Lesional Elastase Activity in Psoriasis, Contact Dermatitis, and Atopic Dermatitis

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Human leukocyte elastase (HLE) is a broad spectrum serine protease derived from neutrophils and macrophages. We developed an assay to determine HLE activity on the skin surface in patients with inflammatory skin diseases. HLE activity was absent in the skin of healthy controls. A massive increase of HLE activity was found in lesional skin of psoriasis (31 times), allergic contact dermatitis (55 times), and atopic dermatitis (35 times), but not in uninvolved skin of diseased patients. Therefore, this assay appears to represent a useful biochemical marker of epidermal inflammation. The presence of proteolytically active HLE in diseased epidermis, which is known to contain specific inhibitors of this enzyme, suggests a pathophysiologic role of this enzymatic activity in psoriasis, contact dermatitis, and atopic dermatitis. J Invest Dermatol 99:306–309, 1992

In contact dermatitis, atopic dermatitis, and psoriasis, disturbance of epidermal cohesion results in the formation of spongiosic vesicles (dermatitis) or focal spongiosis and microabscesses (psoriasis). In both diseases keratinocyte dissociation takes place in the presence of leukocytes. Mechanisms leading to the formation of these disease hallmarks are not well understood. Whereas lymphocytes and macrophages are found in contact dermatitis and atopic dermatitis, polymorphonuclear leukocytes (PMN) and macrophages are the predominating cells within psoriatic spongiform pustules and microabscesses (references given in [1]). Whereas loss of intercellular contact of keratinocytes could suggest proteolysis of intercellular adhesive proteins (e.g., linked to desmosomes), the enzyme(s) involved in these tissue alterations are not known.

Human PMN are known to contain abundant amounts of human leukocyte elastase (HLE), a serine protease located within azurophilic granules [2]. This enzyme acts as a powerful proinflammatory agent, not only by causing degradation of a broad spectrum of matrix components [2–4], but also by its ability to generate chemotactic factors and to cleave antithrombin III (for review see [5]). Next to neutrophils as the primary source for HLE, freshly isolated monocytes and U937 monocyte-like cells have been shown to secrete HLE [6,7]. As a further source, human mast cells have been observed to release HLE when specifically stimulated [8].

In this report we describe an in vivo assay that allows quantitative determination of HLE activity on the surface of diseased human skin. The results obtained with this assay demonstrate excess HLE activities in psoriasis and atopic dermatitis as well as in allergic contact dermatitis. Our observations point toward a protease-antiprotease imbalance that could be related to proteolytic epidermal cell dissociation seen in these conditions, similar to the well-established concept of a protease-antiprotease imbalance leading to pulmonary emphysema [9].

MATERIALS AND METHODS

Patients We investigated 34 patients suffering from chronic plaque-type psoriasis. Their mean age was 44 years, and the average body involvement approximately 15%. None of the patients had received specific treatment for at least 14 d prior to our investigations. The control group consisted of 51 healthy volunteers from the hospital staff. The contact dermatitis group consisted of 14 patients with positive patch tests against nickel sulphate (n = 7) or formaldehyde (n = 7). The diagnosis of atopic dermatitis in 23 patients was based on a family history of atopy, typical skin involvement, and the presence of allergic rhinitis or allergic bronchial asthma.

Sampling Before sampling, the lesions of the patients were cleaned with tap water to remove water-soluble components. For each patient, two filter papers (3 × 3 cm, Schleicher und Schüll, FRG) were placed on the affected skin surface, soaked with 200 μl 1 M sodium chloride, and covered with a polyethylene foil. After 10 min, the filter papers were removed and placed in a vial for storage at −30°C for up to 4 weeks. On average, 90% of the applied sodium chloride solution could be recovered. The storage regime did not diminish the enzymatic activity.

Determination of HLE Solubility To determine the optimal sodium chloride concentration for elution of HLE activity from psoriatic skin, the digits of a patient with both hands totally covered by psoriasis were eluted with sodium chloride solutions of increasing concentration. Each finger was separately eluted by placing it in a vial filled with the sodium chloride solution for 10 min (10 fingers, five different concentrations) and subsequently the eluted HLE activity was determined using 100 μl eluate and 900 μl substrate solution as described below. The total volume of the recovered sodium chloride solution and the total eluted skin surface (calculated as the surface of a cylinder using the length and diameter of

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Abbreviations:
EDTA: ethylenediamine tetraacetic acid
ELISA: enzyme-linked immunosorbent assay
HEPES: n-[hydroxyethyl]piperazine-N-[2-ethane sulfonic acid]
HLE: human leukocyte elastase
MeO-Suc-Ala-Ala-Pro-Val-NA: methoxy-succinyl-alanyl-alanyl-prolyl-valine-p-nitroanilide
PMSF: phenylmethylsulfonyl fluoride
PMN: polymorphonuclear leukocytes

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each finger) were estimated for standardization of the HLE activity per area (cm²) of eluted skin.

**Determination of Lesional HLE Activity** For the determination of HLE activity, 1.8 ml of a specific substrate solution was added to each vial containing the filters. The substrate was freshly prepared by dilution of a 0.1 M stock solution of methoxy-succinyl-4-allyl-4-alkyl-prolyl-valine-p-nitroanilide (MeO-Suc-Ala-Ala-Pro-Val-NA, Sigma) in Me₂SO to a 0.5 mM solution in 0.1 M (HEPES) 0.5 M NaCl, pH 7.5. The substrate solution showed no detectable spontaneous hydrolysis within 48 h. The release of p-nitroaniline was determined after 10, 100, or 1000 min depending on the activity that could be observed by the development of a visible yellow color. Released p-nitroaniline was spectrophotometrically quantitated at 405 nm in comparison with a substrate control. The molar extinction coefficient of p-nitroaniline was found to be 10,690 1 X mol⁻¹ under these assay conditions. Below an absorbance of 2.0 the reaction was linear up to 48 h, as proved by the use of purified HLE (Elastin Products Corporation, Pacific, Mo, USA). Enzymatic activity was standardized to mU/cm² eluted skin, where 1 U releases 1 µmol p-nitroaniline per min.

**Inhibitor Profiles** To establish the inhibitor profiles of HLE-like activities derived from psoriatic lesions, we produced skin eluates from the whole body except the head by wrapping psoriatic patients in a watertight polyethylene foil filled with 21.25% sodium chloride solution. The patients were then placed in a bath filled with tap water to allow the counter-pressure to moisten the whole-body surface [10]. After a 10-min bath the eluates were recovered and concentrated by Amicon YM5 ultrafiltration. The concentrated eluates were centrifuged for 30 min at 6000 X g, filtered (5 µm), and then dialyzed against 0.1 M HEPES, 0.5 M NaCl, pH 7.5. Aliquots (100 µl) and purified HLE of comparable activity (Elastin Products Corporation, Pacific, Mo, USA) were incubated with 100 µl of each inhibitor solution for 30 min at 21°C before adding 800 µl substrate solution (0.5 mM MeO-Suc-Ala-Ala-Pro-Val-NA in 0.1 M HEPES, 0.5 M NaCl, 10% Me₂SO, pH 7.5). All inhibitors were purchased from Sigma, except recombinant eglin C (kindly provided by Dr. Schnebli, Basel, Switzerland) and elafin, which was prepared as described previously [11]. After 30 min incubation, released p-nitroaniline was determined spectrophotometrically at 405 nm versus enzyme-free controls. Inhibition was expressed as percent of inhibitor-free controls.

**Statistical Analysis** Comparisons between patients and healthy controls were made by evaluating the median and the 95% confidence interval of the median, especially because in case of measurements in psoriasis a bell-shaped distribution curve could not be obtained. Statistical significance of differences was determined by the use of the x²-test for medians. Significance was assumed for p < 0.05.

**RESULTS**

Human leukocyte elastase is known to be nearly insoluble in water. The enzyme, however, can be solubilized with concentrated salt solutions [12,13]. To establish the optimal salt concentration necessary for elution of HLE from human skin, test areas symmetrically affected by psoriasis were chosen. Data shown in Fig 1 were obtained from a patient with all digits totally affected by psoriasis. As can be seen, elution of HLE activity from the psoriatic skin surface increases almost linearly from 0.1 to 1.0 M NaCl, whereas with higher concentrations (4.6 M NaCl) no further activity can be eluted.

To ascertain substrate specificity, we determined the inhibition profile of psoriatic skin enzyme activities. This was compared with the inhibition profile of native HLE (Table I). As can be seen from this table, well-known inhibitors of HLE (e.g., recombinant eglin C, α₁-proteinase inhibitor, soybean trypsin inhibitor, PMSF) blocked the enzymatic activity of the skin eluate as well as HLE in a nearly identical manner. Metalloprotease inhibitors such as ethylenediamine tetracetic acid (EDTA) or 1,10 o-phenanthroline were without inhibitory activity for both enzyme preparations. Aprotinin showed only slight inhibition of the enzymatic activity from psoriatic skin and native HLE activity.

Because HLE represents a highly stable protease and the peptide substrate MeO-Suc-Ala-Ala-Pro-Val-NA shows no spontaneous hydrolysis within 48 h, we were able to demonstrate a linear enzymatic reaction for up to 48 h under optimal assay conditions for purified HLE (Fig 2) and psoriatic eluates (data not shown). Using this assay for ex vivo determination of HLE activity on the skin surface the lower detection limit was found to be 0.3 mU/cm² skin (data not shown).

Results from quadruple HLE activity determinations in eight patients with psoriasis vulgaris are shown in Fig 3. All patients examined revealed increased HLE activities. Interestingly, nearly half of the patients showed only moderately elevated elastase levels (approximately 1 mU/cm² skin, Fig 3), whereas the other half (patients E – H) averaged approximately 30 mU/cm² skin.

Subsequently, HLE activity was determined in 34 unselected psoriasis patients (Fig 4) and showed a 31 times increase compared with healthy volunteers. In uninvolved psoriatic skin, no increased HLE activity could be observed. Furthermore, as with psoriatic patients

![Figure 1. Separate elution of 2 X 5 fingers affected by psoriasis with 0–1 M NaCl. Spectrophotometric determination of eluted HLE activity (MeO-Suc-Ala-Ala-Pro-Val-NA hydrolysis).](image)

**Table I. Inhibition of Elastase Activity in Eluates of Psoriatic Skin Compared with Purified HLE**

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Eluates</th>
<th>HLE</th>
</tr>
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<tbody>
<tr>
<td>1,10 phenanthroline</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>EDTA</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SBTI</td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>Rec. eglin C</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PMSF</td>
<td>74</td>
<td>95</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>36</td>
<td>19</td>
</tr>
<tr>
<td>α₁-proteinase inhibitor</td>
<td>100</td>
<td>99</td>
</tr>
</tbody>
</table>

* Final concentrations of 34 ng HLE/ml and psoriatic skin eluates (activity equivalent to 8.8 ng HLE/ml) were pre-incubated with protease inhibitors, 100 µg/ml for 30 min at 21°C before adding the substrate (Meo-Suc-Ala-Ala-Pro-Val-NA).

* 125 µg/ml protease inhibitors.
the affected skin in atopic patients or in acute contact dermatitis showed significantly increased elastase activities (Fig 5). The average enzyme activities recovered in these three skin conditions were close to 10 mU/cm² skin.

**DISCUSSION**

Human leukocyte elastase is contained within the azurophilic granules of PMN, which are released from activated cells leading to biologically active extracellular HLE [2]. This proteolytic serine proteinase is able to degrade a number of extracellular matrix proteins. Besides elastin, HLE cleaves collagen type III [3] and type IV [4] as well as proteoglycans (for review see [5]). Recently it could be shown that HLE is also able to degrade human keratins [14].

In inflammatory skin diseases, such as psoriasis, atopic dermatitis and allergic contact dermatitis dissociation of keratinocytes takes place, leading to the hallmarks of these diseases such as spongiosis (dermatitis) and spongiform pustules (psoriasis) as well as scaling. The loss of intercellular contacts suggests an involvement of proteases. Therefore, we became interested in the question whether HLE, which seems to be a very good candidate for extracellular matrix proteolysis, is present on the lesional skin surface in enzymatically active form.

We have developed an assay for the determination of HLE activity on the surface of human skin using a newly developed salt extraction method and spectrophotometric quantification of the enzymatic activity. This assay represents a sensitive, specific and simple procedure for the determination of HLE activity on the surface of human skin. The tetrapeptide substrate MeO-Suc-Ala-Ala-Pro-Val-NA is known to react specifically with HLE and not with other neutrophil-derived serine proteases [15,16]. The identity of skin elastase activity with HLE has been further substantiated by a comparable inhibition profile (Table I) and by a HLE-enzyme-linked immunosorbent assay (ELISA) using a polyclonal anti-elastase antibody (data not shown). The unusual stability of both substrate and HLE made it possible to follow a linear enzymatic reaction for up to 48 h with purified HLE (Fig 2) as well as with patients' samples. This resulted in a high sensitivity of this spectrophotometric assay.
which is comparable with the sensitivity of the fluorometric HLE determination reported by Lammers et al [12].

In a clinical study, we revealed highly increased HLE activity on the affected skin surface of patients with psoriasis, contact dermatitis, and atopic dermatitis in contrast to healthy controls as well as the unaffected skin of these patients.

In psoriasis, HLE activity was recovered from lesional skin surface in a 31 times excess as compared with healthy control skin (Fig 4). The highest activity obtained from psoriatic lesions was approximately 10,000 times the normal value. HLE activity determinations of separate lesions from the same patient varied within one order of magnitude (Fig 3), which seems to reflect a variable distribution pattern of inflammatory foci within psoriatic lesions. Earlier investigations conducted in our laboratory showed that spot-by-spot clution of psoriatic plaques (approximately 40 mm²) resulted in highly variable enzyme activities within one plaque [10].

In patients suffering from allergic contact dermatitis and atopic dermatitis, HLE-like activities on the surface of affected skin were 55 and 35 times elevated as compared with healthy skin (Fig 5). Because neutrophils do not play a major role in these conditions, other sources of increased HLE-like activities need to be considered.

In a recent report Campbell et al [7] have demonstrated rapid release of HLE from human monocytes. However, the contents of elastase present in these cells amounted to 6.2% of the enzyme content present in a comparable number of PMN. Although great numbers of macrophages are seen in skin of allergic contact dermatitis and atopic dermatitis, the comparatively small amount of HLE contained in these cells as compared with PMN make it difficult to explain the high HLE-like activity detected in these skin conditions. Therefore, other sources need to be considered. It is possible that HLE derived from mast cells [8] may contribute to the enzymatic activity on the skin surface. However, the presence of HLE in mast cells is not unanimously accepted [17].

In contrast to affected skin, no significant amounts of elastase were detected in uninvolved psoriatic skin or healthy skin from control individuals (Fig 4). As in psoriasis, non-affected sites in patients suffering from contact dermatitis and atopic dermatitis revealed no increase in elastase activities (data not shown). These findings suggest that in lesional skin the proteolytic activity of elastase escaped the control of known tissue inhibitors of HLE, e.g., α1-proteinase inhibitor [18], antileukoprotease [18,19], or elastin [19–21].

In consequence, it appears likely that due enzyme activities detected in lesional skin could be related to epidermal damage (e.g., spongiosis, formation of vesicles and microabscesses) known to be present in the conditions studied. Studies by Dubertret et al [22] revealed that, in contrast to healthy individuals, psoriatic biopsies without fixation undergo marked epidermal proteolytic degradation with time. Determination of the inhibitor profile showed that these proteolytic changes were caused by a serine protease similar to HLE. On the other hand, by incubating freshly prepared human skin strips with purified HLE, Briggaman et al [23] observed that this enzyme is able to degrade the basement membranes only. These results, although they may appear contradictory, do not exclude the possibility that under such experimental conditions intracellular inhibitors of HLE (e.g., elastin) may be released into the epidermal extracellular space, thereby preventing epidermal proteolysis.

In addition, the HLE assay described here uses proteolytic activity to quantify epidermal inflammation irrespective of its pathophysiologic nature. The assay may be helpful to quantitate epidermal inflammation with the further advantage that it can easily be applied without harming the patient.

REFERENCES


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