This study describes the changes in number and distribution of somatostatin- and factor XIIIa-immunoreactive dendritic cells in the epidermis and dermis of psoriatic lesional skin during topical treatment with clobetasol propionate or calcipotriol. Immunohistochemical analysis showed that the number of each cell type was increased in lesional skin as compared to normal skin. Investigation of serial biopsies from psoriasis lesions revealed a significant reduction in the number of somatostatin- and factor XIIIa-positive dendritic cells during the treatments. The reduction rate of the somatostatin-positive cells differed between the two groups and closely paralleled the healing process induced by the two treatments. These findings and the fact that somatostatin has been used in several studies as treatment for psoriasis may indicate that the somatostatin-positive cells are specifically involved in the healing process of psoriasis. The reduction of the factor XIIIa-positive cells was associated with the healing process as a whole, but showed no relation to either treatment. Key words: dendritic cells; epidermal thickness; immunohistochemistry; psoriasis.

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Somatostatin is a 14 amino acid peptide with inhibitory action on many neuropeptides and hormones (1). Its synthetic analogue, octreotide, is utilized as treatment for certain neuroendocrine tumours and after pancreatic surgery (2). In several studies (most of them open and one controlled), somatostatin has been used as treatment for psoriasis with a clearance rate of lesions after 12-18 weeks in the majority of patients (3-7). Immunohistochemical analysis showed that the number of somatostatin-positive cells was elevated in psoriatic plaques as compared to healthy human skin (8). These dendritic cells do not coexpress CD1a, CD35, CD45, CD68, factor XIIIa or S-100. However, a subgroup of the cells located in the psoriatic epidermis coexpresses HLA-DR (8). Consequently, these cells probably represent a specific population of dendritic cells in human skin, distinct from other dendritic cells such as Langerhans' cells and factor XIIIa-positive cells (8). In earlier communications we have shown that treatment with peptide T (a synthetic octapeptide) induced changes in the number of the dermal somatostatin immunoreactive cells in psoriasis lesions, indicating that these cells play a role in the pathogenesis and/or healing process in psoriasis (9, 10).

Subunit A of the clumping factor XIII (FXIIIa) is expressed in a population of dendritic cells distributed mainly in the upper dermis and around superficial blood vessels. These cells occur in increased numbers in psoriatic skin (11) and seem to be of bone marrow origin, sharing some features common to monocytes/macrophages (11).

The aim of this study was to investigate deviation over time in the number and distribution of somatostatin- and FXIIIa-positive cells in psoriatic lesional skin during healing accomplished with two commonly used topical therapies, clobetasol propionate and calcipotriol ointments.

MATERIAL AND METHODS

Twenty patients (9 women, 11 men, aged 24 - 64) with moderately severe chronic plaque psoriasis participated in the study. There was a 2-week washout period prior to enrolment. Ten of the patients were treated with a topical corticosteroid, 0.05% clobetasol propionate ointment (Glaxo Wellcome Operations, Greenford, UK), applied BID the first week, once daily the second, every second day the third and for two days the fourth week; 10 patients were treated with a topical vitamin D analogue, 50 μg/g calcipotriol ointment (Lövens Kemiske Fabrik, Ballerup, Denmark) BID for 4 weeks. One lesion was chosen as the site for evaluation and biopsies (in 18 cases the elbow, in 1 case the leg and in another the back). The clinical course was scored according to desquamation, erythema and infiltration as follows: 0 = visible lesion, 1 = mild, 2 = moderate and 3 = severe.

Punch biopsies (4 mm in diameter) from lesional psoriatic skin were taken before and after 1, 2 and 4 weeks of treatment. Lidocain without epinephrine (Astra, Södertälje, Sweden) was used as local anaesthetic. Biopsies from uninvolved skin just below the elbow from 6 patients (1 woman, 5 men, aged 43 - 64) and from 10 healthy volunteers (3 women, 7 men, aged 22 - 50) served as controls.

The biopsies were immersed for 3 h in 4% paraformaldehyde containing 14% saturated picric acid in 0.1 M 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 staining was performed by an indirect immunofluorescence technique according to Coons (12). As primary antisera, we used polyclonal rabbit antibodies raised against human somatostatin diluted 1:200 (Peninsula, St. Helens, UK), and against FXIIIa diluted 1:800 (Calbiochem, San Diego, CA, USA).

The skin sections were incubated with the primary antibodies in a humid atmosphere at 4°C overnight. After rinsing in phosphate buffer (pH 7.4), the sections were incubated for 60 min at room temperature (21°C) with fluorescein isothiocyanate (FITC)-labelled swine anti-rabbit IgG(fab) diluted 1:100 (Dako, Glostrup, Denmark). To prevent fading of the fluorescence, paraphenyldiamine was added to the mounting medium (13). The material was examined under a fluorescence microscope (Zeiss Axiosplan) equipped with an MC 100 camera.

The somatostatin- and FXIIIa-labeled cells were counted per linear millimeter (mm) in the epidermis, papillary dermis and upper 0.25 mm of the reticular dermis in two different sections per biopsy. The healing process was also followed by measuring the thickness of the living epidermis from the granular layer to the tips of the rete ridges (epidermal thickness) using an ocular micrometer. Twentyrete ridges per section were measured in two different sections per biopsy. All readings and measurements in the microscope were performed on coded sections.

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The specificity of the somatostatin antibody was tested by preabsorption with somatostatin-14, giving a final peptide concentration of 30 nM. In addition, omission of the primary antisera served as control.

### Statistical methods

Results were analysed using two-way analysis of variance (ANOVA) with repeated measures on one factor. In case 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 according to the Bonferroni procedure. The distribution for some of the variables was skewed and the data have been log-transformed in order to meet requirements for an adequate ANOVA. When the compound symmetry assumptions were not valid in ANOVA, the F-test was adjusted according to the method of Kirk (14).

For the variables somatostatin- and FXIIIa-positive cells, the differences from day 0 in lesional skin and healthy skin were analysed by Wilcoxon signed rank test within group and with Mann-Whitney U test between groups.

### RESULTS

#### Clinical course

The clinical scores improved significantly during treatment with clobetasol and calcipotriol ointment (p < 0.0001) (Fig. 1), but the improvement in the clobetasol group was faster and more pronounced (p < 0.01).

We also followed the healing process by measuring the epidermal thickness (Fig. 2). Initially, the epidermis was significantly thicker in the lesional psoriatic skin as compared with skin from healthy volunteers (p < 0.0001) and uninvolved skin of psoriasis patients (p < 0.001). Both treatments resulted in a significant reduction over time of the epidermal thickness (p < 0.0001), but the reduction was slower in the calcipotriol group as compared with the clobetasol group (p < 0.001) (Fig. 2).

#### Somatostatin-positive cells

In lesional psoriatic skin, dendritic somatostatin-positive cells were found mainly in the papillary and upper reticular dermis (Fig. 3A). Some cells, located just under the basement membrane, extended their dendritic processes into the epidermis. A few cells were also present in the spinous and basal layers of the epidermis. Preabsorption of the somatostatin antiserum with somatostatin-14 or omission of the primary antisera resulted in an absence of somatostatin-like immunoreactivity.

The number of somatostatin-positive dendritic cells in lesional psoriatic skin varied from 21–90 cells per mm (dermis) and 0–2 cells per mm (epidermis). In uninvolved skin of psoriasis patients and in healthy controls, the somatostatin-positive cells were only seen in the dermis. The number varied from 5–14 cells per mm (dermis) in uninvolved skin of psoriasis patients and from 2–12 cells per mm (dermis) in healthy controls. The number of somatostatin-positive cells in the psoriatic lesions was thus significantly increased in both the dermis and epidermis as compared to skin from healthy controls (dermis p < 0.0001, epidermis p < 0.001) as well as uninvolved skin from psoriasis patients (dermis p < 0.01, epidermis p < 0.05).

The number of somatostatin-positive cells was significantly reduced in the dermis (p < 0.0001) by the two treatments (Figs. 3A, B, 4A), but the reduction of the somatostatin-positive cells was faster in the clobetasol group (p < 0.001) (Fig. 4A). In the epidermis, the somatostatin-positive cells were significantly reduced only in the clobetasol group after 14 and 28 days of treatment (p < 0.05) (Fig. 4B).

#### FXIIIa-positive cells

The FXIIIa-positive dendritic cells were more numerous than the somatostatin-positive dendritic cells in psoriatic lesional skin both in the dermis and in the epidermis (p < 0.0001). The cells were observed mainly in the upper dermis and around superficial blood vessels (Fig. 5A). Their number varied from 2–26 cells per mm (epidermis), and 78–184 cells per mm (dermis). In uninvolved skin of psoriasis patients and in healthy controls, the FXIIIa-positive dendritic cells were only observed in the dermis, their number varying from 48–69 cells/mm in uninvolved skin of psoriasis patients and 26–63 cells/mm in healthy controls. The number of FXIIIa-positive cells in the psoriatic lesions was significantly increased in both the dermis and epidermis as compared to skin from healthy controls (dermis p < 0.0001, epidermis p < 0.0001) as well as uninvolved skin from psoriasis patients (dermis p < 0.05, epi-
The number of FXIIIa-positive cells significantly decreased over time in the dermis \((p < 0.001)\) during treatment with clobetasol and calcipotriol (Figs. 5A, B). The reduction of the FXIIIa-positive cells over time did not differ between the two treatments. In the epidermis, the FXIIIa-positive cells were significantly reduced by both treatments after 14 and 28 days \((p < 0.05)\).

**DISCUSSION**

Somatostatin- and FXIIIa-immunoreactive cells constitute two cell populations displaying a dendritic morphology, which are present in elevated numbers in lesional psoriatic skin (8, 11). Whether and how the somatostatin- and FXIIIa-positive dendritic cells affect adjacent cells such as T-lymphocytes, endothelial cells and keratinocytes in the psoriasis lesion is, at present, unknown. It is also an open question whether these cells are bystanders in the inflammatory reaction or whether they can contribute to the healing process.

In the present open-controlled study, we have investigated changes in the number and distribution of these two cell types in psoriatic skin during two well established topical treatment alternatives of psoriasis, clobetasol propionate and calcipotriol. The therapy and observation lasted 4 weeks, and biopsies were collected before and 3 times during the treatment. The healing process was followed in two ways: first with clinical scoring, which was performed openly, and secondly by microscopic measurement of the epidermal thickness, which was made on blind-coded specimens.

The number of somatostatin-positive cells in the dermis was significantly reduced during the healing of psoriasis induced by the topical therapies. This reduction occurred earlier in the clobetasol-treated group than in the calcipotriol-treated one. The different time course between the two drug treatments paralleled the rates of clinical improvement, especially when measured as the reduction in epidermal thickness. A significant reduction of the number of somatostatin-positive cells in the epidermis could be seen in the clobetasol-treated group, although these cells were much fewer in that skin compartment. Somatostatin affects a variety of immunocompetent cell functions, such as inhibition of T-lymphocyte proliferation (15), immunoglobulin production by B-lymphocytes (16) and neutrophil chemotaxes provoked by substance P (17). Somatostatin may participate in the anti-inflammatory actions of glucocorticoids, since somatostatin immunoactivity in inflammatory sites is stimulated by dexamethasone, while in parallel, the local inflammatory exudates are suppressed (18).
The increase in somatostatin-positive cells in psoriasis may thus be a local host defence mechanism.

Merkel cells are constituents of normal skin mainly located in the basal layer of the epidermis in close contact with nerve endings, but with unknown function. Several peptides have been identified in the granulae of Merkel cells. Merkel cells have been observed in increased amounts in the psoriatic epidermis, and, in contrast to normal skin, a subgroup of these cells (7%) expresses somatostatin (19). In fact, the somatostatin-positive cells localized in the epidermis described in this study may be Merkel cells.

The number of FXIIIa-positive dendritic cells in the epidermis and dermis was reduced equally by the two treatment modalities. This may reflect that changes in the FXIIIa-positive cells are not specifically linked to the topical medicaments used. Factor XIIIa is a fibrin stabilizing factor which covalently cross-links fibrin monomers to a protein that forms a stable clot. FXIIIa is found intra- and extracellularly. FXIIIa-expressing dermal dendritic cells has been linked to various dermatological conditions, including psoriasis (20). FXIIIa-positive cells belong to one of the three most proliferative cell types in psoriatic lesional skin. The other two are T-cells and endothelial cells (21). Since this population of dendritic cells is concentrated around papillary blood vessels, it has been suggested that FXIIIa can be released locally, when necessary, to stabilize clots (22). FXIIIa can also mediate adhesion of various cells (23). FXIIIa-positive cells express MHC class II antigen, and may thus be capable of presenting antigen to T-cells (11).

In conclusion, somatostatin- and FXIIIa-positive cells represent two specific dendritic cell populations with multiple potential, but so far unknown functional properties. Topical treatment with clobetasol propionate and calcipotriol significantly reduces both cell populations during the healing of psoriasis. The reduction rate of the somatostatin-positive cells differs between the two treatments and closely parallels the healing process, while the reduction of the factor XIIIa-positive cells shows no relation to either treatment. Perhaps the somatostatin-positive cells are more sensitive to corticosteroid treatment than the FXIIIa-positive cells. The correlation between the clinical improvement and the reduction of the somatostatin-positive cells, in addition to the fact that somatostatin has been used in several studies as treatment for psoriasis, also indicates that the somatostatin-positive cells may participate specifically in the healing process of psoriasis.

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