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An Immunohistochemical Study of Lysozyme in the Skin of Psoriatic Patients

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The distribution of lysozyme was investigated in psoriatic skin lesions, perilesional skin and in skin from healthy controls, using the immunoperoxidase techniques avidin-biotin complex and alkaline phosphatase-anti alkaline phosphatase. Lysozyme was identified in polymorphonuclear leukocytes present in the Munro microabscesses and also occasionally in other parts of the skin, as shown by very strong cytoplasmic staining. Stratum corneum, stratum granulosum and stratum spinosum were weakly stained. In some cases positive staining along the dermal collagen bundles was demonstrated and is most likely to be related to the number of inflammatory cells in the papillary dermis. Psoriatic skin lesions stained significantly stronger for lysozyme than did perilesional skin ($p < 0.0001$), whereas skin from healthy controls stained weakly positive or was lysozyme negative. Lysozyme may be of some importance in the psoriatic disease process. By comparison the alkaline phosphatase-anti-alkaline phosphatase was found to be the most specific of the two techniques. ***Key words: psoriasis vulgaris; muramidase; polymorphonuclear leukocytes; immunohistochemistry.***

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Intraepidermal (Munro) microabscesses are the most characteristic findings of psoriasis and highly diagnostic. Polymorphonuclear leukocytes (PMNLs) migrate into the epidermis from dilated capillaries in the dermal papillae and may play an important role in psoriasis.

Lysozyme (muramidase) is an enzyme with a low molecular weight (15 kd) synthesized and secreted by the monocytes-macrophage series and some epithelial cells (1). This enzyme is considered to be a constituent of a non-specific defence mechanism, especially against infections, not only through its direct bacteriolytic action but also through its stimulatory effect on PMNLs and macrophages, facilitating phagocytosis (2, 3). Lysozyme can also act synergistically with complement to accelerate complement-mediated lysis of bacteria. Another important function is the stimulation of lymphocyte proliferation (4).

An elevation of serum and salivary lysozyme in myeloproliferative diseases has been reported (5, 6). Markedly increased serum lysozyme levels combined with decreased saliva levels (7, 8) have been found in active plaque psoriasis, whereas healthy individuals have equal lysozyme levels in saliva and skin (9).

The aim of the present study was to localize lysozyme in cells and tissue of psoriatic skin and compare it with that found in the skin of healthy subjects. Since lysozyme is difficult to extract from the skin, we examined the presence of lysozyme using immunohistochemical techniques.

MATERIAL AND METHODS

Formalin-fixed paraffin-embedded skin biopsy specimens from 12 patients suffering from active plaque psoriasis were studied immunohistochemically for the occurrence of lysozyme. A clinical diagnosis of psoriasis was confirmed by histopathological investigation in routine sections stained with haematoxylin and eosin and examined blindly by different pathologists. All specimens were from adult patients, aged 24–51 years (average 38 years), with active skin lesions but without complications such as arthritis. None of the patients studied had received systemic steroids or immunosuppressive drugs during 6 months prior to the study. No other systemic therapy, e.g. photo- or photodermatotherapy or local therapy, had been given during the last 4 weeks. The biopsies were taken freshly from psoriatic lesions and perilesional skin on the abdominal area under local anaesthesia. Biopsy specimens from the abdominal area of 8 healthy subjects with clinically normal skin served as controls.

The tissue sections (5–8 μm) were processed according to standard methods, i.e. dewaxed in xylene and rehydrated through graded alcohols for 30 min. The immunoperoxidase procedure was carried out in accordance with the standard avidin-biotin complex (ABC) technique (10), using a commercial ABC kit (Vector Laboratory, Inc. Burlingame, USA) consisting of a secondary antibody (biotinylated anti-rabbit goat IgG), avidin-biotin conjugated horseradish peroxidase and blocking serum. The primary antibody used was rabbit anti-human lysozyme/muramidase (DAKO, Copenhagen, Denmark), diluted 1:1000. We also prepared a separate set of slides from the specimens using the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method (11), the DAKO APAAP kit system and a mouse anti-rabbit immunoglobulin as a second stage reagent (DAKO, Copenhagen, Denmark). Formalin fixed paraffin-embedded biopsy specimens from intestinal mucosa with Paneth's cells and skin with granulomatous inflammation related to a ruptured epidermal cyst served as positive controls for lysozyme.

The immunohistochemical evaluation was performed by the same investigators throughout the study, and all sections were re-examined blindly. We used a score system to demonstrate the occurrence of lysozyme in cells and tissue where 2 is identical with a strong positive staining, 1 is a moderate positive staining, 0.5 is a weak positive staining and 0 is a negative finding. Statistical significance was calculated using Student's *t*-test.

RESULTS

The results of immunohistochemical staining for lysozyme using the ABC method are summarized in Table I. In psoriatic skin the intensity of the staining varied from one section to another and also between different parts of the skin and was always weaker than the strong staining of PMNLs present in the Munro microabscesses. Occasionally other parts of the skin showed intense brown staining which appeared as granular intracytoplasmic deposits. Stratum corneum showed focally a positive staining and was particularly strong around the Munro microabscesses (Fig. 1). In the granular and spinous layer a moderate to weak positive staining in the cytoplasm of the keratinocytes was detected. Moreover, in some cases a weak immunoreactivity was demonstrated along the dermal collagen bundles.

Perilesional psoriatic skin sections showed a somewhat

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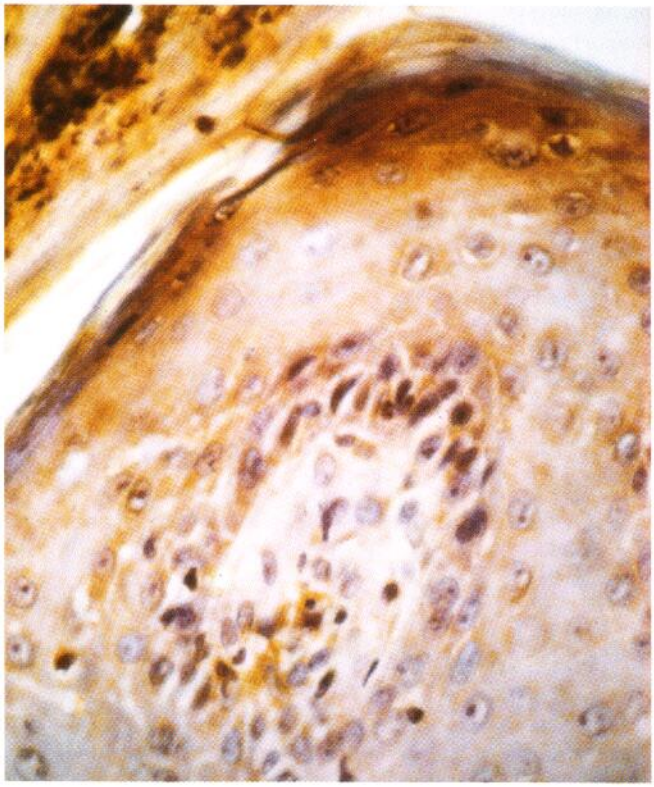

Fig. 1. Section from psoriatic skin showing lysozyme immunoreactivity (brown) in and around a Munro microabscess and in keratinocytes in the underlying epidermis (avidin-biotin-complex method, ×360).

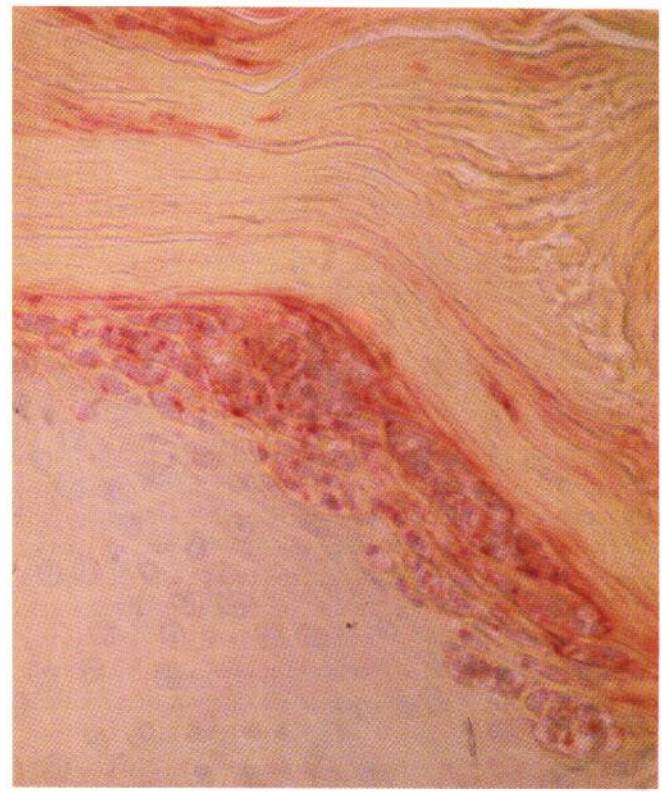

Fig. 2. Section from psoriatic skin showing lysozyme immunoreactivity (red) in both a Kogoj pustule and in a Munro microabscess (alkaline phosphatase-anti-alkaline phosphatase method, ×360).

weaker staining than sections from active skin lesions without, however, reaching statistical significance (Table I).

In specimens from the skin of healthy individuals a weak positive staining was present in keratinocytes of the granular and the spinous layer. The horny layer and cells of the basal layer were lysozyme negative in all specimens, whereas a slight positive staining was present along some of the collagen bundles in the dermis.

When the APAAP method was used, lysozyme stained pink-red and a strong staining was found only in the cytoplasm of PMNLs, whereas a weak positive staining was present in areas around the Munro microabscesses (Fig. 2). In the control group there was no lysozyme staining in any part of the skin when applying the APAAP technique.

DISCUSSION

Several authors have shown the presence of lysozyme in human skin using different methods (12–15). Liss (14) described lysozyme activity in homogenates of psoriatic scales, and Chen et al. (12) suggested that lysozyme is synthesized from human epidermal cells. Lysozyme concentration in the epidermis is three times greater than that of the dermis, and the enzymes account for over 200 μg per g of human epidermis (15). Our findings of

Table I. *Immunostaining of lysozyme in psoriatic lesions, perilesional skin and skin from healthy controls*

Strong positive = 2, moderate positive = 1, weak positive = 0.5, negative = 0.

	Epidermis					Dermis	
	PMNLs	Str. corneum	Str. granulosum	Str. spinosum	Str. basale	P. papill.	P. reticul.
Psoriasis vulg. ($n \times 12$)	2.00[a]	0.25[b]	0.79	0.54	0	0.21	0.21
Perilesional psoriatic skin ($n \times 12$)	0	0	0.67	0.50	0	0.17	0.08
Skin from healthy controls ($n \times 8$)	0	0	0.75	0.25	0	0.19	0.06

[a] $p < 0.0001$.
[b] $p < 0.0069$.

lysozyme in the cytoplasma of keratinocytes are in accordance with the findings of Ogawa (16) who, using the immunofluorescence technique, found lysozyme in the cytoplasm of epidermal keratinocytes. Moreover, stratum spinosum and stratum granulosum expressed a much stronger specific fluorescence for lysozyme than the basal cell layer and the horny layer. In addition, lysozyme was found in the dermis in association with collagen fibres.

When immunohistochemistry was applied to formalin-fixed biopsy specimens from the skin of healthy subjects no lysozyme staining was found in the horny and basal layers. Only stratum spinosum and stratum granulosum showed a weak staining. In the dermis a slight positive staining was related to collagen fibres.

In cases of active plaque psoriasis, PMNLs in the Munro microabscesses and also scattered PMNLs in the dermis showed very strong cytoplasmic staining for lysozyme with both methods (ABC and APAAP). These cells are known to contain lysozyme in either the primary or secondary lysosomal granules (17). This enzyme accounts for 0.5% to 2.5% of the total protein content of the lysosomal granules (18) and is released only during degranulation of the cells. In psoriasis the horny layer was focally lysozyme positive whereas stratum spinosum and stratum granulosum stained weakly for lysozyme. The basal layer was, however, lysozyme negative. In some cases positive staining along the dermal collagen bundles was present, especially in the upper dermis, which is in all likelihood at least partly related to the number of inflammatory cells in the papillary dermis. All in all the staining for lysozyme was found to be significantly stronger in psoriatic skin lesions than in perilesional psoriatic skin and even more so than in skin from healthy controls. This might to some degree be caused by release of the enzyme from the accumulation of PMNLs' in the Munro microabscesses to the surrounding area of stratum corneum. There were, however, no significant differences in the staining intensity in stratum granulosum or stratum spinosum in any of the groups.

When the two techniques were compared, the ABC method was considered to be the most sensitive and, therefore, best at detecting staining in the cytoplasm of keratinocytes. However, a stronger background staining was present when using this technique which, therefore, makes it less specific than the APAAP technique. Thus the staining associated with keratinocytes and dermis could be non-specific and hence give false positive reactions.

These observations suggest that the stronger lysozyme staining in the skin of psoriatic patients could be promoted by chemoattractants (18) or leukotactic factors present in psoriatic epidermis (19).

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