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Topographic Relation between Skin-derived Antileukoproteinase (SKALP) and Leukocyte Elastase in a Case of Annular Pustular Psoriasis

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We have assessed the distribution pattern of a new epidermal elastase inhibitor, skin-derived antileukoproteinase (SKALP) and polymorphonuclear leukocytes (PMN) in a patient with annular pustular psoriasis, using immunohistochemical methods. In clinically uninvolved skin SKALP was not expressed and only occasionally a few PMN could be identified. In the erythematous distal margin of the lesion, expression of SKALP was shown in suprabasal keratinocytes. Although PMN were present in the dermis, no transepidermal PMN migration had occurred at this stage. In the pustular region a dense SKALP-positive zone was demonstrated in the suprabasal compartment. In the centrally healed area neither SKALP expression nor PMN accumulation was shown. The present case report suggests that the induction of SKALP expression does not result from the passage of PMN through the epidermis. The offswitch of SKALP expression coincides with the disappearance of the PMN infiltration and not with the resolution of the mononuclear infiltrate. ***Key words: proteinase inhibitor; immunohistochemistry; polymorphonuclear leukocyte.***

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Several data suggest a role for polymorphonuclear leukocytes (PMN) in the pathogenesis of psoriasis (1). Especially in early and active psoriatic lesions, intraepidermal accumulation of PMN is a characteristic feature. Activated PMN can cause tissue damage and contribute to the inflammatory response by the release of proteinases (elastase, proteinase 3 and cathepsin G), reactive oxygen intermediates and several other mediators of inflammation (2). Elastase is a neutral proteinase that is found predominantly in PMN and can therefore be used as a marker enzyme for PMN (3). Also monocytes contain elastase, though only 5% of the activity found in PMN (4). Elastase is putatively involved in the extravasation of PMN and the migration of PMN through the dermo-epidermal junction into the epidermis (5).

In chronic plaque psoriasis the PMN accumulate in the epidermis forming the so-called micro-pustules of Kogoj, while in pustular psoriasis infiltration of PMN results in the formation of macroscopic pustules. Chronic plaque psoriasis as well as pustular psoriasis are both representants of the psoriasis spectrum, and transition may occur from one into the other variant (6).

In previous papers we have described an epidermal proteinase inhibitor, skin-derived antileukoproteinase (SKALP). SKALP is identical to elafin, an elastase-inhibitor described by others (7). SKALP inhibits the activity of the enzymes elastase and proteinase 3 (8), released by PMN during the process of extravasation. We have characterized SKALP at the biochemical and molecular level (9, 10), showed its presence in the urine of psoriatic patients (11) and localized the SKALP gene to chromosome 20q (12). Immunohistochemical staining located the expression of SKALP in the upper layers of the suprabasal compartment in psoriatic skin, whereas normal skin showed no expression (13).

Because of the functional relationship between SKALP and PMN-derived proteinases, studies on the topographic relation between SKALP and PMN are indicated. It is a practical approach to study this relation in pustular forms of psoriasis in which PMN are abundantly present. Pustular psoriasis is a spectrum which comprises localized forms (psoriasis pustulosa palmoplantaris, acrodermatitis continua of Hallopeau) and generalized forms (acute generalized pustular psoriasis von Zumbusch type, annular pustular psoriasis). Annular pustular psoriasis (APP) is a special form of generalized pustular psoriasis (GPP). Wheras the dynamics of GPP is phasic in time, APP is phasic in place and characterized by a centrifugal expansion of the lesions and a central healing (14).

In this study simultaneous assessment of the distribution of PMN and SKALP was performed. Using a double labelling method we investigated the topographic relation between SKALP and PMN during the induction, the summit and the regression of pustulation in APP.

CASE REPORT

A 71-year-old woman presented with recurrent episodes of APP, complicated by psoriatic arthropathy of the right knee and the distal interphalangeal joints of both hands. From the age of 55 years she had had several episodes of APP, accompanied by fever and general body weakness. The lesions were composed of a sharply demarcated polycyclic erythema with peripheral pustules and expanded by centrifugal spreading within a few days. Central healing of the lesions occurred, and the zone of central healing appeared to have the same dynamics as the centrifugal expansion of the lesions. At the inner margin between lesional and centrally healed skin a colerette scaling was visible.

Relapses of chronic plaque psoriasis and inverse psoriasis interchanged with the episodes of APP.

Besides local therapy, treatment subsequently consisted of several systemic treatments, including methotrexate, PUVA therapy and etretinate in dosages between 25 and 75 mg per day.

MATERIAL AND METHODS

Biopsies

From a typical lesion (Fig. 1) 4-mm punch biopsies were taken. In total 5 biopsies were taken from different zones: (a) clinically uninvolved skin, (b) the distal erythematous margin of the lesion, (c) the pustular region, (d) the paracentral erythematous zone with colerette desquamation and (e) the clinically healed centre. Biopsies were fixed in buffered 4% formalin for at least 24 h and processed for routine histology. Tissue was embedded in paraffin and 5-μm sections were cut.

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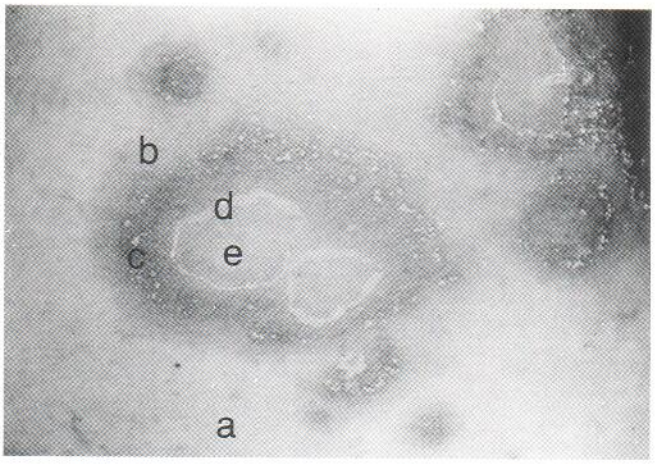

Fig. 1. A typical lesion of annular pustular psoriasis from the presented patient. The different zones for histological examination are indicated (*a–e*).

Antibodies

In the present study the following antibodies were used: monoclonal mouse antibody to human neutrophil elastase and swine-anti-rabbit Ig conjugated with horseradish peroxidase (SWARPO), both obtained from Dakopatts, Glostrup, Denmark. Polyclonal rabbit anti-serum against recombinant elafin was raised as described before (13). Recombinant elafin was a kind gift from Dr. Norman Russell, ICI Pharmaceuticals, UK. Goat-anti-mouse Ig conjugated with alkaline phosphatase (GAMAP) was obtained from TAGO, Burlingame, CA, USA.

Immunohistochemistry

Immunodouble staining was performed, according to standard protocols. After deparaffinizing and rehydration, pre-incubation was performed using 10% normal swine serum and 10% normal goat serum dissolved in phosphate-buffered saline (PBS) to prevent aspecific staining. The sections were then incubated for 60 min with anti-SKALP serum (at a dilution of 1:500) and anti-elastase (at a dilution of 1:250) in PBS with 1% bovine serum albumin (BSA).

After washing in PBS, sections were incubated for 30 min with SWARPO and GAMAP at a dilution of 1:50 and 1:20, respectively, in PBS with 1% BSA and 5% human AB serum. Subsequently, sections were washed in 100 mM NaCl, 5 mM $MgCl_2$ and 10 mM Tris in distilled water (AP buffer) and developed using a mixture of 0.33% 5-bromo-4-chloro-3-indolyl-phosphate-toluidine and 0.66% nitro-blue-tetrazolium in AP buffer. Finally, sections were washed in PBS and sodium-acetate buffer and development was performed, using amino-ethylcarbazole as chromogenic substrate.

RESULTS

The immunohistochemical findings observed in the 5 zones of APP are illustrated in Fig. 2a–e.

In clinically uninvolved skin no significant SKALP expression could be demonstrated and only few PMN could be identified, scattered as single cells (Fig. 2a).

In the erythematous distal margin conspicuous changes were observed. SKALP expression was markedly present in the two uppermost cell layers of the stratum spinosum (Fig. 2b). The density of PMN had increased in the dermis. However, no single PMN or elastase-positive material or any material which could be suspected to be a PMN remnant was identified in the stratum corneum. Occasionally, some PMN were observed in the basal and midepidermal compartments.

In the pustular region (Fig. 2c) a dense accumulation of PMN was present in the dermis and a massive accumulation of PMN was observed within the stratum corneum. In contrast, only occasionally was an isolated PMN observed in the basal zones of the epidermis. The suprabasal compartment was characterized by a continuous SKALP-positive zone which was at least 5–6 cell layers thick.

In the paracentral erythematous zone, a SKALP-positive suprabasal compartment and dermal PMN accumulation were observed (Fig. 2d). The intracorneal PMN accumulation was difficult to identify as such, due to degeneration of the PMN.

In the centrally healed zone, neither SKALP expression nor PMN accumulation was observed (Fig. 2e). A perivascular mononuclear infiltrate was the last testimony of the pustular wave.

Control staining, performed with pre-immune serum of the same animal instead of anti-SKALP and control isotype monoclonal antibodies instead of anti-elastase, was negative with all the 5 zones (not shown).

DISCUSSION

In normal human skin and non-lesional psoriatic skin, SKALP is not expressed. However, in lesional skin of patients with chronic plaque psoriasis a cytoplasmic distribution of SKALP, restricted to the upper layers of the suprabasal compartment, has been reported (13). Also in lesional skin of patients with a pustular form of psoriasis SKALP expression is present, but it is less pronounced than in plaque psoriasis. Functional measurement of SKALP activity in epidermal scales from patients with chronic plaque psoriasis and pustular psoriasis, using a sensitive micro-assay (3), revealed that SKALP activity in pustular psoriasis was significantly lower than in plaque psoriasis (unpublished data).

In scales of patients with atopic dermatitis and the non-inflammatory disorders of keratinization, functional measurement of SKALP showed a moderate presence. In scales of patients with erythrodermic autosomal recessive lamellar ichthyosis and Netherton syndrome a pronounced presence of SKALP, comparable to psoriasis, could be demonstrated (15). Also after sellotape stripping of psoriatic non-lesional skin and normal skin of healthy individuals, SKALP was induced in equal amounts (16). Other conditions in which SKALP expression was shown are keratinocyte culture (17) and several epidermal tumours (18). Therefore, SKALP induction cannot be regarded as specific for psoriasis.

SKALP was shown to be a potent inhibitor of human leukocyte elastase, which belongs to the PMN neutral proteinases. Elastase can damage extracellular matrix proteins, basement membranes and desmosomal structures (2) and is putatively of relevance for the migration of PMN into the skin (5). Speculatively, SKALP functions as an inhibitor of PMN migration and provides protection against elastase-mediated damage.

How induction of SKALP expression by keratinocytes is initiated is not yet clear. An interesting point is whether or not PMN play a role in this process. The present study in a patient with APP provides the possibility to study the kinetics of the topographic relation between PMN trafficking and SKALP expression. In the section of the peripheral margin of the lesion (Fig. 2b), a very clear positive cell layer with SKALP expres-

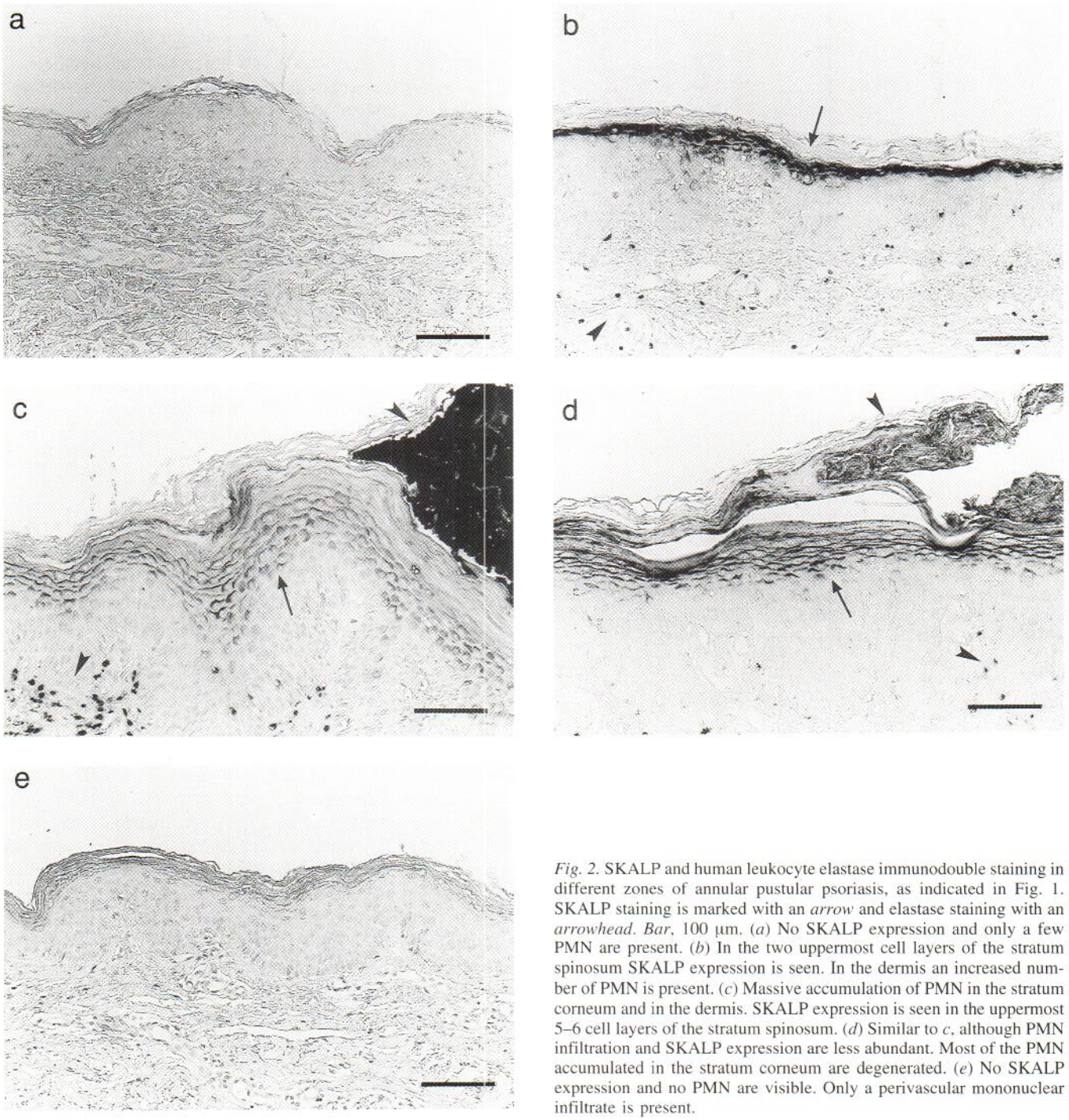

Fig. 2. SKALP and human leukocyte elastase immunodouble staining in different zones of annular pustular psoriasis, as indicated in Fig. 1. SKALP staining is marked with an *arrow* and elastase staining with an *arrowhead*. *Bar*, 100 μm. (*a*) No SKALP expression and only a few PMN are present. (*b*) In the two uppermost cell layers of the stratum spinosum SKALP expression is seen. In the dermis an increased number of PMN is present. (*c*) Massive accumulation of PMN in the stratum corneum and in the dermis. SKALP expression is seen in the uppermost 5–6 cell layers of the stratum spinosum. (*d*) Similar to *c*, although PMN infiltration and SKALP expression are less abundant. Most of the PMN accumulated in the stratum corneum are degenerated. (*e*) No SKALP expression and no PMN are visible. Only a perivascular mononuclear infiltrate is present.

sion was present. In the same section no single PMN was detected in the stratum corneum or in the suprabasal layers of the stratum spinosum. This fact suggests that, in the order of events in the pathogenesis of the psoriatic lesion, the presence of PMN in the epidermis is not obligatory for SKALP transcription. Our observations are compatible with the finding that cultured human epidermal keratinocytes express SKALP in the absence of PMN (17, 19). We therefore conclude that not the presence of PMN but another, hitherto unknown factor is responsible for induction of SKALP expression.

At the area of pustulation and the area with paracentral erythema the SKALP expressing zone is remarkably free from PMN, whereas dermis and stratum corneum are full of PMN (Fig. 2c, d). Despite the presence of SKALP in several cell layers, PMN showed a maximal transgression into the stratum corneum, resulting in pustule formation. In contrast to the situation in chronic plaque psoriasis, with a limited invasion of PMN, in pustular psoriasis PMN invasion and elastase release is massive. This could lead to the situation that in pustular psoriasis all SKALP is saturated with elastase and free elastase is active

despite the abundant presence of SKALP. Presence of free elastase may lead to the formation of macroscopic pustules, characteristic of pustular psoriasis.

In the central area of the clinically healed skin, mononuclear infiltration is present in the absence of PMN and SKALP (Fig. 2e). Downregulation of SKALP was found to correlate with the disappearance of PMN.

The present study indicates that the induction of SKALP expression in psoriatic skin does not result from the passage of PMN through the epidermis but is manifest before epidermal transgression of PMN has taken place. The expression of SKALP in psoriatic skin might be an attempt to downmodulate PMN migration. Furthermore the expression of SKALP and PMN transgression are coupled processes. SKALP appears in the first stages of PMN infiltration and disappears when the composition of the infiltrate changes. We hypothesize that the offswitch of PMN migration is caused by SKALP expression. The ultimate reality test for this hypothesis is to answer the question whether (synthetic) elastase inhibitors would be an effective therapeutic modality in the treatment of pustular psoriasis.

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