Up-Regulation of Elafin/SKALP Gene Expression in Psoriatic Epidermis

Kyoko Nonomura, Kiyofumi Yamanishi, Hirokazu Yasuno, Kiyomitsu Nara,* and Shigehisa Hirose*

Department of Dermatology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto; and *Department of Biological Sciences, Tokyo Institute of Technology, Nagatsuda-cho, Midori-ku, Yokohama, Japan

The expression of mRNA for elafin/SKALP, an inhibitor of leukocyte elastase and proteinase 3, in human normal and psoriatic epidermis was examined by in situ hybridization. In normal epidermis, elafin/SKALP mRNA was detected in the granular layer, but not in the spinous or basal layers. In fully developed psoriatic lesions, elafin/SKALP mRNA was found in the suprabasal layers of the ridges, and in the upper two thirds of the stratum malpighii at the elongated rete ridges. Intense staining was noted near the subcorneal microabscess in psoriasis vulgaris and under the subcorneal pustule in localized pustular psoriasis. In the marginal psoriatic epidermis, elafin/SKALP mRNA was expressed from the middle or upper spinous layer to the subcorneal layer, and the cells expressing elafin/SKALP mRNA increased especially under the parakeratotic corneal layer intermingled with pyknotic nuclei of neutrophils. These findings suggest that the induction of elafin/SKALP gene expression is related closely to the infiltration of neutrophils into the epidermis in psoriasis and plays an important role in protecting the skin components against the tissue damage caused by the infiltrated leukocytes. Key words: proteinase inhibitor/leukocyte elastase/in situ hybridization. J Invest Dermatol 103:88–91, 1994

H
uman leukocyte elastase (HLE) is a serine proteinase contained in azurophilic granules of polymorphonuclear leukocytes (PMNs) [1,2]. At the site of inflammation, the enzyme is released with other proteinases, proteinase 3, cathepsin G, and collagenase, and causes severe tissue injury [1–3]. The degradation products of various matrix components act as chemoattractants and further recruit a greater number of PMNs [4]. The HLE activity is, like other proteinases, regulated by endogenous specific inhibitors [1,5]. Recently, a novel inhibitor for HLE, named elafin (elastase specific inhibitor) [6], SKALP (skin-derived antileukoproteinase) [7,8], and ESI (elastase-specific inhibitor) [9], has been found independently, from psoriatic epidermis, psoriatic scales, and sputum of patients with chronic bronchitis, respectively. Elafin/SKALP, also has potent inhibitory activity against proteinase 3, but not against cathepsin G [10]. The cDNA and genomic DNA for elafin/SKALP have been cloned and the deduced amino acid sequence indicated a 9.9-kDa mature protein with a unique structure of four repeats of putative transglutaminase substrate motifs as well as a proteinase inhibitor domain [11,12].

PMNs are the predominant cells infiltrating in psoriatic microabscesses and spongiform pustules. In the lesional skin of psoriasis, the activity of HLE is increased and is a sensitive marker for infiltration of neutrophils into skin [13,14]. In addition, a large amount of elafin/SKALP activity is detected in the psoriatic lesional epidermis as well as cultured keratinocytes [7,8], and elafin/SKALP is localized in the suprabasal layers of lesional psoriatic epidermis [15]. Hence, the induction of elafin/SKALP gene expression by HLE is suggested to play a crucial role in the pathophysiology of psoriatic lesions.

In this study, we examined the localization of elafin/SKALP mRNA in normal and psoriatic epidermis by in situ hybridization. We show the expression of the gene in normal epidermis and its dysregulation in psoriatic lesions.

MATERIALS AND METHODS

Materials Normal skin specimens were obtained from patients undergoing plastic surgery. Biopsies were taken from typical plaques of patients suffering from psoriasis vulgaris (n = 5), from non-lesional skin of psoriatic patients, from the palmar lesion of localized pustular psoriasis, and from the lesional skin of patients suffering from atopic dermatitis. They had not received any treatment for at least 2 weeks prior to sampling. Half of the specimen was snap frozen in OCT compound (Miles Laboratories, U.S.A.) using liquid nitrogen, and the other half was fixed in formalin and paraffin embedded for routine hematoxylin-eosin staining. Frozen sections (5–6 μm thick) were made to adhere to glass slides coated with Vectabond (Vector Laboratories Inc., U.S.A.) and were rapidly air dried. The sections were fixed in phosphate-buffered saline (PBS) containing 4% (w/v) paraformaldehyde for 15 min at room temperature, air-dried, and stored at −70°C until use.

Construction of Plasmids and Synthesis of RNA Probes The 0.3-kb second exon of the human elafin/SKALP gene (nucleotides: 1184–1457) [12] was inserted into pBluescript II (Stratagene) and pGEM3Z (Promega Biotec) to construct pBSheg1.4r1 and PGEM-heg, respectively. The pBSheg1.4r1 plasmid or pGEM-heg plasmid was linearized with BclI to synthesize the antisense cRNAs or the control sense cRNAs for elafin/SKALP mRNA by T3 or SP6 RNA polymerase, respectively, using a DIG RNA Labeling Kit (Boehringer Mannheim) as described previously [16]. The full-length transcription of the probes was confirmed by Northern hybridization. The cRNAs were fragmented by limited alkaline hydrolysis [17] and used for in situ hybridization.

In situ Hybridization In situ hybridization was performed as described previously [16]. The sections were rehydrated in PBS containing 0.3% (w/v) Triton X-100 for 15 min, then treated with 0.2 N HCl for 20 min. After proteinase K digestion (1 μg/ml), the sections were post-fixed with 4% (w/v) paraformaldehyde in PBS for 5 min. Following acetylation for 10 min, they were soaked in PBS containing 0.1 M glycine and equilibrated in
Figure 1. Localization of elafin/SKALP mRNA in normal epidermis. In situ hybridization was performed using the antisense (a,c,d) or the sense (b) elafin/SKALP cRNA probes as described under Materials and Methods. a) Normal epidermis from the face. Staining was observed in the subcorneal layer. No signal was detected in the basal, spinous and corneal layers. b) A negative control for a. No signal was detected. c) Normal epidermis of the arm. Staining was present in the subcorneal monolayer focally. d) Normal epidermis from the sole. Two to three subcorneal layers were stained. Scale bars, 50 \mu m.

RESULTS

Figure 1a, c, and d shows the normal skin specimens of healthy individuals hybridized with elafin/SKALP antisense cRNA probe. In the normal epidermis from the face, elafin/SKALP mRNA was detected in the subcorneal layer, but not in the spinous or basal layers. In the perifollicular epidermis, subcorneal mRNA expression was increased (Fig 1a). In the normal epidermis from the arm, mRNA positive cells were found in the subcorneal monolayer focally. In the normal epidermis from the sole, two to three subcorneal layers were stained.

50% formamide containing 2 X SSC (1 X SSC: 15 mM NaCl, 15 mM sodium citrate) for 1 h. The sections hybridized in a mixture consisting of 20 mM Tris-HCl (pH 8.0), 2.5 mM ethylenediaminetetraacetic acid (EDTA), 50% formamide, 0.3 M NaCl, 1 X Denhardt's solution (Sigma), 1 mg/ml yeast tRNA (Sigma), 10% PEG6000, and 0.2 ng/\mu l probe. After overnight incubation at 45 °C, they were washed for 1 h at 45 °C in 50% formamide and 2 X SSC, digested with 10 \mu g/ml RNase (Sigma) in 0.5 M NaCl and 10 mM Tris-HCl (pH 8.0) for 30 min at 37 °C. Hybridization signals were detected using a Nucleic Acid Detection Kit (Boehringer Mannheim). Negative control sections hybridized with a sense probe were also examined.

Figure 2. Localization of elafin/SKALP mRNA in non-lesional and lesional skin of psoriasis vulgaris and in lesional skin of localized pustular psoriasis and chronic eczema. Sections were hybridized antisense elafin/SKALP cRNA probe (a-c,e–i). a) Non-lesional epidermis of psoriatic patients. Elafin/SKALP mRNA was expressed in the subcorneal monolayer focally. b) Transitional zone from uninvolved to involved psoriatic epidermis. The involved psoriatic epidermis (the right-hand side) expressed more abundant elafin/SKALP mRNA than the uninvolved epidermis (the left-hand side). In the marginal psoriatic epidermis, hybridization was seen from the middle or upper spinous to subcorneal layer. c) High magnification of (b). Under the parakeratotic corneal layer intermingled with nuclear dust of PMNs, elafin/SKALP mRNA expressing cells increased markedly. d) Hematoxylin-eosin staining of the field corresponding to (c). Expression of elafin/SKALP mRNA in a fully developed psoriatic epidermis. Arrow-head, epidermal-dermal junction. e) All suprabasal layers were stained at the ridge. f) Upper two thirds of elongated rete ridge were stained. g) Maximal staining was seen near the subcorneal microabscess. h) Expression of elafin/SKALP mRNA in the palmar lesion of a localized pustular psoriasis. Staining was found in the spinous layers under the subcorneal pustule. i) Localization of elafin/SKALP mRNA in lesional skin of atopic dermatitis. One or three subcorneal layers were stained. j) Hematoxylin-eosin staining of the field corresponding to (i). Scale bars, 100 \mu m.
cally (Fig 1c). In the normal epidermis of the sole, elafin/SKALP mRNA was expressed in two to three subcorneal layers (Fig 1d), which were identified as granular layers by hematoxylin-eosin staining.

We next examined the distribution of elafin/SKALP mRNA in psoriatic epidermis. In non-lesional epidermis of psoriatic patients, elafin/SKALP mRNA expression was seen in the subcorneal monolayer focally (Fig 2a). Figure 2b shows a transitional zone from uninvolved to involved psoriatic epidermis hybridized with the elafin/SKALP antisense cRNA probe. The involved psoriatic epidermis expressed more abundant elafin/SKALP mRNA than the uninvolved epidermis. In the marginal psoriatic epidermis, mRNA expression was found from the middle or upper spinous to the subcorneal layer. Under the parakeratotic corneal layer intermingled with pyknotic nuclei of PMNs, the cells expressing elafin/SKALP mRNA increased markedly (Fig 2b,c,d). In fully developed psoriatic lesions, hybridization was seen in the suprabasal layers of the ridges (Fig 2e), and in the upper two thirds of the stratum malpighii at the elongated rete ridges (Fig 2f). Near the subcorneal microabscess, maximal staining was observed (Fig 2g). Similarly, in palmar lesions of localized pustular psoriasis, elafin/SKALP mRNA was distributed in the spinous layers under the subcorneal pustules (Fig 2h). In chronic dermatitis with acanthosis of the epidermis (Fig 2i), elafin/SKALP mRNA expression was found in the one to three subcorneal layers (Fig 2j) that roughly corresponded to the granular layers. Neither the acanthotic spinous layer nor the basal layer was stained.

DISCUSSION

A recent immunohistochemical study using antiserum against purified elafin/SKALP has shown that the elastase inhibitor is present in many epidermal cells of lesional skin of psoriasis [15]. This localization is consistent with that of elafin/SKALP mRNA determined by in situ hybridization. In psoriatic lesions, proteolysis of keratinocytes has been observed under parakeratotic scales and in the area surrounding the subcorneal microabscess [18]. Interestingly, cells expressing elafin/SKALP mRNA are increased with downward extension under the parakeratotic corneal layers containing pyknotic nuclei of PMNs. Moreover, strong signals of the mRNA are found around the subcorneal microabscess filled with PMNs. The pattern of the mRNA expression is similar to that of localized pustular psoriasis. Thus, the up-regulation of elafin/SKALP gene expression in psoriasis is closely correlated with the infiltration of PMNs into the epidermis. Because the epidermis of chronic eczema shows acanthosis without PMN infiltration into the epidermis, elafin/SKALP gene is expressed only in the thickened granular layer despite the presence of acanthosis. A factor, or the proteinases released from the infiltrating PMNs, may strongly induce the elafin/SKALP gene expression in keratinocytes in psoriasis.

Previous studies have shown that no elafin/SKALP is produced in normal epidermis [7]. Immunohistochemical staining also could detect the proteinase inhibitor neither in normal epidermis nor in non-lesional epidermis of psoriasis [15]. However, in situ hybridization revealed that elafin/SKALP mRNA is synthesized in the granular layer of both normal and non-lesional epidermis. The localization of elafin/SKALP gene expression corresponds with that of transglutaminase 1 (keratinoceyte transglutaminase), a cross-linking enzyme to form the cornified envelope [16,19]. The substrates of the enzyme, such as involucrin, loricrin, and cystatin-α, are also expressed in the granular layer [20–22]. In addition, elafin/SKALP is cross-linked by transglutaminases [11]. These findings suggest that elafin/SKALP is a constituent protein of the cornified envelope. The reason for the discrepancy in the expression of the mRNA and protein of elafin/SKALP remains unknown. The translation of elafin/SKALP may be highly suppressed by a post-transcriptional mechanism in normal and uninvolved psoriatic epidermis.

In the development of psoriatic lesions, the gene expression of elafin/SKALP, which has been limited to the granular layer, becomes up-regulated, probably as a result of infiltration of PMN into the epidermis. Keratinocytes may thus protect themselves and other skin components against the tissue damage caused by PMNs, resulting in the formation of a subcorneal microabscess.

This work was supported in part by a grant from the Ministry of Health and Welfare of Japan.

REFERENCES