Elastase-inhibiting Activity in Scaling Skin Disorders

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Elastase inhibiting activity (EIA) has been observed in normal skin as a response to surface trauma, immediately following the intra-epidermal accumulation of polymorphonuclear leukocytes (PMN). In order to elucidate the relation between EIA and inflammation, the inhibiting activity was assessed in skin samples of scaling dermatoses (*a*) without significant inflammation: erythrodermic autosomal recessive lamellar ichthyosis (EARLI), non-erythrodermic autosomal recessive lamellar ichthyosis (NEARLI), X-linked recessive ichthyosis (XLRI) and X-linked dominant chondrodysplasia punctata (XLD-CDP); (*b*) with predominantly mononuclear cell infiltration: atopic dermatitis; (*c*) with mixed infiltration of PMN and mononuclear cells: psoriasis and Netherton syndrome. All skin disorders investigated showed an increased EIA as compared with normal skin. Scales from psoriatic lesions, EARLI and Netherton syndrome showed a statistically significant increase in EIA above that observed in other monogenic disorders of keratinization NEARLI, XLRI XLD-CDP and above atopic dermatitis. EIA proved to be an indicator for abnormal keratinization with a marked expression when a mixed infiltrate is present in the skin. *Key words: Polymorphonuclear leukocytes; Scaling dermatoses.*

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Human polymorphonuclear leukocytes (PMN) contain the proteolytic enzyme elastase, which is unique and specific for these cells (1, 2). Briggaman and co-

workers suggested that elastase might disrupt the dermo-epidermal junction in order to permit the penetration of PMN into the epidermis (3).

Inhibiting activity directed against elastase has been demonstrated in the epidermis of normal skin immediately after the transepidermal migration of PMN induced by surface trauma (4). Therefore it is attractive to hypothesize that the presence of elastase-inhibiting activity (EIA) might be indicative of an acute infiltrate with PMN.

The aim of the present study was to elucidate further the role of EIA in inflammation in a variety of scaling skin disorders. The spectrum of diseases included disorders of keratinization without significant inflammation, scaling skin disorders with a predominantly mononuclear infiltrate, and disorders of keratinization with a mixed infiltrate of mononuclear cells and PMN. EIA was quantified in scales and biopsies of lesional skin.

PATIENTS AND METHODS

Patients

A total of 34 patients with a variety of keratinization disorders were included in the study. The monogenic disorders of keratinization comprised: erythrodermic autosomal recessive lamellar ichthyosis (EARLI, $n=5$), non-erythrodermic autosomal recessive lamellar ichthyosis (NEARLI, $n=6$), X-linked recessive ichthyosis (XLRI, $n=4$), Netherton syndrome ($n=3$), and X-linked dominant chondrodysplasia punctata (XLD-CDP, $n=3$). For the purpose of comparison, the following polygenic disorders characterized by inflammation and scaling were included: psoriasis ($n=6$) and atopic dermatitis ($n=7$).

Diagnosis was established by clinical and histological criteria. Patients had not used any therapy for at least 1–2 weeks before collecting specimens. As controls, 18 healthy volunteers participated in the study.

Sampling procedure

Scales were collected from the clinically involved skin of all patients. The scales were taken from various parts of the body, with the exception of the palms, the soles and the head. In 6 healthy controls, normal stratum corneum was collected from the back by gentle scraping with a scalpel blade. From 6 other healthy volunteers, scrapings of normal callus were obtained from the feet. Bleeding was avoided during collection of scales and scrapings.

Keratotome biopsies (Castroviejo) were taken from the clinically involved skin of 6 psoriatic patients and in 3 of them from the symptomless skin at least 20 cm from the adjacent lesion. In 6 healthy controls, keratotome biopsies were taken from the skin on the back. For biopsies from lesional skin the setting for depth was 0.3 mm and for the non-lesional skin 0.2 mm.

Scales and biopsies were stored at −20°C prior to analysis.

Analytical procedures

Scales and biopsies were processed for determination of EIA as described previously (4, 5). In brief, biopsies were rinsed in phosphate-buffered saline, and all samples were homogenized at appropriate concentrations in buffer containing cetrimide (0.3% cetrimide, 0.1 M Tris, 1 M NaCl; pH 8.5) and centrifuged (15 min, 35000 *g*). Serial dilutions were made from the clear supernatant and a standard preparation of elastase (1 ng enzyme, equivalent to 500 PMN) was added.

Elastase activity was measured as the release of the fluorescing product (4-methyl-7-aminocoumarin) from the fluorogenic substrate MeOSuc-Ala-Ala-Pro-Val-N-methyl-coumarin.

Inhibition of the elastase preparation was determined for every sample dilution with the following formula:

$$\text{Percentage inhibition} = \left(1 - \frac{E_{\text{sample}}}{E_{\text{max}}}\right) \times 100$$

where E_{sample} = elastase activity with sample
E_{max} = elastase activity without sample

EIA is expressed as the weight of the sample (μg) required for 50% inhibition of the standard elastase preparation under the experimental conditions described.

Statistical analysis

Statistical evaluation was carried out using the Wilcoxon ranking test for two independent samples.

RESULTS

The EIA of biopsies from lesional and non-lesional psoriatic skin are given in Fig. 1. A highly significant increase in EIA was observed for the psoriatic lesions compared with the skin of normal volunteers ($p<0.005$). EIA in clinically uninvolved skin of psoriatic patients was in the same range as for healthy volunteers.

The EIA of scales from disorders of keratinization are given in Fig. 2. EIA in scales of psoriatic lesions was higher than that in stratum corneum from normal volunteers ($p<0.005$). Compared with the inhibition in biopsies, scales from psoriatic lesions showed an even greater EIA. Plantar callus also showed an increased EIA compared with the activity in normal stratum corneum from healthy volunteers ($p<0.005$), though the inhibition was less than that observed for psoriatic scales. Scales obtained from monogenic disorders of keratinization without inflammation, and from atopic dermatitis, were characterized by EIA of the same order of magnitude as normal callus. In contrast, two monogenic disorders of keratinization (Netherton syndrome and EARLI) with inflammatory changes showed pronounced EIA in same range as scales from psoriatic lesions.

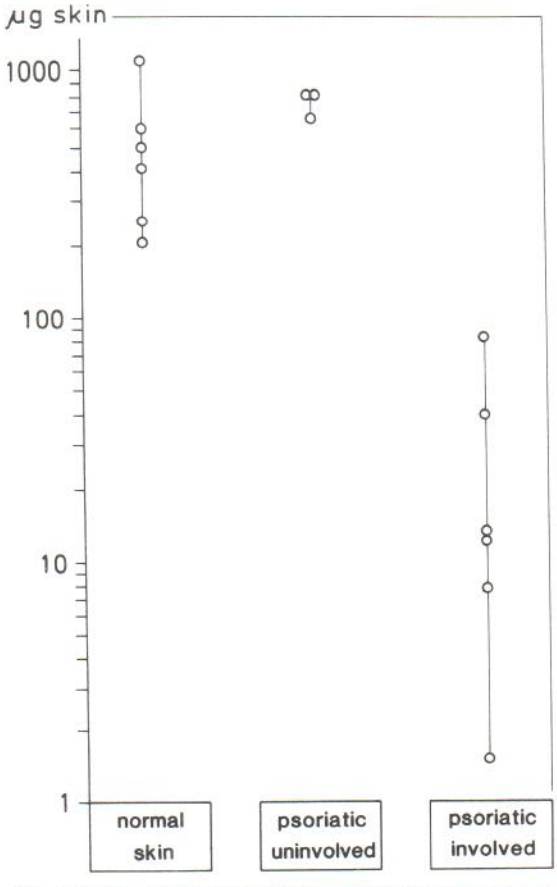

Fig. 1. Individual elastase inhibiting activity in biopsies from psoriatic and normal skin. Inhibiting activity is expressed as the weight of the skin (µg) required for 50% inhibition of the standard elastase preparation; thus the lower the point, the greater the inhibiting activity.

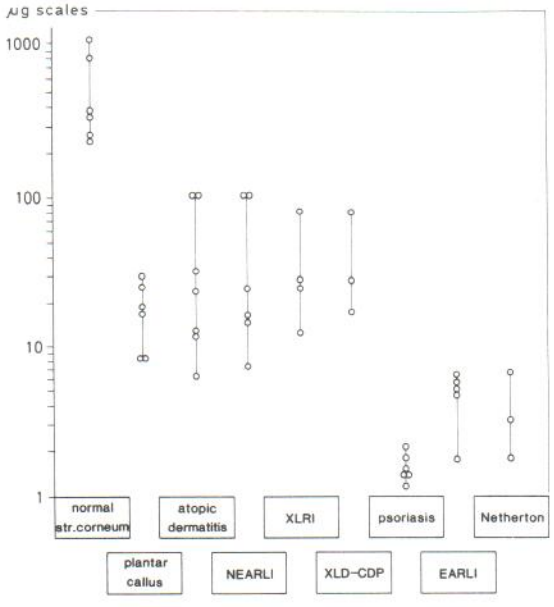

Fig. 2. Individual elastase inhibiting activity in scrapings of normal skin and callus and scales of several dermatoses. Units as in Fig. 1. XLRI = X-linked recessive ichthyosis; NEARLI = non-erythrodermic autosomal recessive lamellar ichthyosis; XLD-CDP = X-linked dominant chondrodysplasia punctata; EARLI = erythrodermic autosomal recessive lamellar ichthyosis.

DISCUSSION

The present study demonstrates that scales of various scaling skin disorders display some EIA, whereas pronounced EIA occurs in inflammatory conditions. EIA in scales from psoriatic lesions was compared with EIA of keratotome biopsies from the lesions. It is of importance that EIA in the scales was even more marked than EIA in the biopsies, indicating that the topographical distribution of EIA is not limited to the dermis or viable layers of the epidermis, but persists in the horny layer. The preservation of EIA in the horny layer may be explained by its remarkable stability, one of the reported characteristics of EIA in psoriasis (6).

A slight but statistically significant inhibition was measured in scales of patients with some monogenic disorders of keratinization: XLRI, XLD-CDP and NEARLI. In these disorders the abnormalities in the epidermis are not accompanied by a significant inflammatory infiltrate (7). The magnitude of EIA was in the same range as EIA of normal plantar callus. Therefore, the induction of slight EIA might be an aspecific event in abnormal keratinization.

Scales of chronic eczematous lesions of patients with atopic dermatitis also showed a moderate EIA, of the same order of magnitude as scales from the non-inflammatory disorders of keratinization. The inflammatory infiltrate in uncomplicated atopic dermatitis is mononuclear with an admixture of some eosinophils (8). This sort of infiltrate was not accompanied by an induction of EIA above the range already reached by abnormal keratinization.

However, scales of lesional skin of patients with psoriasis, scales of patients with EARLI and scales of patients with Netherton syndrome showed a pronounced EIA. In psoriasis, intra-epidermal accumulation of PMN is classical feature in the early and active phases of the disease (9). In the central zone of chronic, stable lesions, intra-epidermal accumulation of

PMN occurs in an intermittent cyclic pattern (10, 11). In Netherton syndrome the histological picture shows features similar to psoriasis, with prominent PMN accumulation (12). In this syndrome, capillary dilation is seen with PMN invading the formation of micropustules (13). Therefore infiltrates in which PMN participate seem to be associated with EIA levels well above that found in non-inflamed disorders of keratinization or atopic dermatitis.

Scales from EARLI show a highly significant increase in EIA as compared with its non-inflammatory counterpart, NEARLI. Based on clinical, histological, cell-cycle kinetic and biochemical criteria (*n*-alkanes, lamellar body enzymes) differentiation between these two forms of autosomal recessive lamellar ichthyosis is possible (14, 15, 16). The present observation further substantiates the differences between EARLI and NEARLI from the point of view of inflammation controle. The association between the marked EIA and the composition of the inflammatory infiltrate can only be speculative, as the infiltrate present in EARLI has so far not been studied extensively. Although the histological picture of EARLI shows striking similarities to psoriasis (12), an intra-epidermal accumulation of PMN has not been reported.

The source of EIA is not known. However, the fact that EIA is slightly increased in scales of disorders of keratinization without any infiltrate present, suggests that it is synthesized in the epidermis. The fact that EIA induction does not coincide with, but has a delay with respect to intra-epidermal accumulation of PMN following standardized injury (3), also implies that EIA is not carried into the epidermis by PMN but rather is induced in the epidermis itself.

The biochemical nature of EIA is not known. Preliminary characterization of the elastase inhibiting factor in psoriatic scales showed that we are dealing with a heat-stable, acid- and alkaline-stable substance with a molecular weight of approximately 10,000 Dalton (6). This elastase inhibitor does not inhibit human cathepsin G or bovine trypsin. The inhibition of human leukocyte elastase is stoichiometric and, assuming equimolar kinetics, the concentration of the inhibitor in psoriatic epidermis averages 25/μg/g. These properties suggest that the inhibitor described here is distinct from any previously reported antiproteinase (17, 18, 19).

Further characterization of elastase inhibitors in diseases with intra-epidermal invasion of PMN is in progress. Pharmaceutical compounds with EIA might open up a new approach to treatment of skin diseases in which intra-epidermal invasion of PMN is of significance.

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