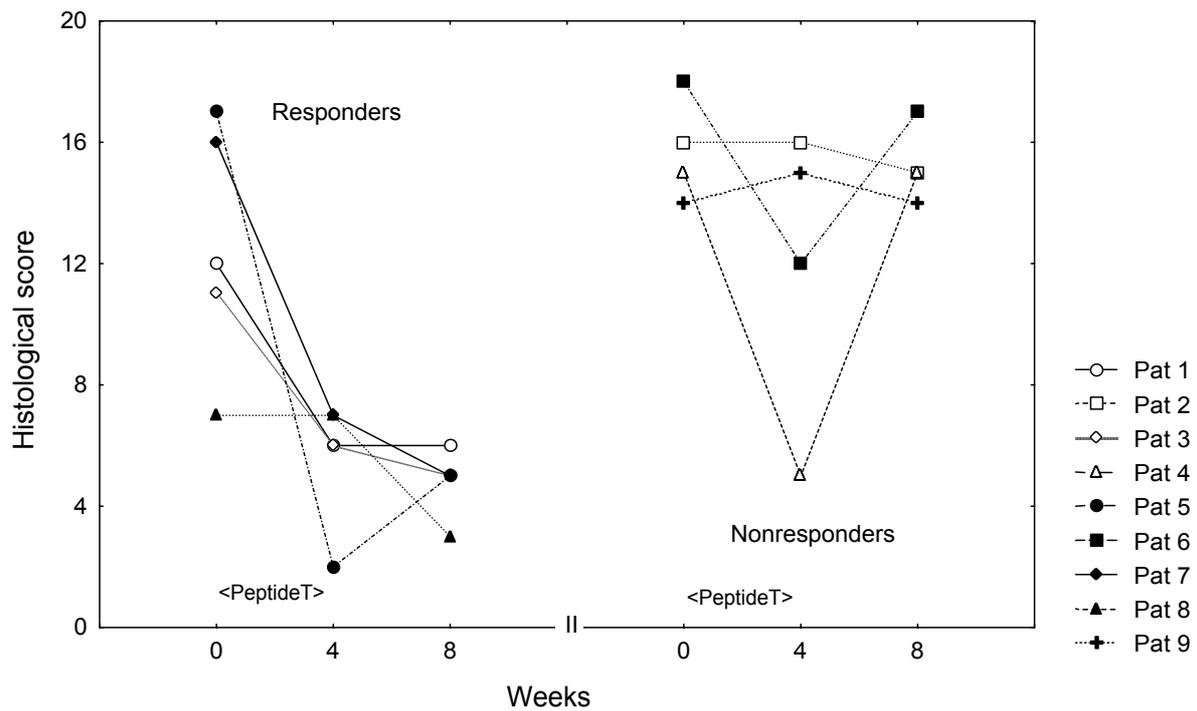


Fig 2. Histological score before treatment, after 4 weeks treatment with peptide T, and after 8 weeks. All 5 patients (nos. 1, 3, 5, 7, 8) who improved clinically also showed a major reduction of the histological score (responders). Among the 4 patients (nos. 2, 4, 6, 9), who did not respond to the treatment (nonresponders), 2 patients (nos. 14 and 6) showed an initial improvement of the histologic score, but deteriorated after cessation of peptide T.

Side-effects

(I). Patient no. 4 showed a reduction in the WBC (white blood cell count) from $3.8 \times 10^9/l$ to $2.2 \times 10^9/l$ (reference value $3.0-9.0 \times 10^9$) 2 weeks after starting peptide T, and $2.5 \times 10^9/l$ at the end of treatment. The differential count showed relative lymphocytosis (60%) during this period. Three weeks after the last dose, the WBC and differential count had normalized. No such hematological changes occurred in other patients. The blood pressure fell slightly, (mean 140/82 before the study, mean 125/79 after 4 weeks of peptide T treatment). There was no increase in susceptibility to infections. Three patients complained of headache, 2 of fatigue, 2 of dizziness, 2 of flatulence, and 3 of euphoria. The subjective side-effects were mild and none of the patients discontinued treatment because of them.

Histopathological and immunohistochemical changes in psoriasis lesions during peptide T treatment (II)

The infiltrating lymphocytes were mainly located in the upper dermis, often in conjunction with the superficial vascular plexus. All patients with clinical improvement showed a marked reduction in the number of infiltrating lymphocytes after peptide T therapy. Two of the nonresponders (nos. 6 and 9) showed no change in the number of lymphocytes, in no. 2 the number decreased, and in no. 4 an initial reduction was followed by an increase. The CD3+ and CD4+ cells tended to decrease among the responders, but since we had no pretreatment values in two of them, no definite conclusions can be drawn. Most infiltrating lymphocytes were HLA-DR+. The CD1a+ dendritic cells increased in the epidermis in all those who were improved by peptide T. Although fewer HLA-DR+ dendritic cells were seen in the epidermis, they also increased in responders during the observation period.

The percentage of HLA-DR+ keratinocytes was below 10 in most cases, but 3 patients had much higher values (no. 6 - 75%, no. 7 - 40% and no. 9 - 35%). These patients also differed clinically from the rest. Patient no. 6 had severe arthritis and no. 9 had widespread psoriasis. The psoriasis in patient no. 7 initially deteriorated before it healed almost completely. During the same period, the percentage of HLA-DR+ keratinocytes decreased from 40% before treatment, to 5% 4 weeks after treatment.

Somatostatin-immunoreactive cells (III, IV, V)

In the psoriasis lesions, somatostatin-immunoreactivity was seen in dendritic cells, which were mainly located in the papillary and upper reticular dermis, and surrounded by lymphocytic infiltrates (*Fig. 3A*). Some of the dermal

A

B

Fig. 3. Somatostatin-immunoreactive dendritic cells in psoriasis lesions were mainly found in the papillary and upper reticular dermis (x200) (A). Some of these cells were located just below the basement membrane and stretched their dendritic processes into the epidermis (x400) (B). A few somatostatin-positive cells were also seen in the epidermis (B).

somatostatin immunoreactive cells located just below the basement membrane, stretched their dendritic processes into the epidermis (*Fig. 3B*). We also found a few somatostatin-positive cells in the spinous and basal layers of the epidermis, and most of these cells were found close to other HLA-DR+/somatostatin-negative dendritic cells. The number of somatostatin-positive cells in the psoriasis lesions was significantly higher in both the dermis and epidermis than in the skin from healthy controls (dermis $p < 0.0001$, epidermis $p < 0.001$) (IV).

Somatostatin-immunoreactive cells in psoriasis lesions during peptide T treatment (III)

The number of somatostatin-immunoreactive cells differed considerably from one patient to the other in the pretreatment biopsies. Nevertheless, changes in the somatostatin-positive dermal cell populations were seen in all cases during the treatment. In patients with only a few cells (nos. 4,6,7,8), the population increased and then decreased. In those cases (nos. 1,2,9,10) with more immunoreactive cells initially, a decrease was seen. Patients nos. 3 and 5 had a fluctuating pattern.

Somatostatin- and factor XIIIa-immunoreactive cells in psoriasis lesions during clobetasol propionate and calcipotriol treatment (IV)

Clinical course

Twenty patients with plaque psoriasis (10 patients in each group) were treated topically with either clobetasol propionate or calcipotriol. The clinical score, rating degree of erythema, desquamation and infiltration of the target lesion, improved significantly during treatment with clobetasol or calcipotriol ointment ($p < 0.001$), but the improvement in the clobetasol group was faster and more marked ($p < 0.01$). Although both treatments significantly reduced the epidermal thickness ($p < 0.001$), the reduction occurred more rapidly in patients treated with clobetasol than with calcipotriol ($p < 0.001$) (*Fig. 4*).

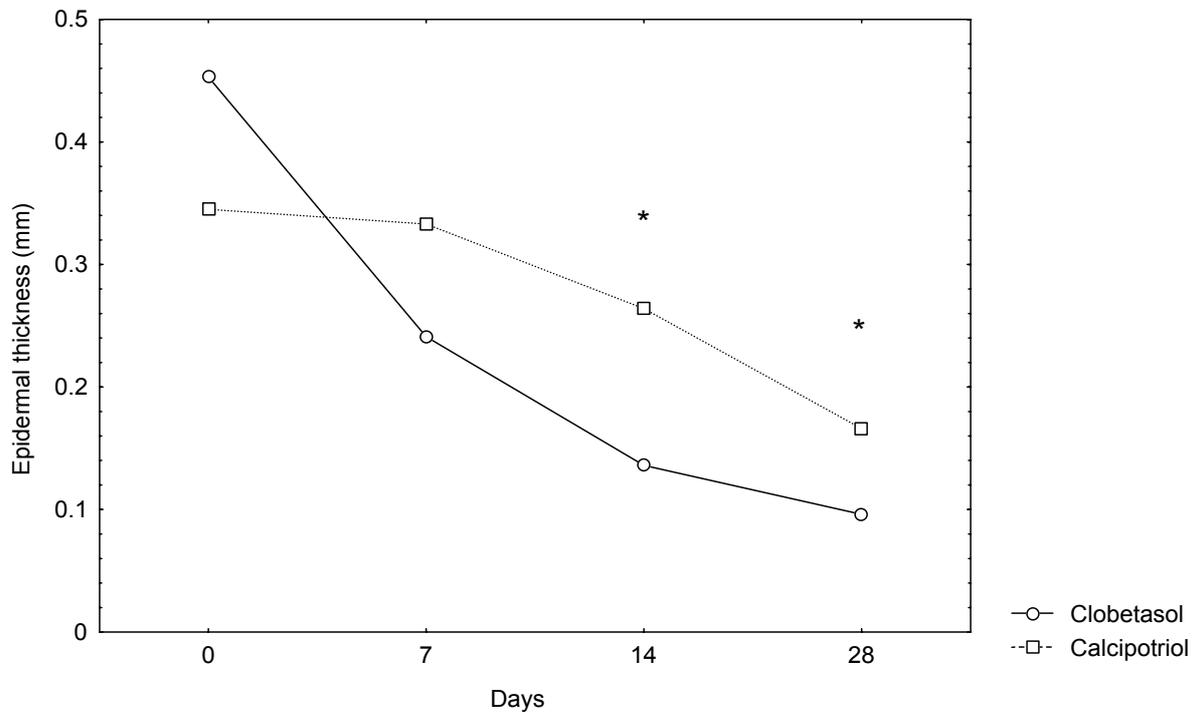


Fig. 4. Epidermal thickness (plot of means) during treatment with clobetasol propionate or calcipotriol. Significant differences between the groups are indicated (* $p < 0.05$).

Somatostatin-immunoreactive cells

The number of somatostatin-positive cells was significantly reduced in the dermis ($p < 0.001$) by the two treatments, but the reduction was more marked and rapid in the clobetasol group ($p < 0.001$) (Fig. 5). In the epidermis, which had fewer somatostatin-positive cells, a significant reduction occurred only in the clobetasol group after 14 and 28 days of treatment ($p < 0.05$).

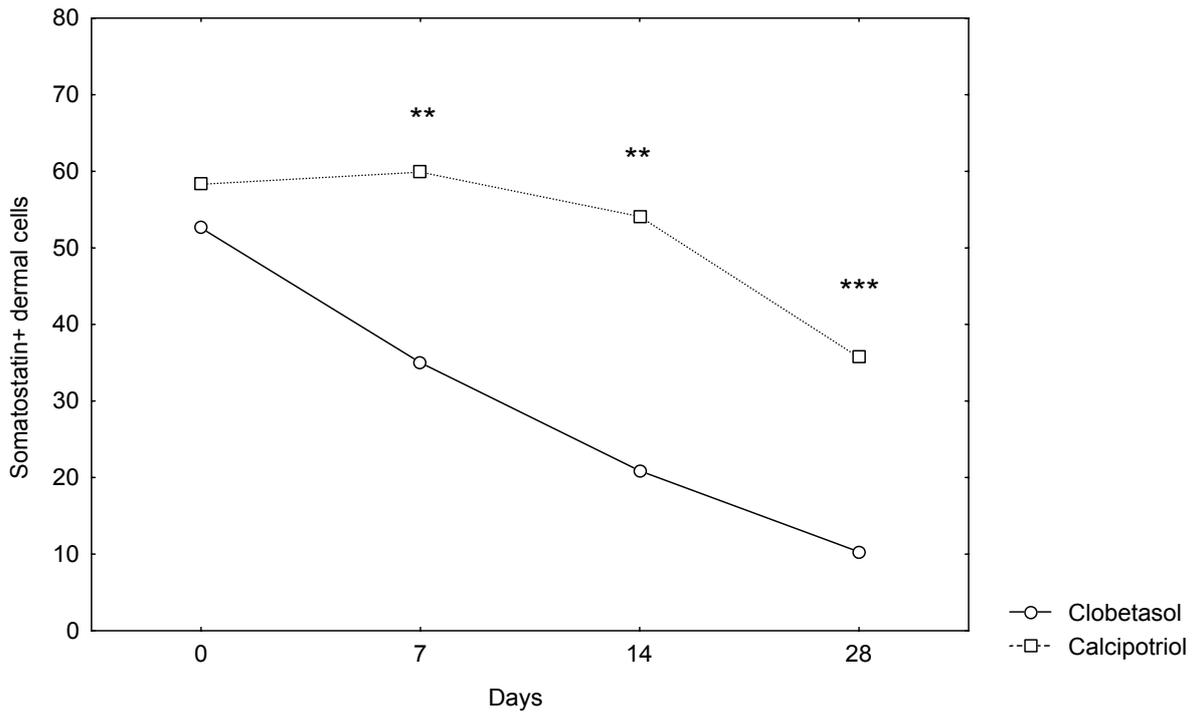


Fig. 5. Somatostatin-positive cells in the dermis (plot of means) during treatment with clobetasol propionate or calcipotriol. Significant differences between the groups are indicated (** $p < 0.01$, *** $p < 0.001$).

FXIIIa-immunoreactive cells

The FXIIIa-positive dendritic cells were mainly located in the upper dermis and around superficial blood vessels. These cells were more numerous than the somatostatin-positive cells in the dermis of untreated psoriasis lesions ($p < 0.001$). The number of FXIIIa-positive cells in the dermis significantly decreased during treatment with clobetasol or calcipotriol ($p < 0.001$), but this reduction did not differ between the two treatments (*Fig. 6*). In the epidermis, which had fewer FXIIIa-positive cells, the number of cells was reduced by both treatments after 14 and 28 days ($p < 0.05$).

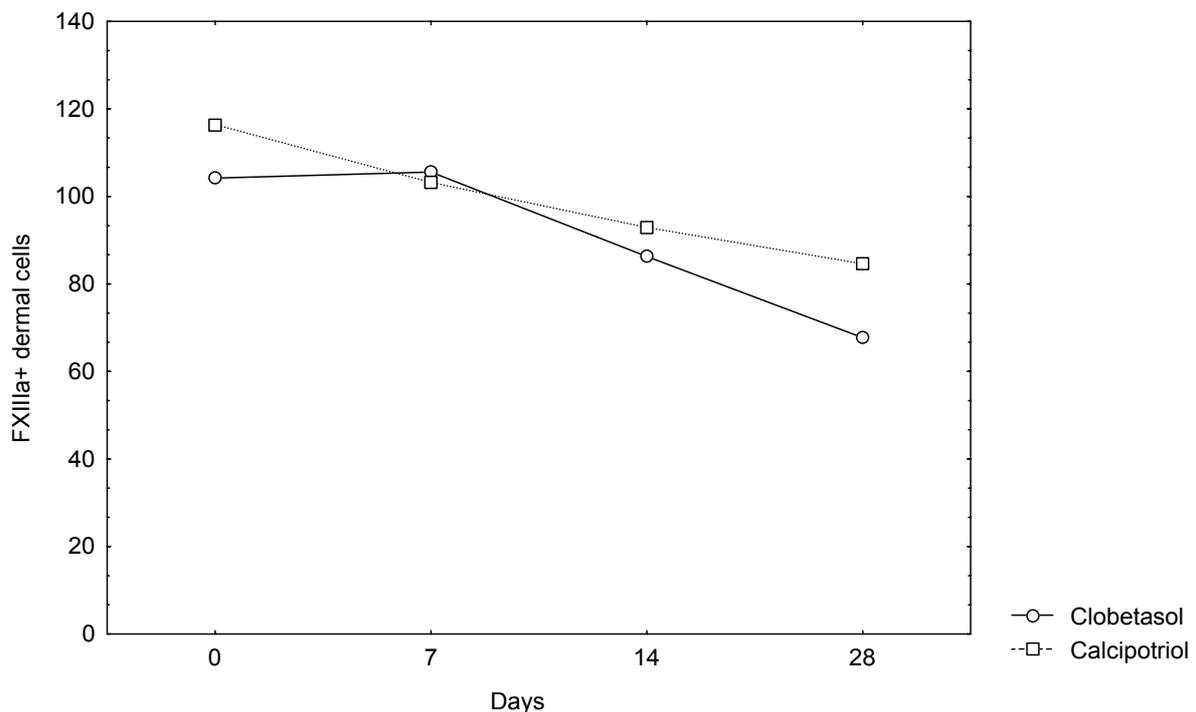


Fig. 6. FXIIIa-positive cells in the dermis (plot of means) during treatment with clobetasol propionate or calcipotriol. The differences between the groups were not significant.

Immunohistochemical characterization of the somatostatin-immunoreactive cells in psoriasis (V)

In order to describe further the somatostatin-positive dendritic cells in psoriatic skin we studied the colocalization of somatostatin and antigens known to be expressed by other dermal dendritic cells and macrophages (*Table 1*). Double-labeling experiments revealed a group of dendritic cells in the epidermis that were immunoreactive for both somatostatin and HLA-DR in all 5 cases examined. The number of these cells varied between 0.2 and 5 per mm

epidermis, which represented 0.3 - 14 % of the total population of HLA-DR+ dendritic epidermal cells. Only a few of the somatostatin-positive cells in the dermis coexpressed HLA-DR. No double-labeling was seen with somatostatin and CD1a, CD35, CD45RB, CD45R0, CD68, FXIIIa or S-100.

Lack of somatostatin mRNA expression in T-cells (VI)

The expression of somatostatin mRNA in non-activated and activated CD2+ blood T-cells and in the T-cell lines listed in *Table 3* was analyzed with RT-PCR and PCR. The somatostatin primers were designed to detect mRNA for both somatostatin-14 and somatostatin-28 (Takeba et al 1997). We found no somatostatin mRNA expression in blood lymphocytes and in the T-cell lines.

mRNA expression of SSTR subtypes in human leukemic and normal T-cells (VI)

The expression of mRNA for the five SSTR subtypes by the human leukemic T-cell lines CCRF-HSB2, CCRF-CEM, HUT-78, Jurkat, Molt-4, P30 and Peer, the human T-cell clone AF 24, and in non-activated and TPA-activated CD2+ blood T-cells was analyzed using RT-PCR and PCR (*Table 3*). The leukemic T-cell lines expressed mRNA for SSTR2, 3 and 4, of which the amplified products for SSTR2 and SSTR4 were pronounced in all cell lines. Non-activated CD2+ cells from healthy blood donors expressed mRNA for SSTR1-4 of which the amplified product for SSTR3 showed the highest intensity, while activated CD2+ cells showed an intense SSTR5 band, but a weak expression of SSTR1-4. We found that normal blood T-cells and T-cell clones express SSTR1 and 5, while the leukemic T-cell lines show a weak or negligible expression of these receptors. It is also obvious that SSTR2, 3 and 4 are expressed in all T-cell lines, but vary in expression in normal T-cells in relation to activation.

Table 3. Expression of mRNA for somatostatin receptor subtypes by human T-lymphocytes^a

| Cell type | SSTR 1 | SSTR 2 | SSTR 3 | SSTR 4 | SSTR 5 |
|--------------------|--------|--------|--------|--------|--------|
| P30 | - | +++ | + | ++ | - |
| CCRF-CEM | - | +++ | + | + | - |
| Molt-4 | - | +++ | + | ++ | + |
| Jurkat | - | +++ | +++ | ++ | + |
| CCRF-HSB2 | - | +++ | + | ++ | - |
| Peer | - | +++ | ++ | ++ | - |
| HUT-78 | - | +++ | + | ++ | - |
| AF24 | ++ | + | ++ | ++ | +++ |
| CD2 | + | + | +++ | + | - |
| CD2(activate d) | + | + | + | + | ++ |

^a Relative expression of mRNA for somatostatin receptor subtypes in human leukemic T-cell lines (P30, CCRF-CEM, Molt-4, Jurkat, CCRF-HSB2, Peer and HUT-78), the normal T-cell clone AF24 and normal nonactivated and activated CD2⁺ blood T-cells was determined by RT-PCR and PCR. The table summarizes the results of two identical experiments. Definition of symbols used in table: - no expression; + weak signal; ++ moderate-to-strong signal; +++ strong signal.

Somatostatin and T-cell adhesion to ECM proteins (VI)

To determine whether the expression of SSTR could have functional effects on T-lymphocytes, we evaluated the effect of somatostatin-14 on the adhesion of T-cells to surfaces coated with the ECM proteins FN, CIV and LAM. Somatostatin in a concentration of 10^{-4} M and 10^{-5} M significantly increased the adhesion of peripheral blood T-lymphocytes from healthy donors (n=10) to FN (p<0.001). Somatostatin 10^{-4} - 10^{-6} M tended to increase T-lymphocyte adhesion also to CIV and LAM.

Adhesion experiments with somatostatin were performed using the T-cell lines listed in *Table 3*. The leukemic T-cell lines showed a high spontaneous adhesion to FN, which was only slightly augmented by somatostatin. The leukemic T-cell lines showed weak "spontaneous" adhesion to CIV and LAM. Somatostatin markedly increased the adhesion of the T-cell lines to these ECM components. In repeated experiments with the S ezary T-cell line HUT-78, somatostatin

significantly increased the adhesion to FN in concentrations of 10^{-4} M ($p < 0.01$) and 10^{-5} M ($p < 0.05$), and also to CIV at 10^{-5} M ($p < 0.05$).

Adhesion experiments with somatostatin analogs having different receptor affinities (VI)

Somatostatin-14 has a high affinity for all five SSTR, which would suggest that it can enhance adhesion via all these receptors. To determine whether a specific SSTR subtype is mainly responsible for the increased T-cell adhesion to ECM components induced by somatostatin, we did experiments with somatostatin analogs selective for various SSTR (*Table 2*). NC 8-12 (highly selective for SSTR2 and 3) in a concentration of 10^{-4} M increased T-cell adhesion to FN, CIV and LAM more effectively than the other analogs. The somatostatin analogs octreotide, RC 160, and somatostatin-28, increased T-cell adhesion almost to the same extent as somatostatin-14.

Thus, NC 8-12 and octreotide, which both have a high affinity for SSTR2 and 3, effectively increased T-cell adhesion. This finding and the observation that all the T-cell lines and normal T-cells, as shown by RT-PCR, expressed SSTR2 and 3 (*Table 3*), support the conclusion that the enhancement of T-cell adhesion to ECM induced by somatostatin is probably mediated via SSTR2 and/or 3.

DISCUSSION

The occurrence of psoriasis is due to an interaction between a genetically predisposed individual and environmental influences, but the exact cause of the disease remains unknown. Several types of therapy can induce a temporary remission, but there is no known permanent cure. Most treatments today are time-consuming and not cosmetic. Many patients are obliged to attend day-care centers several times a week. Systemic treatment is given only to the most serious cases because of its side effects. Thus, there is a great need for more research about the pathogenic mechanisms of this disease in order to develop new effective medications without severe adverse effects and which are easy for the patient to handle.

Peptide T

Peptide T is a synthetic octapeptide designed to block the CD4 receptor and originally intended as medication for HIV-infections (Pert et al 1986). After peptide T had been given to 3 HIV-negative cases of psoriasis with promising results (Marcusson et al 1989), we performed an open phase 1 study, where 9 patients with chronic plaque psoriasis were treated with peptide T during 4 weeks (I). During the study, 5 patients showed an improvement in their PASI score of at least 50% (*Fig 1*). One of them had a marked deterioration before his psoriasis cleared. The latter had used a potent topical steroid continuously over a long period, and the deterioration that preceded healing was perhaps a rebound phenomenon after stopping topical therapy. Notably, 2 patients with chronic psoriasis for several years, were free from lesions for more than 6 months after the peptide T therapy (unpublished data).

We also followed the activity of psoriasis by histological scoring (*Fig 2*) and assessing epidermal thickness on serial biopsies taken from one selected lesion. These examinations confirmed the clinical findings (II). 4 weeks after discontinuing of peptide T, all the patients who had improved clinically also showed a significant reduction in their epidermal thickness and histologic score. In addition, 2 of 4 of the clinical non-responders showed an improved histologic score and epidermal thickness directly after peptide T treatment (*Fig 2*). However, their values returned to their original score 4 weeks after discontinuing the therapy. We might perhaps have achieved a more substantial clinical improvement also in these cases if the peptide T treatment had been prolonged or if higher doses had been used. We found that use of the histopathological score and epidermal thickness gave valuable additional information about the clinical activity. These methods also permitted a more unbiased assessment of healing in this small, open, clinical study, especially

since they were done on blind coded specimen. Both these evaluation methods have also been used in other clinical studies of psoriasis (Farber et al 1991, Gottlieb et al 2000).

After our initial report, other authors have reported their results with peptide T as treatment for psoriasis. In a trial by Farber et al, two comparable psoriasis lesions in 14 patients were infused for 2 weeks with saline or a saline solution containing peptide T (Farber et al 1991). They evaluated the lesions clinically and scored them in a double-blind fashion. They also took biopsies before and after treatment from both sites and scored them for histopathological features of psoriasis. The lesions infused with peptide T significantly improved both as regards the clinical score ($p < 0.01$) and the histological score ($p < 0.05$). In another open study with peptide T treatment of 5 cases of severe psoriasis, Delfino et al reported a complete remission in 1 patient and good improvement in 3, while no effect was seen in 1 case (Delfino et al 1992). Gulliver et al treated 10 psoriasis patients with an initial dose of 24 mg peptide T i.v., followed by intranasal administration of 6 mg/day for 55 days (Gulliver et al 1999). On completion of the treatment, the mean PASI had fallen from 16.4 to 4.0, but 3 patients had withdrawn from the study.

The mechanism by which peptide T may affect psoriasis is still unknown, but there are several possible mechanisms. Peptide T has been demonstrated to bind to CD4 (Ramsdale et al 1993). It may therefore interfere with the interaction between CD4+ T-cells and antigen-presenting cells, which is necessary for T-cell activation. Peptide T may also influence a putative interaction between CD4+ T-cells and HLA-DR+ keratinocytes found in psoriasis lesions. In our study, peptide T treatment resulted in an increase in the epidermal dendritic CD1+ cells in patients who had improved during treatment (II). CD1 is a marker for Langerhans cells and these cells also express CD4. Peptide T induces production of IL-10 and inhibits IFN- γ production by peripheral blood monocytes (Raydchaudhuri 1999). IL-10 is a potent anti-inflammatory cytokine which resolves psoriatic lesions (Assadullah et al 1999), and psoriasis can be induced by IFN- γ therapy (O'Connell et al 1992). Peptide T has also sequence similarities with VIP, and VIP can stimulate keratinocyte proliferation (Pincelli et al 1992).

Psoriasis is characterized by a clustering of activated T-cells in the skin lesions. The presence of these cells is believed to be one of the features that controls psoriasis since treatments that inhibit T-cell activation (e.g. cyclosporine and tacrolimus) cause the lesions to resolve. Substances which target various surface proteins that are needed for the interaction between T-lymphocytes and antigen-presenting cells and abolish subsequent T-cell activation provide a novel rational

approach for the treatment of psoriasis (Griffiths 1998). Advanced clinical trials with such substances are currently in progress (Abrams et al 1999, Krueger et al 1999, Gottlieb et al 2000). In the case of peptide T, the observations made so far indicate that peptide T may affect psoriasis, but there is, of course, a need for larger controlled clinical studies before we know whether peptide T can be used to treat psoriasis.

Somatostatin expression in dendritic cells

Neuropeptides including somatostatin, are probably present in sufficient amounts in human skin to affect various cell functions. In addition to their neurotransmitter and neuroendocrine functions, neuropeptides have mitogenic properties and can modulate responses of the immune system (Payan 1992). Evidence is accumulating that neuropeptides play an important role in the pathogenesis of inflammatory skin diseases (Pincelli et al 1992, Eedy 1993, Raydchaudhuri and Farber 1998). It has been hypothesized that stressful events and local trauma cause the release of neuropeptides, such as substance P from sensory nerves in the skin which, in turn, may initiate the development of psoriasis lesions in predisposed individuals (Farber et al 1986). This theory is supported by case reports of patients in whom cutaneous nerve damage resulted in clearance of their psoriasis at that site, but with reappearance of the skin lesions after recovery of cutaneous sensation (Farber et al 1990).

Our immunohistochemical evaluation of serial biopsies from psoriasis lesions during peptide T treatment revealed changes in a population of cells immunoreactive for somatostatin and with dendritic morphology (III). These cells were mainly found in the dermis. In the biopsies taken before treatment, we found variations in the number of immunoreactive cells in the patients. During the treatment, major changes in cell number (both increases and decreases) occurred in all cases. Nevertheless, the number of somatostatin-positive dendritic cells was lower in 7 of the 10 patients after the 4 weeks treatment with peptide T, than before treatment. The large fluctuations in numbers of the somatostatin-positive cells during peptide T treatment suggests that peptide T may affect these cells. As previously mentioned, peptide T has been shown to induce IL-10 production in monocytes (Raydchaudhuri 1999), and IL-10 reportedly induces somatostatin expression in macrophages and splenocytes (Elliott et al 1998).

The findings in paper III raised the question whether the somatostatin-positive cells are also affected by other established treatments for psoriasis. Serial biopsies were therefore taken from psoriasis lesions during topical treatment with clobetasol (a potent corticosteroid) and calcipotriol (vitamin D₃) (IV).

They showed a significant reduction in the number of somatostatin-positive cells in the dermis during healing induced by both therapies. This reduction occurred earlier in the clobetasol-treated group than in those treated with calcipotriol (*Fig 4*). A significant reduction in the number of somatostatin-positive cells in the epidermis was only seen in the clobetasol-treated group, although far fewer of these cells were found in this skin compartment. Higher numbers of dendritic cells that express FXIIIa have been reported to occur in psoriasis lesions (Cerio et al 1989). Both treatments reduced the number of FXIIIa-positive cells in the dermis and epidermis, but unlike the somatostatin-positive cells, the reduction in FXIIIa-positive cells showed no relation to either topical treatment (*Fig 5*). The close correlation between clinical improvement and the reduction in somatostatin-positive cells (*Fig 3,4*), as well as the fact that somatostatin has been used in several studies to treat for psoriasis, may indicate that the somatostatin-positive dendritic cells play a role in healing of psoriasis. It may also suggest that somatostatin-positive cells are more sensitive to corticosteroid treatment than FXIIIa-positive cells.

The number of somatostatin-immunoreactive cells in the psoriasis lesions before treatment differed between the patient groups in Papers III, IV and V. Four of 10 patients in Paper III had relatively few somatostatin-positive cells before peptide T, while in Papers IV and V, all 28 patients had a high numbers of somatostatin-positive cells before treatment. We believe that these initial differences in cell number might reflect variations in the activity of the disease or were caused by previous treatments given. The patients in the peptide T study had more severe psoriasis, and were therefore more intensely treated until the 2-week long wash-out period before starting of the peptide T treatment (III). One patient, who had been treated with PUVA and etretinate, had extremely low levels of somatostatin-positive cells throughout the study. Another patient was given UVB treatment until a couple of weeks before he entered the study. All 4 patients with low initial levels of somatostatin-positive cells had used potent local corticosteroids before the peptide T study. Glucocorticoids exert a dose-dependent biphasic effect on somatostatin secretion, with an inhibitory effect in high doses (Papachristou 1994). Treatment with PUVA and corticosteroids are known to reduce the number of Langerhans cells (Baker et al 1985), and may perhaps also affect other populations of dendritic cells, such as the somatostatin-positive cells. The treatments before peptide T administration may therefore have affected the number of somatostatin-positive cells, especially in those cases where only a few cells were seen in the lesions. A wash-out period of 2 weeks is commonly used in clinical studies of psoriasis. It is un-ethical to oblige patients with severe psoriasis to go without treatment for a longer period before a study, because it can cause a marked deterioration in their disease. Unlike the patients in Paper III, those in Papers IV and V had mild-to-moderate psoriasis, and

several had not used any therapy at all for several months before the initial biopsy was taken. This means that the pre-treatment biopsies in Papers IV and V probably give a more representative picture of the number of somatostatin-positive cells in psoriasis lesions before initiation of active treatment.

To characterize the somatostatin-positive dendritic cells in psoriatic skin further, we studied the colocalization of somatostatin and other antigens reported to be expressed by dermal dendritic cells and macrophages. In the immunohistochemical double-labeling study, we found no co-expression of somatostatin and FXIIIa, CD1a, CD35, CD45RB, CD45RO, CD68 or S-100, but a small subgroup of these cells co-expressed HLA-DR (V). Since Merkel cells have been reported to express somatostatin (Wollina 1992), it was suggested after publication of our study that the somatostatin-positive cells might in fact be Merkel cells (Wollina 1998). To test this hypothesis, we also did a double-staining experiment with somatostatin and neuron-specific enolase (NSE). NSE is a well-accepted marker for Merkel cells (Masuda et al 1986, Fantini and Johansson 1995). We found that hardly any somatostatin-positive dendritic cells in psoriasis also co-expressed NSE (Talme and Schultzberg 1999).

In conclusion, somatostatin-positive cells seem to represent a specific population of dendritic cells, which differ from Langerhans cells, FXIIIa-positive dendritic cells and Merkel cells (IV,V). They are found in greater numbers in psoriasis lesions than in normal skin of patients with psoriasis and healthy subjects (IV,V). The exact role of somatostatin and somatostatin-positive cells in psoriasis is not known. Somatostatin may affect various functions of immunocompetent cells involved in psoriasis. It has been shown to inhibit lymphocyte proliferation induced by mitogens, antibody production and cytokine production in CD4+ T-cells (Payan et al 1984, Blum et al 1993), as well as neutrophil chemotaxis induced by substance P (Kolasinski et al 1992). Nerve-induced vasodilatation and release of substance P are also inhibited by somatostatin (Gazelius et al 1981). A subgroup of somatostatin-positive cells found in the psoriatic epidermis co-expresses HLA-DR, which suggests that these cells are able to process and present antigens to T-cells.

Somatostatin receptor expression and function in T-cells

Most somatostatin-positive dendritic cells in psoriasis are found in the papillary and upper reticular dermis where they are surrounded by lymphocytic infiltrates. Since somatostatin is able to modulate responses of the immune system, it is conceivable that the somatostatin-containing dendritic cells in psoriatic skin can regulate the function of T-lymphocytes located in the same compartment. Somatostatin exerts its effects by binding to five distinct receptors, which have

recently been cloned (Rens-Domiano et al 1992, Yasuda et al 1992, Raynor et al 1992, Raynor et al 1993, Patel 1997, Hocart et al 1998). Although somatostatin affects several fundamental lymphocyte functions very few direct studies have been done on the expression of somatostatin receptors on T-cells (Tsutsumi et al 1997, Cardoso et al 1998). Paper VI provides new information regarding SSTR expression and function in T-lymphocytes. By using RT-PCR, we have mapped the mRNA expression of the five different somatostatin receptors in normal blood T-lymphocytes, in eight human leukemic T-cell lines and in a normal human T-cell clone (AF24) (*Table 3*). The normal T-lymphocytes and the leukemic T-cell lines, showed some consistent differences and similarities with respect to SSTR expression. Thus, the normal T-cells expressed mRNA for SSTR1 and SSTR5 while the leukemic cell lines did not have SSTR1 and 5. The leukemic T-cell lines showed a strong expression of SSTR2, while normal T-cells had poor SSTR2 expression at the mRNA level. SSTR3 and 4 were generally present in all the normal and leukemic T-cells examined. Normal T-lymphocytes and all the T-cell lines studied were negative with respect to somatostatin mRNA expression. Although T-cells can respond to somatostatin, they do not seem to produce the neuropeptide themselves.

Controlled lymphocyte motility is a vital feature of the immune system and lymphocytes undergo a continuous transition between an intravascular and an extravascular state (Picker and Butcher 1992, Picker 1994). A prerequisite for the localization of T-lymphocytes at specific tissue sites, such as the skin, is that they can adhere specifically to endothelial cells and extravasate (Hauzenberger et al 1995). ECM components are present in basement membranes, perivascular connective tissue and connective tissue in interstitial compartments of various organs. After extravasation, interactions with ECM components, such as FN, CIV and LAM, play a crucial role in the ability of T-lymphocytes to migrate in to tissues and localize in specific areas. To further clarify the function of the somatostatin receptors in this context, we studied the effect of subtype specific signaling on T-cell adhesion, using somatostatin analogs specific for various receptors (*Table 2*). We found that somatostatin analogs specific for SSTR2 and/or SSTR3 enhanced adhesion of T-cells to FN and, to a certain extent, to CIV and LAM.

T-lymphocytes express multiple SSTRs and somatostatin may therefore regulate cell functions via distinct receptor subtypes. It is now an important task to identify the exact function of each SSTR subtype in T-lymphocytes. Treatments, which interfere with the recruitment of skin-homing lymphocytes by inhibiting cell adhesion, and can prevent T-lymphocytes trafficking into the skin, should be useful as therapy for inflammatory skin disorders. The present study indicates that somatostatin may be a major regulator of the capacity of T-lymphocytes to

adhere to ECM components. Our findings suggest that different SSTR subtypes may be useful targets for therapy in disorders involving T-cells. The development of selective SSTR receptor antagonists should provide more information in this regard.

CONCLUSIONS

- In an open study with peptide T treatment of psoriasis, 5 of 9 patients improved their PASI score >50%. The clinical results were confirmed by assessing the histopathological score and epidermal thickness on biopsies taken during the treatment. Peptide T treatment caused an increase in CD1+ dendritic cells and a reduction in infiltrating lymphocytes in those patients who improved during the treatment.
- Somatostatin-immunoreactivity was seen in cells with a dendritic appearance. The majority of these cells were located in the dermis. More somatostatin-positive dendritic cells were present in psoriasis lesions than in normal skin.
- Colocalization of somatostatin- and HLA-DR-immunoreactivity was found in a subgroup of dendritic cells in psoriatic skin mainly located in the epidermis. No double-labeled cells could be detected in uninvolved skin. It is probable that these cells can process and present antigen to T-cells.
- The somatostatin-positive dendritic cells did not co-express CD1a, CD35, CD45RB, CD45RO, CD68, factor XIIIa or S-100, antigens, which have been reported to be expressed by other dendritic cells in human skin. These cells therefore probably represent a specific population of dendritic cells, distinct from Langerhans cells and factor XIIIa-positive cells.
- The number of somatostatin- and factor XIIIa-immunoreactive dendritic cells was significantly reduced by topical treatment with clobetasol propionate and calcipotriol. The rate of reduction in the somatostatin-positive cells differed between the two groups and closely paralleled the healing induced by both treatments.
- T-lymphocytes express somatostatin receptors (SSTR 1-5) related to activation and stage of differentiation and SSTR expression distinguishes normal and leukemic T-cells. Normal T-cells expressed SSTR1 and SSTR5 while T-leukemia cell lines did not. The leukemic T-cell lines showed a strong expression of SSTR2 while the normal T-cells showed a weak one at the mRNA level. SSTR3 and 4 were present in all the normal and leukemic T-cells examined. SSTR5 was selectively expressed in activated normal T-cells.
- Normal T-lymphocytes and all the T-cell lines studied were negative with respect to somatostatin mRNA expression. Thus, although T-cells can

respond to somatostatin they do not seem to produce the neuropeptide themselves.

- Somatostatin and somatostatin analogs specific for SSTR2 and/or SSTR3 enhanced adhesion of T-cells to fibronectin and, to a certain extent, also to collagen type IV and laminin. Thus, somatostatin regulates T-cell adhesion to extracellular matrix components via distinct receptor subtypes.

ACKNOWLEDGEMENTS

I wish to express my warmest gratitude to all those who have helped me throughout this study, and especially to:

Jan Marcusson, my supervisor, for introducing me to the field of dermatological science, for all your good and fruitful ideas, for always believing in me, and for constant support during my experiments.

Karl-Gösta Sundqvist, my co-supervisor, for introducing me to the field of immunology and cell biology, never-failing positive attitude and ability to arouse enthusiasm, and for providing me with excellent laboratory support.

Marianne Schultzberg, for teaching me immunohistochemical techniques and microscopy, for your support during my experiments, and for always being helpful.

Eva Bergdahl, for excellent technical collaboration in the laboratory, for patiently teaching me laboratory methods, and for always being kind and obliging.

Olle Johansson and Marita Hilliges, for introducing me in the exciting field of neuropeptides and somatostatin in human skin.

Barbro Lundh Rozell, for introducing me to the histopathology of psoriasis.

Lennart Wetterberg, for having taken the initiative and given us the opportunity to perform the peptide T study.

Anna Ivanoff, Jyrki Ivanoff, Maria Hägglund and Joost van Neerven for your collaboration in the somatostatin receptor study.

Inga Volkman, for skilled technical laboratory help.

Peter Lidbrink, for friendship, support and encouragement during these studies.

Lennart Emtestam, for friendship, all good advice and for always kindly answering my questions about various matters during this project.

All my colleagues and co-workers in the Department of Dermatology, for creating a friendly and encouraging environment.

My wife *Lotta*, for loving and faithful support during all these years of hard work.

This study was supported by grants from the Edvard Welander and Finsen Foundation, the Swedish Psoriasis Association and the Swedish Society for Medical Research.

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