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Colocalization of Somatostatin- and HLA-DR-like Immunoreactivity in Dendritic Cells of Psoriatic Skin

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This study presents an immunohistochemical characterization of somatostatin-positive dendritic cells in psoriatic lesions. Somatostatin is a neuropeptide with inhibitory action on several neuropeptides and hormones, but also with immunomodulating properties, and has been used in several studies as treatment for psoriasis. The number of somatostatin-positive dendritic cells was found to be larger in psoriatic lesions than in normal skin of psoriasis patients and healthy controls. Colocalization of somatostatin and HLA-DR immunoreactivity was demonstrated in a subgroup of dendritic cells of psoriatic skin, whereas double-labelled cells were not found in uninvolved skin. The somatostatin-positive cells in the epidermis and dermis did not co-express CD1a, CD35, CD45RB, CD45RO, CD68, factor XIIIa or S-100. On the basis of these findings, the somatostatin-positive cells seem to represent a specific population of dermal dendritic cells, distinct from Langerhans' cells and factor XIIIa-positive cells, which are found in elevated amounts in chronic plaque psoriasis. ***Key words: immunohistochemistry; Langerhans' cells; major histocompatibility complex class II; psoriasis.***

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Close cell-cell apposition of epidermal Langerhans' cells with infiltrating lymphocytes has been demonstrated ultrastructurally in psoriasis plaques (1), indicating a role for these cells also in the pathogenesis of psoriasis. The therapeutic effects of cyclosporin A (2), anti-CD4 antibodies (3) and peptide T (4) support the notion that the T lymphocyte plays a key role in psoriasis.

In the psoriatic dermis, there are large numbers of dendritic cells which are located beneath the hyperproliferating keratinocytes and surrounded by T cells. The subunit A of the clotting factor XIII (FXIIIa) is expressed in a population of dermal dendritic cells, distributed mainly in the upper dermis and around superficial blood vessels (5). The FXIIIa-positive dermal dendritic cell has been reported to be one of the three most proliferating cell types in psoriatic skin, the other two being T cells and endothelial cells (6).

Somatostatin is a 14 amino acid peptide with inhibitory action on many neuropeptides and hormones, for example growth hormone (7). Its synthetic analogue octreotide is now used for the suppression of the symptoms of different neuroendocrine tumours (8). Somatostatin-immunoreactive nerve fibres and dendritic cells are found in human skin (9, 10). Somatostatin has been used in several studies (most of them open, one controlled) as treatment for psoriasis, with a clearance rate between 30–80% (11–15). Peptide T is a synthetic octapeptide, which was designed as a ligand for the CD 4 receptor (16). Serial biopsies from psoriatic skin during 4 weeks of peptide T treatment showed major changes in the number of somatostatin-immunoreactive dermal dendritic cells. The changes entailed both increases and decreases during the course of the treatment (17), indicating that the regulation of somatostatin in the dendritic cells may be part of the healing process of psoriasis.

This study is an attempt to further characterize the somatostatin-positive dendritic cells seen in psoriatic skin. The colocalization of somatostatin with other factors expressed in dermal dendritic cells/macrophages, such as factor XIIIa, HLA-DR, CD1a, CD35, CD45RB, CD45RO, CD68 and S-100, was investigated.

MATERIAL AND METHODS

Patients with a chronic plaque psoriasis of moderate severity (untreated for 4 weeks) were chosen for this study. Skin biopsies from 5 patients (4 men and 1 woman, age range 38–74 years) were analysed in a double-labelling study, and biopsies from 8 patients (5 men and 3 women, age range 28–74 years) were used in an elution-restaining study.

Punch biopsies (4 mm) from lesional psoriatic skin, uninvolved skin of psoriasis patients and healthy controls were taken in a 10-cm radius around the elbow, after local injection of lidocaine without epinephrine. The specimens were immersed for 3 h in 4% paraformaldehyde and 14% saturated picric acid in 0.1M Sörensen phosphate buffer (pH 7.4) at 4°C and then rinsed in the same buffer containing 10% sucrose for at least 24 h. Sections (12 μm) were cut on a cryostat (Microm HM 500 M) and stored at −20°C. The sections were stained with indirect immunofluorescence technique according to Coons (18). The *primary antisera* are specified in Table I. All experiments were repeated twice or three times.

Double-labelling experiments

Double-labelling experiments were performed with a mixture of somatostatin antiserum (diluted 1:200) and mouse monoclonal antibodies against one of the following antigens: HLA-DR (1:50), CD1a (1:25), CD35 (1:10), CD45RB (1:25), CD45RO (1/50) or CD68 (1:25). The occurrence of somatostatin and HLA-DR double-labelling was also studied in biopsies (4 mm) of uninvolved skin near the elbow region from 5 psoriatic patients and from 5 healthy volunteers. The sections were incubated with the primary antibodies overnight in a humid atmosphere at 4°C. The sections were then rinsed and incubated for 60 min at room temperature (21°C) with a mixture of TRITC-labelled swine anti-rabbit antibodies (Dako) and FITC-labelled goat anti-mouse antibodies (Dako). The mounting medium contained para-phenylenediamine to prevent fading of the fluorescence (19). The material was examined in a fluorescence microscope (Zeiss Axioplan, equipped with a MC 100 camera).

The somatostatin-labelled cells were counted per linear millimeter (mm) in the epidermis and whole dermis in 4

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Table I. *Primary antibodies*

Antigen	Species	Cellular distribution	Source
Somatostatin	Rabbit (polyclonal)	Somatostatin-positive nerve fibres and dendritic cells	Peninsula S: t Helens, UK
Factor XIIIa	Rabbit (polyclonal)	FXIIIa-positive dendritic cells	Calbiochem San Diego, CA, USA
S-100	Rabbit (polyclonal)	Melanocytes Langerhans' cells Schwann cells	Dako Glostrup, Denmark
HLA-DR	Mouse (monoclonal)	Class II MHC	Becton Dickinson Sunnyvale, CA, USA
CD1a	Mouse (monoclonal)	Langerhans' cell	Becton Dickinson Sunnyvale,CA,USA
CD35	Mouse (monoclonal)	Follicular dendritic cells	Dako Glostrup, Denmark
CD45RB	Mouse (monoclonal)	B cells T cell subsets monocytes, macrophages	Dako Glostrup, Denmark
CD45RO	Mouse (monoclonal)	Activated T cells monocytes, macrophages granulocytes	Dako Glostrup, Denmark
CD68	Mouse (monoclonal)	Macrophages monocytes	Dako Glostrup, Denmark

sections per patient. The results from lesional skin ($n=5$) and uninvolved skin ($n=5$), as well as skin from healthy controls ($n=5$), were compared by analysis of variance (ANOVA) and Scheffe's *post hoc* test. The specificity of the somatostatin antiserum was tested by preadsorption with somatostatin, giving a final peptide concentration of 30 nM. In addition, omission of the primary antiserum served as control.

Elution-restaining experiments

The sections were incubated overnight in 4°C with somatostatin antiserum (diluted 1:200), followed by incubation with TRITC-labelled swine anti-rabbit antibodies for 60 min. After photography of the somatostatin- positive dendritic cells, the coverslips were removed and the sections rinsed in PBS for 20 min. The antibody complex was removed by immersion of the sections for 60 s in a solution containing 1.6 ml 3% $KMnO_4$, 2 ml 5% H_2SO_4 and 50 ml distilled water (20). The complete elution of the antibodies was controlled in all sections by reincubation with secondary antibodies. Sections without immunofluorescent cells were rinsed briefly in PBS and incubated overnight with either FXIIIa antibodies diluted 1:800 or S-100 antibodies diluted 1:200, followed by incubation with TRITC-labelled swine anti-rabbit antibodies. New photographs of identical fields were taken and compared with the micrographs of somatostatin-positive cells.

RESULTS

Somatostatin-positive cells

In lesional psoriatic skin somatostatin-positive cells with a dendritic appearance were mainly located in the papillary and upper reticular dermis (Fig. 1A). Some cells were also present in the spinous and basal layers of the epidermis. Many of the latter somatostatin-immunoreactive cells were in close contact with HLA-DR-positive/somatostatin-negative dendritic cells and with lymphocytes. Some of the dermal somatostatin-immunoreactive cells, located just under the basement membrane, stretched their dendritic processes into the epidermis.

Sections incubated with control serum, or with PBS instead of the primary antiserum, displayed no somatostatin immunoreactivity.

The number of lesional somatostatin-positive dendritic cells varied between 16 and 49 cells per mm dermis (mean 37.0 ± 18.3, S.D., $n=5$) and between 1 and 7 cells per mm epidermis (mean 2.2 ± 1.6, S.D., $n=5$). In uninvolved skin of psoriasis patients (Fig. 1B) and in healthy controls the somatostatin-positive dendritic cells were only located in the dermis, and their number varied between 2 and 10 cells per mm (mean 6.4 ± 1.1, S.D., $n=5$ and mean 6.4 ± 3.0, S.D., $n=5$, respectively). The number of somatostatin-positve cells in the psoriatic lesions was thus significantly increased, compared to skin from healthy controls as well as uninvolved skin from psoriasis patients (Fig. 1A,B), both in the dermis and the epidermis

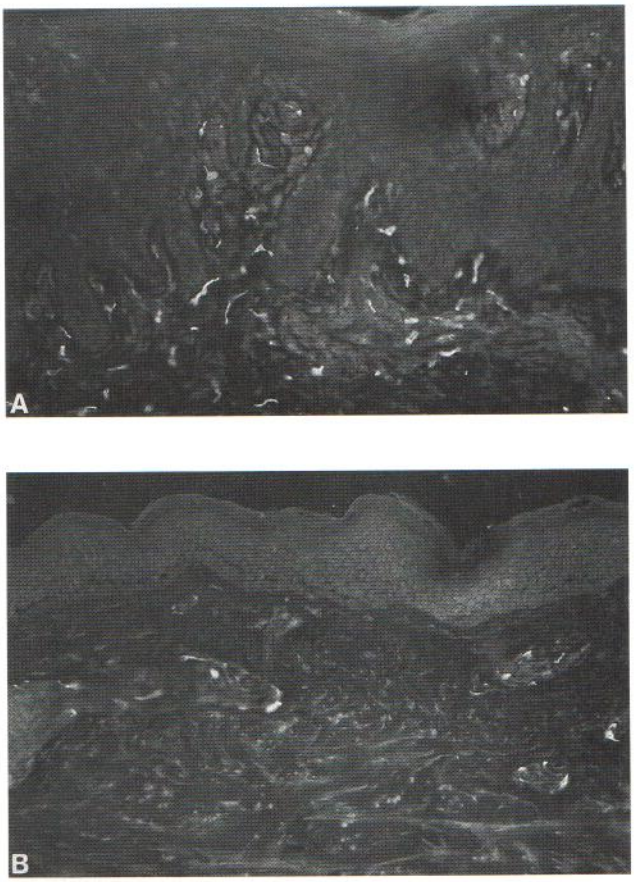

Fig. 1. Somatostatin-immunoreactive dendritic cells in lesional psoriatic skin (A) and uninvolved skin of a psoriasis patient (B). Magnification 400×.

($p<0.01$). No significant difference was seen between nonlesional skin from psoriasis patients and skin from healthy individuals ($p<0.01$).

Double-labelling experiments

Analysis of the epidermis in psoriasis lesions revealed a group of dendritic cells in the epidermis that were immunoreactive for both somatostatin and HLA-DR (Fig. 2A,B). This type of cell was observed in all of the 5 cases examined. The staining for somatostatin and HLA-DR, respectively, was slightly weaker in the epidermis than in the dermis, partly due to a stronger epidermal background staining. The number of dendritic cells costoring somatostatin and HLA-DR varied between 0.2 and 5 per mm epidermis, which represented 0.3–14% of the total population of HLA-DR-positive dendritic epidermal cells. In 3 patients, somatostatin-positive HLA-DR-negative dendritic cells were observed in the epidermis (0.5, 2 and 6 cells per mm, respectively). A few somatostatin-positive HLA-DR-positive dendritic cells could be seen in the dermis just under the basement membrane. None of the somatostatin-positive dendritic cells in uninvolved skin coexpressed HLA-DR. No double-labelled cells were seen in the sections stained with somatostatin and CD1a, CD35, CD45RB, CD45RO or CD68.

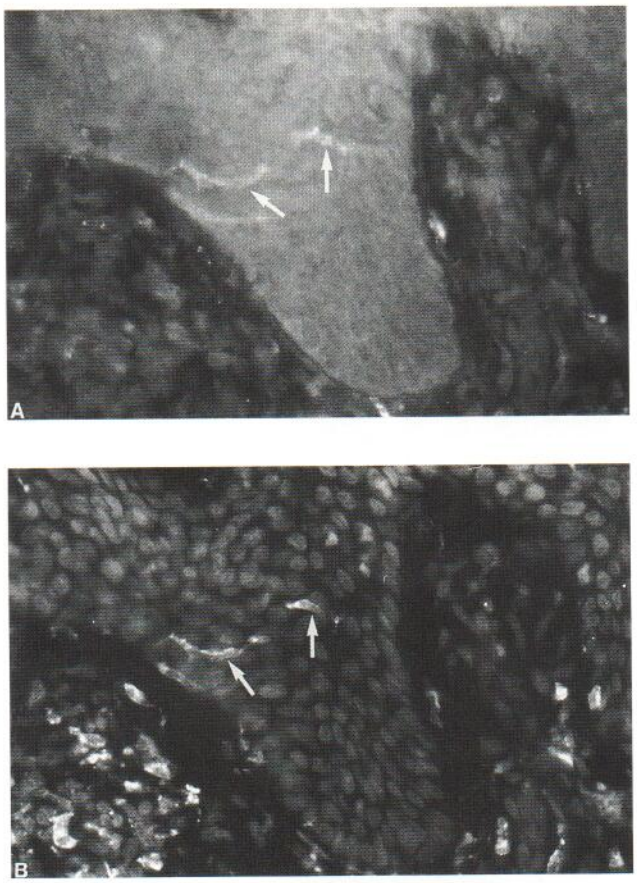

Fig. 2. Immunofluorescence micrographs of a section of psoriatic skin after double-staining with antibodies to somatostatin (A) and HLA-DR (B). Note double-labelling of some dendritic cells (*arrows*) in the epidermis. Magnification 400×.

Elution-restaining experiments

The FXIIIa-positive cells with dendritic appearance were more numerous than the somatostatin-positive dendritic cells in psoriatic skin. The FXIIIa-immunoreactive cells were mainly observed in the upper dermis and around superficial blood vessels. S-100-immunoreactive dendritic cells could be seen in the upper dermis, often in small groups, and in the epidermis they occurred mainly in the spinous and basal layers. Elution and restaining did not reveal coexpression of somatostatin and FXIIIa or of somatostatin and S-100 in the dendritic cells of lesional skin.

DISCUSSION

In this study we have described a group of somatostatin-positive cells with dendritic appearance, present in elevated numbers in lesional psoriatic skin. A subgroup of these cells were found to coexpress HLA-DR. The somatostatin- and HLA-DR-positive cells were mainly located in the epidermis, and their number varied from a few cells up to 14% of the total population of epidermal HLA-DR-positive dendritic cells. Unlike the regular Langerhans' cells, these cells were negative for CD1a. Increased numbers of CD1-negative HLA-DR-positive subpopulations of dendritic cells have been reported in psoriasis (21) and in other inflammatory dermatoses (22). In the dermis, the somatostatin-immunoreactive cells were, with a few exceptions, HLA-DR-negative. In uninvolved skin of psoriasis patients and in healthy controls, the somatostatin-positive cells were fewer and only located in the dermis. None of these cells coexpressed HLA-DR.

FXIIIa-positive dendritic cells have been reported to be increased in inflammatory cutaneous diseases (23). Since this population of dendritic cells is concentrated around papillary blood vessels, it has been suggested that FXIIIa can be released locally, when it is necessary to stabilize clots and cross-linked fibrin (24). FXIIIa-positive cells also express MHC class II antigen and may thus be capable of presenting antigen to T cells (23). However, coexpression of somatostatin and FXIIIa in dendritic cells was not seen in the present material.

S-100, which is a melanocyte marker but also occurs in Langerhans' cells and Schwann cells, could not be found in the somatostatin-positive dendritic cells.

There is accumulating evidence that neuropeptides play an important role in the pathogenesis of different skin diseases (25). In addition to their neurotransmitter and neuroendocrine functions, some neuropeptides have mitogenic properties and can modulate responses of the immune system (26). It has been hypothesized that stressful events and local trauma lead to a release of neuropeptides such as substance P from sensory nerves in the skin, which in turn may initiate the development of psoriatic lesions in predisposed individuals (27). 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 (28). The epidermis and dermis of psoriatic lesions are more densely innervated with substance P-containing nerves, compared to lesion-free skin (29). Notably, nerve-induced vasodilatation and release of substance P are inhibited by somatostatin (30).

T lymphocyte activation is regarded to be of importance in

the initiation and maintenance of psoriatic skin lesions (31). Binding sites for somatostatin were identified on mononuclear leukocytes (32), and somatostatin was shown to inhibit T cell proliferation (33) and interleukin-2 receptor expression in T cells (34).

Other conceivable roles of somatostatin in psoriasis include effects on somatomedins and epidermal growth factor (EGF). Fibroblasts (35) and keratinocytes (36) were shown to express somatomedin C/insulin growth factor 1 (IGF1) receptors. Stimulation of fibroblasts, which results in production of somatomedins (37) and somatomedin C/IGF1, was shown to increase human keratinocyte proliferation (38). The EGF binding and receptor distribution were reported to be altered in psoriatic skin (39). A decrease in plasma EGF was observed in psoriatic patients after somatostatin treatment (13). A direct effect of somatostatin on keratinocytes seems to be less probable, since normal and psoriatic epidermis and skin appendages seem to lack somatostatin receptors (40).

In conclusion, this study shows the presence of elevated numbers of somatostatin-positive dendritic cells in psoriatic lesions, compared to normal skin of psoriatic patients and healthy individuals. These cells are mainly localized in the dermis. The physiological role of these cells is unknown. A subgroup of the somatostatin-positive cells were found to coexpress HLA-DR. These double-labelled cells were mainly located in the epidermis. In view of their expression of class II major histocompatibility complex, it can be expected that they are able to process and present antigens to T cells. Somatostatin is a peptide with inhibitory and immunomodulatory functions. The occurrence of somatostatin-containing dendritic cells in psoriatic skin lesions may indicate a regulatory role in T cell activation and/or the release of neuropeptides such as substance P. Activation of the somatostatin-positive dendritic cells may thus contribute to break the vicious circle of immunhyperstimulation, and to promote the healing of psoriasis.

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