Secretory leukocyte protease inhibitor (SLPI) is a small, cationic protein that is known to be constitutively expressed by several glandular epithelia. SLPI inhibits leukocyte-derived proteinases, has anti-HIV-1, antibacterial, and anti-fungal properties, and interferes with the induction of synthesis of proinflammatory mediators in monocytes and macrophages. We now report that at both the mRNA and the protein level, SLPI shows inducible expression in a nonglandular epithelium. A weak expression of SLPI was found in the stratum granulosum of adult normal human epidermis; however, in lesional psoriatic epidermis and in migrating keratinocytes of healing wounds, a strong cytoplasmic staining was seen in the suprabasal keratinocytes. Remarkably, in the dermis adjacent to SLPI-expressing keratinocytes, SLPI was found extracellularly associated with elastin fibers, whereas the dermis in normal skin was negative. In cell culture, SLPI was hardly expressed in monolayers of proliferating keratinocytes. Differentiating cultures with a phenotype of normal skin expressed low levels of SLPI, whereas cultures with a regenerative/psoriatic phenotype expressed high levels. Functional studies with recombinant SLPI indicated that its antibacterial spectrum and potency are distinct from other anti-microbial peptides such as lysozyme and defensins. In view of the multiple functions of SLPI and the inducibility, we propose that it acts as an important first line defence mechanism in cutaneous injury. **Key words: epidermis/protease inhibitor.**

Induction of SLPI (ALP/HUSI-I) in Epidermal Keratinocytes


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SLPI activity has been demonstrated in stratum corneum extracts using biochemical techniques, although the exact cellular source of SLPI was not identified (Wiedow et al, 1993). Previously we have shown that SKALP is expressed by human epidermal keratinocytes in vivo and in vitro, and that it was induced in human epidermis during inflammation (Alkenade et al, 1994; van Bergen et al, 1996), thereby providing protection against two of the major PMN serine proteinases (elastase and proteinase-3). Analysis of stratum corneum extracts revealed additional anti-protease activity, distinct from SKALP, and we found that psoriatic scales contained large amounts of anti-microbial activity. We were therefore interested to examine whether human keratinocytes
would express SLPI as a potential source of anti-protease and anti-

bacterial activity. Here we report that SLPI is expressed at low

levels in normal human skin but is grossly upregulated in psoriatic skin and in healing wounds. In cultured keratinocytes, SLPI expression was

highly upregulated by the addition of fetal calf serum, which induces a psoriasiform differentiation. The results indicate that SLPI expression is inducible and could act as a multifunctional protein by protecting

against excessive proteolysis and providing anti-microbial activity. SLPI is inducible and could act as a multifunctional protein by protecting

highly upregulated by the addition of fetal calf serum, which induces

in healing wounds. In cultured keratinocytes, SLPI expression was

bacterial activity. Here we report that SLPI is expressed at low levels

innate defence mechanism.

MATERIALS AND METHODS

Skin biopsies To study wound healing in normal skin, four partial thickness

wounds were made under local anaesthesia on the outside of the upper arm

of nine healthy volunteers at day 0 using a 3 mm punch. The wound depth was

≈1 mm. At days 1, 2, 4, 7, 14, and 60, respectively, standard 4 mm punch

biopsies were taken over the previously made wounds.

As a model for superficial epidemial injury we used the tape-stripping model. Briefly, the stratum corneum of normal human skin was removed by consecutive

applications of adhesive tape for a total of 5 passes. This was performed on the back in six subjects. At 12 h, days 1, 2, 4, 7, and 14, respectively, standard 4 mm punch biopsies were taken from the previously tape-stripped areas. To study normal and psoriatic skin, 4 mm punch biopsies were taken from five individuals. All biopsies were taken after informed consent, with permission of the local Medical Ethics Committee.

Preparation and characterization of the anti-sera Two polyclonal rabbit anti-sera (AS-81 and AS-98) and two mouse monoclonal antibodies (clones 5 and 31) against SLPI, developed in our laboratories were used in this study. Their reactivity was compared with that of two of our polyclonal anti-sera (92–1 and 93–1) against the related protease inhibitor SKALP/elafin. Table I summarizes the specificity and reactivity of all anti-sera used in this study. AS-81 was raised against SLPI purified from sputum as described previously (Krams et al., 1981). AS-98 was raised against recombinant SLPI using a protocol described earlier (Schalkwijk et al., 1991). Monoclonal antibodies against SLPI were raised and characterized as described before (de Water et al., 1986). Both clone 15 and 31 were of the IgG1 class. The specificity of AS-81 has been described before. It was demonstrated that when the polyclonal antibody AS-81 was used for affinity chromatography, only a singly component was bound and eluted from sputum, which was identical to SLPI as determined by amino acid analysis (Krams et al., 1984). The specificity of the monoclonal antibodies was determined by immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), and immunoblotting (de Water et al., 1986). All monoclonal antibodies were of the IgG1 class. For this study, additional experiments were performed to exclude any possible cross-reactivity of the anti-SLPI antibodies with SKALP, and vice versa; however, it needs to be remembered that the homology between SLPI and SKALP is not very high, at least at the protein level. Only one linear epitope of six identical amino acids in the second domain (MLNPNP, one-letter amino acid code) is shared between the two molecules. In Table I we have indicated the reactivity of the antibodies against recombinant SLPI and SKALP/elafin. The anti-serum AS-81 and the monoclonal antibodies clone 15 and 31 show no cross-reactivity with SKALP. The anti-serum AS-98 showed some cross-reactivity with SKALP. The polyclonal anti-sera against SLPI and clone 31 gave similar staining patterns in immunohistochemistry, which were quite distinct from the anti-SKALP anti-sera (see Table I). To further check the SLPI specificity of the polyclonal anti-serum, recombinant SLPI was coupled to cyanogen bromide-activated Sepharose 4B according to standard protocols. The reactivity of the anti-SLPI anti-serum in the immunochinostchemical staining could be totally abolished by pretreatment of the serum on the SLPI affinity column. When the anti-SLPI serum was absorbed on a sepharose 4B column coupled with recombinant SKALP, no change in reactivity of the anti-serum was noted.

Immunohistochemical staining were performed using theVectastain ABC kits

for monoclonal and polyclonal antibodies purchased from Vector Laboratories (Burlingame, CA). Recombinant SLPI/ALP was kindly donated by Dr. R.C. Thompson (Synergen, Boulder, CO) and Dr. G. Steffens (Grünenthal GmbH, Aachen, Germany). Cyanogen bromide-activated Sepharose 4B was used for coupling of recombinant SLPI/ALP and immunoadsorption, was obtained from Pharmacia (Uppsala, Sweden).

Immunohistochemistry Each biopsy was rinsed and fixed for 4 h in buffered

4% formalin. The fixation time was found to be critical with respect to the preservation of the immunoreactivity of SLPI. Tissues were embedded in paraffin wax, sectioned, deparaffinized, rehydrated, and preincubated with normal swine serum. The sections were subsequently incubated with anti-SLPI antibodies. Polyclonal anti-SLPI AS-81 serum was used at a 1:2000 dilution, AS-98 at a 1:1000 dilution, and the monoclonal antibodies at ≤5 µg per ml. Following this incubation the sections were washed in phosphate-buffered saline and incubated with biotinylated secondary antibody followed by a complex of avidine and biotinylated horse radish peroxidase for 30 min at room temperature. According to the manufacturer’s instructions sections were developed with aminoethylcarbazole as the chromogenic substrate. For control stainings we used nonrelevant monoclonal antibodies of the same isotype, normal rabbit serum, or preimmune serum, which were all negative. The photographs shown in Figs 1 and 3–5 were taken from sections stained with polyclonal serum AS-81, used at a 1:200 dilution.

Keratinocyte culture Primary human epidermal keratinocytes were obtained

according to the Rheinwald-Green system. For the experiments the human keratinocytes were seeded at 105 cells in keratinocyte growth medium (KGM) in 60 mm culture dishes or in 6 well plates as described before (van Russen et al., 1994). For induction of differentiation, at confluence the culture medium was switched to KGM supplemented with 5% fetal calf serum (Alkemade et al., 1994). We have previously shown that treatment with fetal calf serum induces a psoriasiform differentiation of the keratinocytes, which is independent of the Ca2+ concentration (Pfundt et al., 1996; van Russen et al., 1996). Forty-eight hours after the addition of fetal calf serum the cultures were harvested for RNA extraction. The culture supernatants were used for determination of SLPI by ELISA. In some experiments the cells were cultured on coverslips (Thermanox, LAB-TEK Division, Miles Laboratories, Naperville, IL) in 24 well plates for immunocytochemistry.

Northern blot analysis Total RNA was isolated from cultured keratinocytes and frozen keratome biopsies derived from normal healthy epidermis and psoriatic lesional epidermis. The isolation was performed using RNAzol, as suggested by the manufacturer (Cinna/Biotex Laboratories, Hous-ton, TX). RNAzol was added to either harvested and frozen (∼80°C) keratocyte cultures, viable keratinocyte cultures, or frozen keratome biopsies. RNA concentrations were determined from the absorbance at 260 and 280 nm and equal quantities (10 µg) of total RNA were denatured by glyoxal and DMSO, and fractionated on a 1% agarose gel in 10 mM sodium phosphate buffer, pH 7.0. After blotting by capillary transfer on nylon membrane, RNA was fixed to the membrane by ultraviolet irradiation (312 nm, 0.2 J per cm2). Hybridization was performed using 5′-phosphate-labeled DNA probe (Henzel et al., 1986), which was kindly donated by Dr. R. Henzel-Wieland (Grünenthal GmbH, Aachen, Germany). Hybridizations for equal loading were performed using a probe for human acidic ribosomal phosphoprotein P0 (hARP) (Laborda, 1991). All probes were labeled with 32P by random priming following standard procedures. Autoradiography was done on X-Omat S film (Kodak, France) at –80°C with an intensifying screen.

Quantitative determination of SLPI in culture supernatants by ELISA A sandwich type ELISA was used to determine SLPI levels in supernatants of cultured keratinocytes. Briefly, microtitre plates were coated with monoclonal anti-SLPI (clone 31) overnight. After blocking with bovine serum albumin, samples and standards were incubated in serial dilutions. As a second antibody we used a polyclonal rabbit anti-SLPI serum (AS-81). Finally, peroxidase conjugated swine-anti-rabbit IgG (DAKO, Glostrup, Denmark) was used for detection with orthophenylene diamine (Pierce) as the chromogenic substrate. The SLPI concentrations were read from a calibration curve of recombinant SLPI/ALP. Because fetal calf serum and bovine pituitary extract that are present in the culture media could contain bovine SLPI, which is homologous to human SLPI, we checked the cross-reactivity of our anti-sera. In a concentration range of 0.1%–10% fetal calf serum, or in the presence of bovine pituitary extract, no detectable signal in the ELISA SLPI was found.

Anti-bacterial assays The anti-bacterial activity of SLPI was investigated as described before (Hiemstra et al., 1996). Briefly, logarithmic-phase S. aureus

(strain 42D) and P. aeruginosa (strain 9072) (C.C. 27953) were grown in tryptic soy broth. In mid-logarithmic phase bacteria were harvested and washed in 10 mM phosphate buffer (NAPB, pH 7.4) and the concentration was measured by spectrophotometry. Bacteria were resuspended in NABP/1% tryptic soy broth to a final concentration of 106 colony forming units (cfu)/ml just before the start of the assay. Anti-bacterial proteins tested were hen egg white lysozyme (Sigma, St Louis, MO), defensins purified from human sputum [a mixture of human HNP-1, -2, and -3 (Hiemstra et al., 1996)], and recombinant SLPI/ALP. Anti-bacterial proteins were dialyzed against 0.01% acetic acid and vacuum evaporated in glass tubes. One milliliter of mid-logarithmic phase bacteria was added to the tubes and incubated at 37°C. At the start of the experiment (t = 0) and at 2, 4, and 24 h, 50 µl of the suspension was plated on Columbia III agar with 5% sheep blood (Becton Dickinson, Leiden, The Netherlands)
### Table I. Specificity and reactivity of the antisera used

<table>
<thead>
<tr>
<th>Antiserum code</th>
<th>Animal</th>
<th>Immunogen</th>
<th>Cross-reactivity with SLPI</th>
<th>Cross-reactivity with SKALP</th>
<th>Reference</th>
<th>Epidermal staining</th>
<th>Staining of elastic fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyclonal AS-81</td>
<td>rabbit</td>
<td>SLPI purified from sputum</td>
<td>none</td>
<td>Kramps et al, 1981</td>
<td>normal epidermis: stratum granulosum weakly positive</td>
<td>normal epidermis: negative</td>
<td>psoriatic epidermis: strongly positive</td>
</tr>
<tr>
<td>polyclonal AS-98</td>
<td>rabbit</td>
<td>recombinant SLPI</td>
<td>detectable but 100-fold lower than reactivity with SLPI</td>
<td>not available</td>
<td>normal epidermis: stratum granulosum weakly positive</td>
<td>normal epidermis: negative</td>
<td>psoriatic epidermis: strongly positive</td>
</tr>
<tr>
<td>monoclonal clone 15 IgG1</td>
<td>mouse</td>
<td>SLPI purified from sputum</td>
<td>none</td>
<td>de Water et al, 1986</td>
<td>normal epidermis: stratum granulosum weakly positive</td>
<td>normal epidermis: negative</td>
<td>psoriatic epidermis: negative</td>
</tr>
<tr>
<td>monoclonal clone 31 IgG1</td>
<td>mouse</td>
<td>SLPI purified from sputum</td>
<td>none</td>
<td>de Water et al, 1986</td>
<td>normal epidermis: stratum granulosum weakly positive</td>
<td>normal epidermis: negative</td>
<td>psoriatic epidermis: weakly positive</td>
</tr>
<tr>
<td>polyclonal 92-1</td>
<td>rabbit</td>
<td>recombinant SKAL/elafin</td>
<td>none</td>
<td>Schalkwijk et al, 1991; Alkemade et al, 1992; Schalkwijk et al, 1993</td>
<td>normal epidermis: negative</td>
<td>normal epidermis: negative</td>
<td>psoriatic epidermis: strongly positive</td>
</tr>
<tr>
<td>polyclonal 93-1</td>
<td>rabbit</td>
<td>synthetic peptide amino acid 23 to 36 of SKAL/elafin</td>
<td>none</td>
<td>Pfundt et al, 1996</td>
<td>normal epidermis: negative</td>
<td>normal epidermis: negative</td>
<td>psoriatic epidermis: negative</td>
</tr>
</tbody>
</table>
Positive staining is detected on elastic fibers in the papillary and reticular dermis. Keratinocytes of the acanthotic epidermis. The basal cell layer is negative. Positive staining is detected on elastic fibers in the papillary and reticular dermis. Scale bar: 100 µm.

Figure 2. Detection of SLPI mRNA by northern blot analysis. Ten micrograms of total RNA was loaded and probed with cDNA of SLPI or the constitutively expressed hARP gene, which is used to correct for differences in sample loading. Lanes 1–4, normal skin biopsies; lanes 5–8, lesional psoriatic skin. Quantitation of the SLPI mRNA signal relative to the hARP signal, indicated a significant, nearly 3-fold increase in the psoriatic biopsies (0.88 ± 0.16 arbitrary units) compared with normal skin (0.31 ± 0.09 arbitrary units) (p < 0.05, Wilcoxon signed rank test).

Figure 3. Immunoperoxidase staining of normal wound healing. (a) At day 4, the keratinocytes are migrating from the wound edge over the wound bed and express large amounts of SLPI in the cells of the leading edge, and in the suprabasal cells of the neo-epidermis. s, scab; scale bar: 100 µm. (b) At day 14, re-epithelialization is complete. SLPI expression is downregulated compared with day 4, but is still increased compared with normal skin. w, former wound bed; scale bar: 100 µm.

Figure 4. Immunoperoxidase staining of normal skin 48 h after tape-stripping. SLPI is strongly expressed in the stratum granulosum and the upper layers of the stratum spinosum (arrow). Scale bar: 100 µm.

Figure 5. Immunoperoxidase staining for SLPI in cultured keratinocytes. (a) Keratinocytes cultured in KGM. A monolayer of proliferating keratinocytes is present, with only few differentiated cells. No significant staining for SLPI was noted. Nuclear staining is caused by hematoxylin and eosin counterstaining. Scale bar: 50 µm. (b) Keratinocytes cultured in KGM with fetal calf serum that allows the cells to differentiate. Note that the large polygonal cells show positive cytoplasmic staining for SLPI. No counterstaining was used. Scale bar: 50 µm.

RESULTS

SLPI is upregulated in psoriatic epidermis and specifically associates with dermal elastic fibers Immunohistochemical staining of adult normal human skin specimens showed a weak staining of the stratum granulosum with all anti-SLPI sera tested (Fig 1a). The same was found for the nonlesional skin of psoriatic patients (not shown); however, lesional psoriatic skin revealed a subpopulation of epidermal keratinocytes that was strongly positive for SLPI (Fig 1b), both with the polyclonal and with the monoclonal anti-SLPI antibodies. The upper layers of the suprabasal compartment showed a strong cytoplasmic staining in all specimens studied, with minor interpatient variation (n = 5). No staining of basal cells or dendritic cells was observed in the epidermis. In the reticular dermis a marked staining was seen of the coarse elastic fibers (identified as red staining fibers in the Mason staining) that are oriented parallel to the skin surface. In the papillary dermis delicate fibers with an orientation perpendicular to the skin surface were found weakly positive. Staining of the elastic fibers was strong with both polyclonal anti-sera and gave a moderate staining with one of the monoclonal antibodies (clone 31). Non-relevant control sera were negative. Staining of the sections with anti-sera (92–1 and 93–1) against SKALP, a related protein present in suprabasal keratinocytes, were negative for the elastic fibers (not shown). In normal skin, where SLPI is only weakly expressed in the epidermis, the elastic fibers were negative with the anti-SLPI anti-sera (see Fig 1). This strongly suggested that SLPI secreted by the epidermal keratinocytes, specifically associates with the dermal elastic fibers, a phenomenon described earlier in lung tissue (Kramps et al., 1981). When the polyclonal anti-SLPI serum was absorbed on an SLPI affinity column both the epidermal and the elastin fiber staining could be completely abolished (not shown). Table I summarizes the properties of the antibodies used and the staining patterns observed in human skin. The difference in expression levels between normal and psoriatic skin was also examined at the mRNA level. Northern blot analysis of total RNA isolated from biopsy material, as shown in Fig 2, demonstrates that SLPI is highly expressed in psoriatic skin. Quantitation of the northern blot signal relative to the signal obtained by the reference probe for hARP showed a 3-fold increase in the psoriatic biopsies.
SLPI is upregulated following skin injury  Wound healing in normal human skin was studied in excisional wounds on the upper arm of healthy volunteers. At day 1, a distinct expression of SLPI was seen in the epidermis adjacent to the wound bed, mainly in the stratum granulosum and upper layer of the stratum spinosum (Fig. 3a). From day 4 to day 7, strong SLPI expression was seen in cells of the suprabasal layers of the migrating sheet of epidermal cells and in the original wound edge (Fig 3a). Two weeks after injury, SLPI expression was again restricted to the stratum granulosum, but was still increased compared with normal skin (Fig 3b).

Tape stripping of normal human skin was used as a model for transient superficial epidermal injury, which disrupts the barrier function but leaves the dermo–epidermal architecture intact. In this model, epidermal hyperproliferation is induced and a moderate amount of PMN is found, with a peak influx at 12–16 h. As early as 24 h after tape-stripping, positive keratinocytes were found in the stratum spinosum, whereas in normal skin SLPI is restricted to the stratum granulosum. SLPI expression was highest at 48 h (Fig 4) and was rapidly downregulated to undetectable levels at day 7 and thereafter.

Induction of SLPI expression in cultured keratinocytes  Culture systems for human keratinocytes have provided useful models to study cellular control of epidermal growth and differentiation in vitro. Here we have used a previously described keratinocyte culture model that allows induction of normal and psoriasiform differentiation (Pfändt et al, 1996; van Ruissen et al, 1996). When human epidermal keratinocytes are cultured in KGM, a confluent monolayer of cells is obtained with only a few differentiated cells as assessed with staining for involucrin and transglutaminase (van Ruissen et al, 1996). When these cells are immunostained for SLPI, very few positive cells were found (Fig 5a). Addition of fetal calf serum induces a psoriasiform differentiation within 48 h, and the large suprabasal polygonal cells are positive for SLPI as determined by immunocytochemistry (Fig 5b). Under these culture conditions, the expression of involucrin and transglutaminase, which are associated with normal differentiation, is induced (not shown). With northern blot analysis a strong signal for SLPI was found in the cultures stimulated with fetal calf serum (Fig 6).

This finding was confirmed at the protein level as determined by ELISA. Quantitation of the concentration of SLPI in the supernatant of confluent cultures, indicated a significant, nearly 3.5-fold increase after a 48 h period in the presence of fetal calf serum (645 ± 167 ng per ml) compared with KGM (179 ± 52 ng per ml) (p < 0.0006, Student's t test).

Anti-bacterial assays  SLPI is a well-established proteinase inhibitor for at least two PMN-derived proteinases, and speculatively this would be a major function of the molecule in the acute phase of inflammation. Recently, several other biologic properties of SLPI were discovered, such as anti-viral, anti-bacterial, and LPS-modulating effects. Because the early induction and secretion during wound healing could be relevant for the control of skin infection in this critical phase, we have extended our previous studies (Hienstra et al, 1996) that showed that SLPI effectively inhibits growth of bacteria such as E. coli. Here we have investigated the effect of SLPI on P. aeruginosa, which is a relevant skin pathogen, and we have compared it with the anti-bacterial action of other anti-microbial proteins. Figure 7 shows that P. aeruginosa is killed by SLPI. The potency of SLPI is similar to lysozyme, which was taken as a positive control. SLPI showed a spectrum of activity that was distinct from other known anti-bacterial proteins. Defensins isolated from human neutrophils were very effective in killing S. aureus (90% killing at 3 µM) compared with SLPI (90% killing at 15 µM, data not shown); however, defensins did not show any appreciable killing of P. aeruginosa even at doses of 25 µM (see Fig 7).

DISCUSSION  The normal phenotype of human epidermis includes the expression of cytokeratins 1 and 10, a restricted expression of transglutaminase and involucrin in the stratum granulosum, basal integrin expression, and the absence of cytokeratin 16 and SKALP (for a review see Eckert et al, 1997). Under inflammatory conditions such as psoriasis and injury, the epidermal keratinocytes are hyperproliferative and follow a distinct differentiation pathway (regenerative maturation) characterized by the induction of cytokeratins 6/16 and SKALP expression, the presence of transglutaminase and involucrin in the stratum spinosum, and suprabasal integrin expression (Mansbridge and Knapp, 1987; Michel and Demarchez, 1988; Schalkwijk et al, 1993; Hertle et al, 1997). Here we present evidence that SLPI is also induced under these conditions, suggesting that the regenerative maturation pathway, as seen in wound healing and psoriasis, is basically associated with an anti-inflammatory and anti-microbial response of the epidermis.

PMN-derived neutral proteinases can degrade a broad range of extracellular matrix proteins including elastin, proteoglycans, and basal membrane constituents (Schrijver et al, 1989; Janoff, 1985), and have been implicated in various pathologic processes (Malech and Gallin, 1987). In vitro studies have provided evidence that during PMN migration in connective tissue matrices, elastase is involved in degradation of connective tissue components such as elastin and fibronectin (Rice and Weiss, 1990). In healing wounds, the neo-epidermis is migrating in and over the wound bed, which is filled with PMN and debris. Expression and secretion of SLPI and SKALP would provide protection against the three major neutral serine proteinases from PMN. The inhibitory spectrum of these two molecules is overlapping (both inhibit PMN elastase) and complementary (inhibition of proteinase 3 and cathepsin G by SKALP and SLPI, respectively). Interes-
Vol. 111, No. 6 December 1998 Slpi in Human Epidermis

observed that the spectrum of anti-bacterial activity of SLPI was distinct, which are two potential skin pathogens. In this study we observed that SLPI secreted by epidermal cells associates with dermal elastic fibers. Via this mechanism SLPI is targeted to the dermal compartment and protects these fibers against PMN-dependent proteolysis. The association of SLPI with elastic fibers could easily be demonstrated with a polyclonal serum. Of the monoclonal antibodies only one (clone 31) was positive and the other was negative. Presumably the physical association of SLPI with the elastic fibers shields the epitope from binding with the antibodies. Additional proof for the specificity is the finding that in normal skin the elastic fibers are virtually negative; furthermore, immunohistochemical staining of the elastin fibers was completely abolished by immunosorption of the anti-serum. The association of SLPI with elastic fibers has previously been discovered in lung tissue (Kramps et al., 1989), and it was hypothesized that SLPI binds via electrostatic interactions by virtue of its positive charge, as it was found for other cationic proteins as well.

In addition to its anti-proteinase activity, SLPI was recently shown to have anti-HIV-1 and anti-bacterial properties. In normal skin the rigid structure of the stratum corneum provides sufficient protection against microbial invasion. After injury, however, the epidermal barrier function is destroyed whereas growth and invasion of microorganisms is favored. Teleologically it makes sense that the adjacent epidermis and the neo-epidermis are induced to express anti-microbial proteins. A number of anti-bacterial proteins have been described in various mammalian epithelia, including members of the defensin family (Martin et al., 1995). The expression of two anti-bacterial proteins has recently been shown to be induced in keratinocytes in inflamed skin. An epidermal β-defensin, named hBD-2, was isolated from porcine skin and its expression was found to be induced by microorganisms and proinflammatory cytokines in keratinocytes in vitro (Harder et al., 1997).

In addition, the human cathelicidin LL-37 was found to be expressed in keratinocytes in inflamed skin (Frohm et al., 1997). We now report that SLPI is an inducible, anti-bacterial protein in human keratinocytes, and we found that it effectively inhibits growth of P. aeruginosa and S. aureus, which are two potential skin pathogens. In this study we observed that the spectrum of anti-bacterial activity of SLPI was distinct from that of defensins. Here we used a defensin preparation that was a mixture of human defensins HNP-1, 2, and 3. Although in another study this preparation was found to inhibit growth of P. aeruginosa, our defensin preparation was not effective against P. aeruginosa. A possibility for this apparent lack of activity might be a difference in the bacterial strain we used; however, our defensin preparation was active against S. aureus, which was used as a positive control.

The physiologic mechanisms controlling SLPI expression are not completely understood, and have been studied only in airway epithelial cells. In vitro, SLPI expression is increased in airway epithelial cell lines by a variety of substances, including phorbol ester (Maruyama et al., 1994), the (pro)inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (Sallenave et al., 1994), PMN elastase (Abbinante Nissen et al., 1993; Sallenave et al., 1994), and steroids (Abbinante Nissen et al., 1995). Here we have found that SLPI expression in skin is stimulated by osmotic stress (tape-stripping) and inflammation (psoriasis, wound healing), and in vitro by fetal calf serum. Further studies are required to define the mediators (cytokines, growth factors) that are involved in the induction of SLPI expression in keratinocytes.

In view of its multiple functional properties and the inducibility, we hypothesize that SLPI plays a role in a cutaneous host defense mechanism, e.g., as part of an epidermal stress response, providing anti-microbial activity and an anti-proteinase shield against proteolysis of dermal elastic fibers and structural epidermal proteins.

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