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Case Report

Eosinophilic Fasciitis
Ultrastructural Study of an Early Biopsied Case

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Summary. A 55-year-old woman with eosinophilic fasciitis was biopsied 8 weeks after the onset of her illness. Under the electron microscope the changes were almost exclusively located in the fascia with many active fibroblasts, accumulation of protocollagen fibrils (10–50 Å diameter), elastic fibre remodelling and numerous degranulating mast cells. The inflammatory infiltrate was dense and mostly composed of lymphocytes and plasma cells, with 16% eosinophils.

The connective tissue changes may be part of a healing process following microinjury of the fascia. However, large numbers of lymphocytes and plasma cells are unusual in the healing process and are more common in the cellular reaction of morphea. Nevertheless, the absence of macrophages in subcutaneous fat, together with large number of eosinophils in the fascia may be considered to be distinctive features of eosinophilic fasciitis.

Key words: Eosinophilic fasciitis – Ultrastructural study – Healing process – Morphea

Introduction

Eosinophilic fasciitis (E.F.) was described by Shulman in 1974 (18) in two patients with scleroderma-like skin changes in the absence of Raynaud's phenomenon and visceral involvement and with hypergammaglobulinemia and transient blood eosinophilia. The classification of this syndrome among the various types of cutaneous sclerosis is still debated. The only detailed ultrastructural study of this syndrome was carried out by Tamura and al. (1979) on hyalinized subcutaneous tissue obtained 3 and 5 years after the beginning of the cutaneous symptoms.

We report the ultrastructural features of a case biopsied 8 weeks after the onset of the illness. We focused our study on the cellular reaction and the connective tissue changes.

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Case Report

A 55-year-old woman experienced an erythematous swelling of the thighs, legs and arms, on the morning following a day of strenuous physical exertion with pain in the knee and shoulder joints. The swelling and pain disappeared after a month but the skin of the arms and shoulders felt tight. Fixed flexion was present in all the proximal interphalangeal joints, in the wrists and in the right elbow. On examination, the skin of the arms showed it to be bound down, but it was not atrophic. No lilac ring, hair loss or pigmentary change could be observed in this area. Raynaud's phenomenon and systemic symptoms were not present. The white-cell count was 10,300 with 13% eosinophils. Tests for parasitic diseases were negative. The erythrocyte sedimentation rate was 45 mm/h, and the gammaglobulin level was 10.6 g/l. Ig G, Ig A, Ig M were normal, as were the serum aspartate aminotransferase, lactic dehydrogenase, creatine phosphokinase, and aldolase. Tests for rheumatoid factor, antinuclear antibodies and cryoprecipitate were negative. Electromyography of arm and roentgenography of the chest and oesophagus, together with pulmonary function tests, were normal.

A biopsy was therefore performed on the affected area of the arm.

The patient was treated later with 50 mg of prednisolone per day, the dosage was then gradually decreased over the next six months.

No peripheral blood eosinophilia or skin tightness was found one year after the biopsy.

Materials and Methods

Specimens from the skin, subcutaneous fat, fascia and muscle were removed under local anesthesia, and each one was cut into three parts. One part was fixed in calcium-buffered formaldehyde and processed further for optical microscopy, with haematein-eosin, Weigert and Giemsa stains.

Another part was immediately fixed in 2% glutaraldehyde buffered 0.1 M cacodylate for 2 h, dehydrated in ethanol and propyle oxyde series and embedded in T.A.A.B. medium. Fine sections were stained with uranyl acetate-lead citrate and observed under a Hitashi $HU_{12}A$ microscope.

The third part was rapidly frozen in liquid nitrogen and studied by direct immunofluorescence for the presence of Ig A, Ig G, Ig M, Ig E, C_3 and C_4.

Results

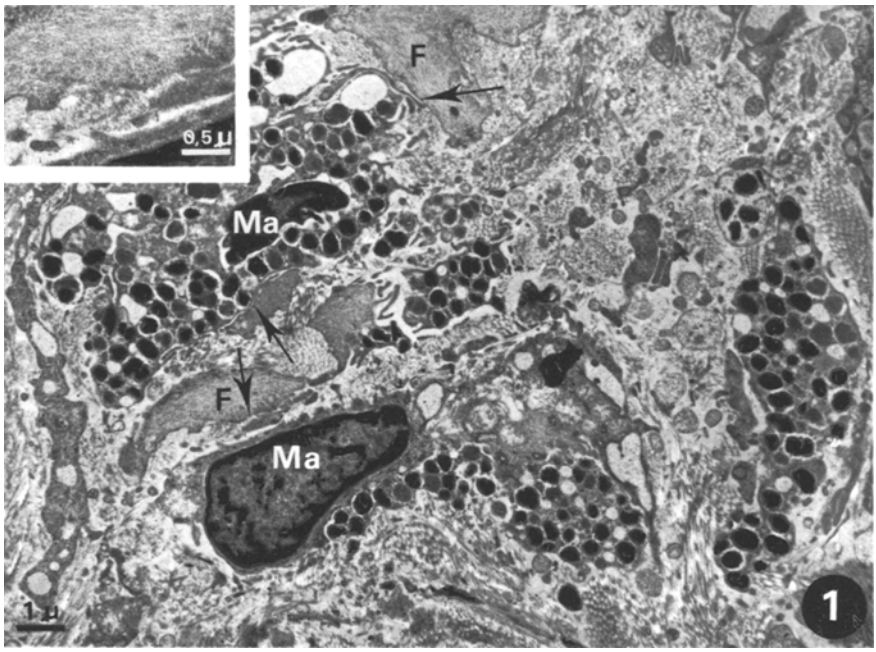
On histological slides, the most striking changes are found in the fascia which is approximately 6 times thicker than a control fascia, with a dense inflammatory infiltrate around the capillaries. The infiltrate is mostly composed of lymphocytes and plasma cells. On the Giemsa stain, mast cells are numerous and many of them appear degranulated. Polymorphonuclear cells with eosinophilic granules are few and scattered around the area of heavy cell infiltration.

The muscle shows mild lymphocytic infiltrates and fibrosis of the epimysium together with focal sclerosis in some subfascial areas. The deeper part of the muscle is intact. In the subcutaneous fat tissue few lymphocytes are observed but there is neither panniculitis with lipophagia, nor thick collagen bundles within fat trabeculae.

The epidermis and dermis have no inflammatory infiltrates. The connective tissue of the dermis is not hyalinized and elastic fibres do not look aggregated or disrupted on the Weigert stain. The epidermal appendages are not atrophic.

Direct immunofluorescence tests for the presence of Ig A, Ig G, Ig M, Ig E, C_3 and C_4 were negative for all the biopsies.

Under the electron microscope, the fascia shows numerous fibroblasts with well developped rough endoplasmic reticulum (R.E.R.), partial thickening of cytoplasmic membrane and many thin protocollagen fibrils. Those secreting



The four electron micrographs were taken in the fascia.

Fig. 1. Narrow contacts (*arrows*) between cytoplasmic process of syntesizing fibroblasts (*F*) and degranulating mast cells (*Ma*). *Inset:* a part of a synthesizing fibroblast

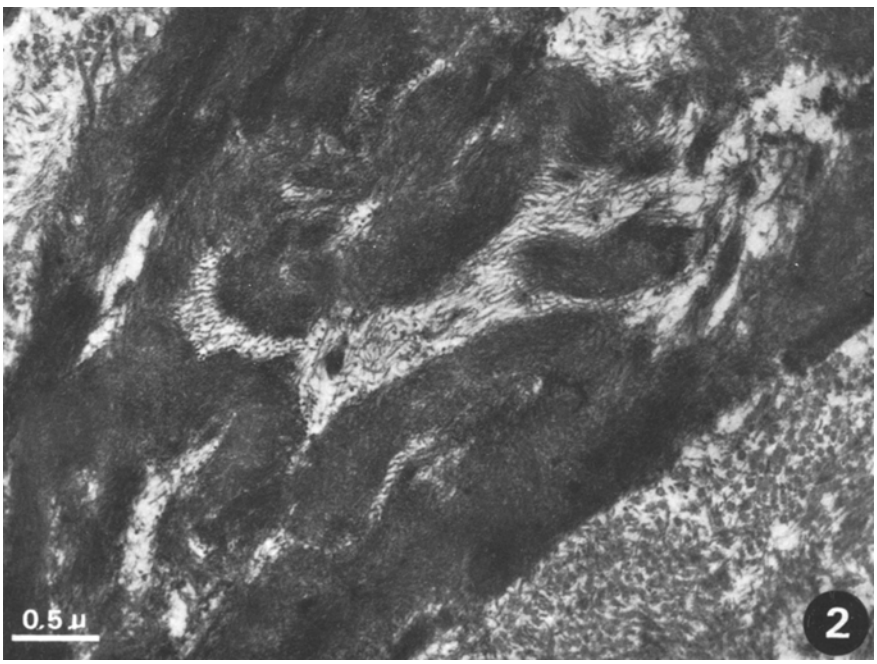


Fig. 2. Clusters of very thin protocollagen fibrils (10–40 Å d)

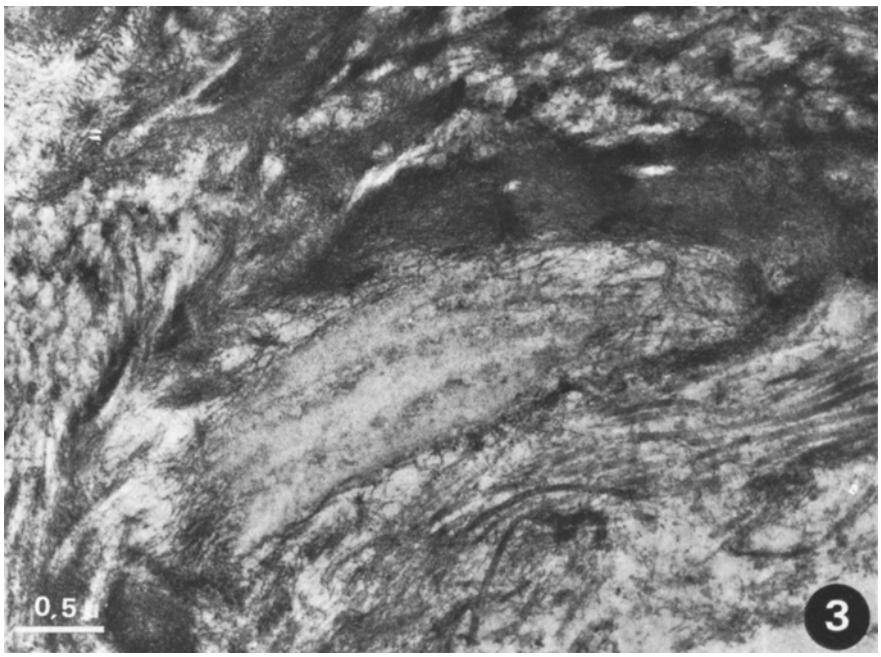


Fig. 3. Active fibrillar remodelling of an elastic fibre

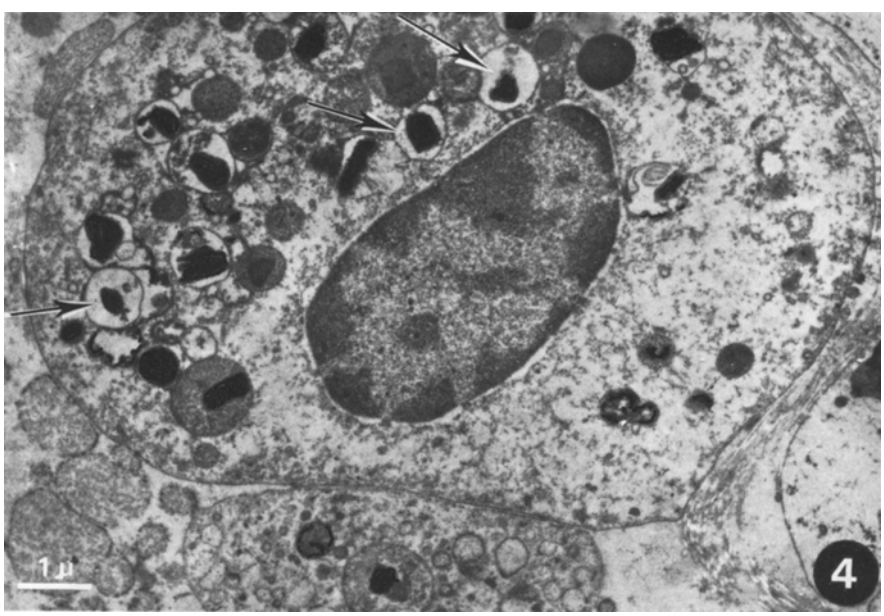


Fig. 4. Polymorphonuclear eosinophil: altered granules (*arrows*) with the dense crystalloid core but without the less dense matrix around

fibroblasts are in close contact with degranulating mast cells (Fig. 1). Accumulation of thin protocollagen fibrils (10–40 Å) are observed in many areas (Fig. 2). Within the collagen bundles, the collagen fibres have normal cross-band pattern and periodicity in longitudinal section, and regular contours in cross section with diameters ranging from 600 to 900 Å. "Beaded" filament or plywood arrangement are not found. The elastic fibres show active fibrillar remodelling (Fig. 3).

The inflammatory infiltrate is dense around the capillaries. In four instances lymphocytes and plasma cells are found within the vessel wall. The endothelial cells are swollen but necrosis or thrombosis is not observed. No electron-dense deposit is observed on the capillary basement membrane.

Among the hundred inflammatory cells counted on different blocks, 47 are lymphocytes, 33 plasma cells with voluminous R.E.R., 16 eosinophils, 3 macrophages and 1 basophil. Half the eosinophils have the crystalloid central core but not the peripheral less dense matrix in their granules (Fig. 4).

A few lymphocytes and eosinophils are scaterred in the deeper part of the subcutaneous fat tissue. Necrotic adipocytes, macrophages, thin collagen fibrils between adipocytes or within fat trabeculae are not observed.

Discussion

In this case the biopsy was performed only 8 weeks after the strenuous exercise which immediately preceded the lesions. The skin, subcutaneous fat, fascia and muscle were removed separately so as to study as precisely as possible the tissue where the damage predominated and probably began. The damage was focused on the fascia, with active connective tissue synthesis and remodelling and marked cell infiltrates with lymphocytes, plasma cells, degranulating mast cells and eosinophils.

Numerous fibroblasts were present and showed signs of intense fibrillogenesis. The close contact of these synthesizing fibroblasts with degranulating mast cells was related to the observations of Eady (1976) who pointed out that no new connective tissue formation takes place without demonstrable activity of mast cells.

Accumulation of very thin protocollagen fibrils (10–40 Å diameter) was observed in our case but we did not find beaded filament of embryonic type reported by Haynes and Rodnan (1971) in scleroderma or thin fibrils having a diameter of 100–200 Å within the collagen bundles, as reported by Braun-Falco and Rupec (1964) in scleroderma. Plywood arrangement of the collagen fibrils as described by Fleishmajer and Prunieras (1972) in the subcutaneous tissue in generalized morphea was not found, either in the fascia or in the subcutaneous fat.

In our case, the intense fibrillogenesis as well as the fibrillar remodelling of elastic fibres may be part of a healing process following fascial microinjury after physical exertion. As Barnes et al. (1979), and Michet et al. (1981) have reported many patients with eosinophilic fasciitis experience rapid onset of the disease after strenuous physical activity. Lewis (1978) reported the case

of a woman with a 17-year-long history of E.F. who had recurrence of pain, stiffness and swelling every time she undertook heavy exertion.

The inflammatory cells which predominated around the capillaries and infiltrated into the vascular wall without associated vascular necrosis, were found in 10 out of 20 cases by Barnes et al. (1979) and in 2 out of 6 cases by Nassanova et al. (1979). These lesions were associated with hyperplasia and/or hypertrophia of the endothelial cells. Interestingly, an autoradiographic study performed by Fleischmajer and Perlish (1977) showed an increased division rate of dermal endothelial cells in scleroderma. No similar study was performed in E.F. Intraendothelial microfilaments or perivascular deposits of amorphous-granular substance as described in scleroderma by Haustein and Klug (1975) were not found in our case of E.F.

Lymphocytes were numerous in the fascia and some of them spilled over into the subcutaneous fat and into the muscle epimysium. As Johnson and Ziff in 1976 demonstrated that lymphokines stimulate in vitro collagen accumulation, the inflamed tissues may become sclerotic as the disease advances. Barnes et al. (1979) concluded that, when studied in late stages, E.F. shares many morphological features with scleroderma.

Plasma cells were also numerous with enlarged R.E.R. though no immunoglobulin deposit could be found by direct immunofluorescence.

According to Asboe-Hansen's study (1973), degranulation of numerous mast cells may be the tissue response to oedema and the stimulus for connective tissue synthesis in the fascia, but Clark et al. (1975) demonstrated that histamine also has a selective chemotactic activity for eosinophils. The rather large number of eosinophils in the fascia could thus be partially explained by the degranulation of mast cells.

Although eosinophilia in the peripheral blood has been described as a characteristic feature of E.F., eosinophils are not inevitably found in the damaged tissue. In our case we found a discordance between the few scattered eosinophils (4 to 5% of the cells) observed on histological sections (Giemsa stain), and the 16 eosinophils out of the 100 inflammatory cells counted on ultrathin sections. Two explanations may be suggested for this discordance: i) the characteristic eosinophilic granules on the Giemsa stain may have been badly identified where the inflammatory infiltrate was the densest, so only scattered and peripheral eosinophils were counted. However, under the electron microscope, very few eosinophils were intermingled with lymphocytes and plasma cells. ii) the altered granules lacking dense matrix observed in half of the eosinophils may lose their affinity for the Giemsa stain.

A relationship between eosinophils and fibrogenesis has been suggested by Bade (1977) in endomyocardial fibrosis and Löffler endocarditis. Furthermore, according to Butterworth and David (1981), after eosinophil degranulation in schistosomal infection, the major basic protein localized in the cristalloid core of the eosinophil granule may induce toxic effects on the host tissue. These hypotheses have not been definitely confirmed, as stated by Weller and Goetzl (1980). E.F. biopsies performed by Moutsopoulos et al. (1980) after treatment on clinically improved patients indicated the disappearance of the eosinophils.

The relationship of E.F. with the various forms of cutaneous sclerosis has to be elucidated. Winkelman in 1975, before Shulman's description, stated that, in morphea, the sclerosis may be superficial (with clinical features of lichen sclerosus and atrophicus or bullous morphea), dermal as in typical morphea, or in the panniculus or fascia. In our early biopsied case of E.F., the sclerosis was limited within the fascia.

Comparing E.F. with the mechanisms of wound healing studied by Ross and Odland (1968) shows, that E.F. lacks the neutrophilic polymorphonuclears and the macrophages observed in the early phases of wound healing. However, fibrillogenesis is active in the final phase of healing as is seen in E.F.

Compared with the cellular reaction in morphea reported by Fleischmajer and Prunerias (1972) (9) EF shows very few macrophages in the fascia, absence of panniculitis with numerous lymphocytes and macrophages in the subcutaneous fat tissue and many eosinophils. These may be the distinctive features of E.F.

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