

## Elastolysis in Lichen Ruber Planus

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**The dermal elastic fiber network was studied in specimens from five patients with lichen ruber planus, using a standard elastin staining procedure (orcein), results being compared with those for the elastin-associated microfibrillar network stained using anti-fibrillin antibodies in an immunofluorescence and an avidin-biotin-peroxidase complex technique. Additional specimens of healed apparently normal skin were taken from two of the patients.**

Orcein-stained fibers were scarce or absent in the inflammatory zone in all the lesions. In contrast, an extensive fibrillin immunoreactive network was present in the papillary zone in all the specimens, in a pattern similar to that of normal skin. In specimens from healed lesions of lichen ruber planus, dermal orcein-stained fibers were present in the papillary dermis.

The findings indicate that the amorphous component of elastic fibers is destroyed during the acute phase of lichen ruber planus. Hypothetically, the elastolysis is caused by elastases released from macrophages known to be present in the lichenoid infiltrate. In contrast, the fibrillin fiber network seems to be less or not at all affected by proteolytic events during the inflammatory phase of lichen ruber planus. **Key words:** *Elastin; Fibrillin; Vitronectin.*

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Lichen ruber planus is characterized clinically by pruritic papules and histologically by a band-like cellular infiltrate in the upper dermis with hydropic degeneration of basal keratinocytes and by the presence of keratin bodies, presumably formed from degenerating keratinocytes undergoing apoptosis (1, 2). Hypothetically, this keratinocyte cell death is due to a T lymphocyte-mediated attack (1–3). The cellular infiltrate in lichen planus has been found to consist mainly of T lymphocytes, but the presence of macrophages has also been noted (4–6). Macrophages, which are known to have the potential to produce an elastase (a metalloprotease) that can be inhibited by alfa-2-macroglobuline but not by al-

pha-1-antitrypsin, have been found more frequently in late lesions than in early lesions of lichen ruber planus (6, 7).

Few reports have been published on changes in the extracellular matrix during a lichenoid inflammatory reaction. Fibrosis of the papillary dermis has been reported to occur during the resolving phase of lichen ruber planus (8).

In the present study, the changes of the elastic fiber network were studied during the acute phase of lichen ruber planus, a standard procedure (orcein staining) being used to stain the elastic fibers. The amorphous elastin is associated with 8–12 nm microfibrils, which form an extensive network that can be immunostained using anti-fibrillin antibodies (9, 10). The microfibrils are thought to have the structural function of attaching the elastic fibers to the surrounding matrix and to the lamina densa (10).

Vitronectin immunoreactivity is normally present at the periphery of elastic fibers in adults (11). It is also associated with keratin bodies, and complement-independent binding of vitronectin to keratin intermediate filaments has been demonstrated (12, 13). Consistent with these findings, phagocytosis of apoptotic polymorphous leukocytes was recently shown to be dependent on the vitronectin receptor of macrophages, indicating the involvement of vitronectin as a scavenger protein (14).

A scarcity of vitronectin immunoreactive fibers in the papillary dermis of lichen ruber planus lesions noted in an earlier study of ours, indicating loss of elastic fibers during the lichenoid inflammatory events, prompted the present study, in which the distribution of the elastic fiber network (stained with a standard orcein elastin staining procedure) was compared with that of the fibrillin immunoreactive network in lesions of lichen ruber planus and in healed such lesions.

## MATERIALS AND METHODS

### *Biological tissue*

The specimens were obtained by biopsy of skin lesions from five patients with lichen ruber planus in the acute phase, the diagnoses being based on the clinical picture and histology. The patients were treated with local application



of betamethasone. Additional specimens were taken from two of the patients of apparently normal skin adjacent to the prior biopsy site, approximately 2 months later. IgM immunoreactive globules, so-called keratin bodies, were found adjacent to the dermal epidermal junction in all five specimens from lesions.

#### Fixation procedure

The specimens were immersed in a transport medium (550 g ammonium sulphate added to 1 liter 25 mM potassium citrate, 5 mM N-ethylmaleimide, 5 mM magnesium sulphate) being washed within 48 h in transport medium lacking ammonium sulphate and then immediately frozen in a propane-butane mixture at the temperature of liquid nitrogen. The specimens were stored at  $-70^{\circ}\text{C}$ . Cryostat sections, between 4 and 10  $\mu\text{m}$  thick, were cut and fixed in acetone for 20 min at  $4^{\circ}\text{C}$ , consecutive sections being treated with anti-fibrillin and anti-vitronectin or with standard elastin stain.

#### Immunohistochemical techniques

The specimens were stained using anti-fibrillin and anti-vitronectin in an avidin-biotin-peroxidase complex technique and in a standard immunofluorescence technique (16).

#### Histochemical stains

*Elastin stain:* Orcein staining without preoxidation was used, according to Pranter (17).

#### Proteins and primary antisera

Monoclonal anti-fibrillin was produced by Sakai et al. (9). The working dilution was 1:3000 (avidin-biotin-peroxidase complex technique) or 1:400 (immunofluorescence). Polyclonal anti-vitronectin antibodies (as earlier characterized) were used at a working dilution of 1:10000 (avidin-biotin-peroxidase complex technique) or 1:1000 (immunofluorescence technique) (10, 11).

## RESULTS AND DISCUSSION

A band-like cellular infiltrate was seen in the papillary dermis in the specimens from active lichen ruber planus lesions, and a high amount of keratin bodies were present. These keratin bodies contained immunoreactivity of IgM and vitronectin, but not of fibrillin as described before (12, 15). Only a few orcein-stainable globules, so-called elastic globules, were seen.

Orcein stainable elastic fibers were scarce or absent in the inflammatory zone of papillary dermis and upper reticular dermis in the active lesions (Fig. 1a). There were also very few or no vitronectin immunoreactive fibers in this area (not shown). However, a distinct fibrillin immunoreactive network was present (Fig. 1b). In the healed lesions, orcein-stained fibers were present in the papillary

dermis, mostly in a pattern consistent with the so-called "eulanin fiber plexus", but there were also some fibers that extended perpendicularly towards the dermal epidermal junction (Fig. 2).

The present results demonstrate vast destruction of the normal elastic network in the upper reticular and the papillary dermis in lichen ruber planus lesions, hypothetically caused by macrophage-derived elastases.

The retained fibrillin immunoreactive network suggests that the elastin-associated microfibrils are not affected by the involved proteolytic enzymes, unless there is a fast ongoing resynthesis of fibrillin. Both amorphous elastin and fibrillin are produced by fibroblasts. The microfibrils have been proposed to constitute a framework upon which amorphous elastin is deposited during elastogenesis (18). Hypothetically the regeneration of elastic fibers during the resolution of lichen ruber planus lesions may be promoted by the presence of an intact microfibrillar network in the papillary dermis.

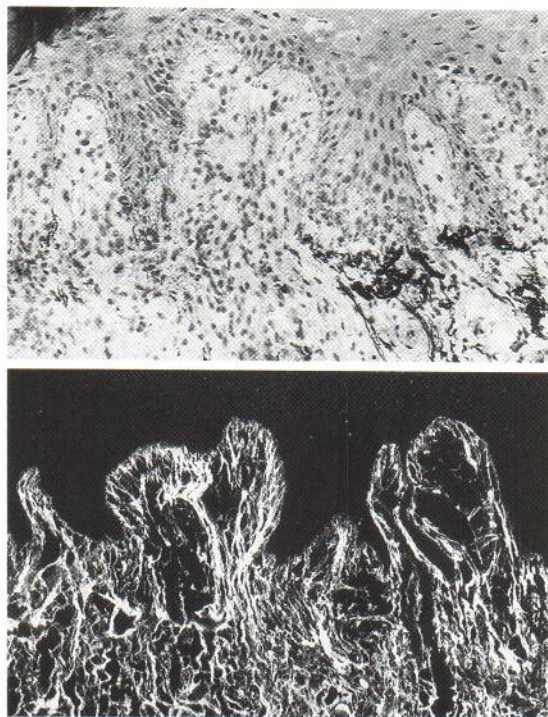


Fig. 1. Consecutive sections from a specimen of an active lichen ruber planus lesion, stained with standard elastin staining procedure (orcein) (a) and with anti-fibrillin in an immunofluorescence technique (b). ( $\times 125$ )





Fig. 2. A section from an apparently healed lichen ruber planus lesion, stained with standard elastin staining procedure (orcein). ( $\times 250$ )

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