Tropical myositis: ultrastructural studies

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SYNOPSIS Specimens of muscle were obtained from non-suppurating lesions of nine patients with tropical myositis. When examined in an electron microscope, these revealed patchy myocytolysis with loss of band structure. Perimysial cells were also degenerate. Sections from two out of nine patients revealed intracellular vesicles, about 80 nm in diameter, some of which contained 10 nm granules. These vesicles were seen budding from cell membranes and resembled virus particles. The focal necrosis with which they are associated and their absence from control sections support the concept that they are related to the disease process.

Inflammatory disease of skeletal muscles is seen infrequently in America (Roston, 1967) and Great Britain (Borman et al., 1963). However, in hot moist areas of the world, tropical myositis may seriously affect the health of the community.

It rarely occurs at high altitudes above 5000 feet (1524 m). Children and young adults are most frequently afflicted but the disease has been seen in all age groups. In Uganda, there appears to be a diminished incidence in those with a high standard of living and it is rare among expatriates. It may occur simultaneously in two members of a family but rarely twice in the same individual (Taylor et al., 1973).

One or more skeletal muscles are involved with a frequency proportional to their bulk, common sites being the biceps, pectoral muscles, glutreal, and quadriceps. Initially, the patient may feel feverish and suffer arthralgia (Taylor and Henderson, 1972) or focal muscle pain may be the presenting feature. The affected area is firm at first and operative examination reveals oedematous grey muscle fibres. In a number of patients, spontaneous resolution may occur at this stage. Alternatively, the site becomes acutely tender and fluctuant, and on incision pus is released. This may be sterile in a few cases, but the majority of specimens yield profuse cultures of staphyloccoci (Foster, 1965).

These findings give rise to speculation that the skeletal muscle is first damaged by an unknown agent and subsequently colonized by bacteria.

Filaria (Buxton, 1928) and guinea worm (Anand and Evans, 1964) have been proposed as the primary pathogen but the geographical distribution of these diseases does not correlate with that of tropical myositis. Leptospira, malaria, and toxacara (Taylor et al., 1973) have been excluded on the basis of serological tests but there remains the possibility that other hookworm larvae may migrate and, by lodging in muscle tissue, induce a tissue reaction (O’Brien, 1963). Because of the high incidence of hookworm infestation in East Africa, this concept is hard to disprove though larvae have never been found on microscopy. Scurvy (Wiseman, 1943) was thought to be important in the pathogenesis but it is now rare in East Africa. However, white muscle disease in calves is now thought to be due to a complex deficiency of selenium and tocopherol (Hungerford, 1967), but similar deficiency syndromes have not been sought in tropical myositis.

Histological sections of non-suppurative lesions have shown patchy myocytolysis and lymphocytic infiltrate (Taylor et al., 1970). The histological and clinical features with a restricted geographical distribution have led us to seek viruses in thin sections of involved muscle.

Patients and methods

Muscle was examined from nine Baganda referred to Mulago Hospital, Kampala for incision and drainage of tropical myositis. A fibre was removed from a site 2-3 mm outside the abscess before the release of pus. Four control specimens were obtained from muscle in the floor of cutaneous ulcers arising from myobacterial infection (two patients), pemphigus, and malignancy. The muscle fibre was immersed in 3% buffered glutaraldehyde at 4°C and cut into...
blocks 1 mm square. These were fixed for one hour, postfixed in 2% osmium tetroxide, dehydrated, and embedded in Araldite. Thin sections were examined in a Zeiss EM9 electron microscope; routine paraffin sections were also examined.

Results

The biopsy site was confirmed by light microscopy. Skeletal muscle adjacent to the abscess showed patchy myocytolysis, the sarcoplasm melting away or showing coagulative necrosis. Degenerative fibres were surrounded by lymphocytes, plasma cells or eosinophils and, in suppurating areas, neutrophils.

The general features of inflammation were seen in thin sections from tropical myositis patients and controls. These included separation of the myofibres, the intervening space being filled by amorphous material interpreted as oedema fluid.

Muscle affected by tropical myositis was characterized by focal abnormality of one or more fibres. The band structure was upset with loss of myofilaments. In sections showing advanced degeneration, 200 nm vacuoles were common, some being interpreted as dilated tubules of the sarcoplasmic reticulum.

Fig 1 A degenerate cell, interpreted as a necrotic myocyte, from patient 1. Vesicles are seen apparently budding from an intracellular membrane. They are 80 nm in diameter and one or two contain granules. × 140 000
Myelin bodies were also seen.

In many micrographs, the endomysial and perimysial cells were seen to be degenerate. Sections from three patients revealed multiple vesicles in these cells and in leucocytes. They were regular and about 80 nm in diameter with an envelope of density equivalent to that of the cell membranes and had no inclusions. They were interpreted as being pinocytotic vesicles.

However, a necrotic myocyte and regenerating endothelial cells seen in micrographs from patient 1 contained different vesicles in their cytoplasm (fig 1). They were 80 nm in diameter, contained two or three 10 nm granules, and lay in groups of three or four together. In several sections they were seen budding or pinching off from intracellular membranes. These features and their consistent size lead us to believe that they are viruses, rather than products of cellular necrosis. In another area, 50 nm vesicles were seen to contain 5 nm granules arranged in a circle around the periphery. It is possible that they were axonal or synaptic vesicles, for though not characteristic of these structures, the cell in which they lay has not yet been precisely identified.

A perimysial cell or fibroblast from patient 3 (fig 2) was found to contain round or oval virus-like particles, some 90 nm in diameter. They were seen to bud from the cell membrane which was less dense than the particle envelope. A few contained 10 nm granules and one has a globular arrangement of the envelope such as that described for virus particles (Caspar, 1962). We have concluded that the particles seen in these two patients represent viruses in the supporting cells. Virus-like particles were not seen in micrographs from control patients.

**Discussion**

The micrographs of muscle affected by tropical myositis revealed widespread non-specific changes including infiltration by oedema and inflammatory cells. There was also focal myocytolysis and disturbance of the band structure which was not observed in control sections taken from muscle adjacent to areas of bacterial inflammation. Though histochemical examination was not undertaken, there was no evidence of preferential involvement of granular (type 1) fibres, as seen in chloroquine myopathy (Hughes et al, 1971). Mitochondrial degeneration, as seen in chloroquine and steroid myopathy, is not a feature of tropical myositis.

Multivesiculation is a well recognized feature of many types of cytopenesis. This has led to difficulty...
in the interpretation of the micrographs from two of our patients which revealed virus-like particles. Their regular size and inclusions indicate that they are not microcincytotic vesicles (Gray, 1973). They bud from internal and external membranes and do not appear to lie in axons or motor end plates, which differentiates them from synaptic vesicles (Babel et al, 1970). These virus-like particles may represent commensals but the focal necrosis with which they are associated, and their absence from control sections, lead us to conclude that the particles and the disease process are related. Their precise nature will be determined only by serological and ultrastructural studies on other specimens. Should these provide further evidence of virus infection, several groups of viruses may have to be considered as possible pathogens.

Of the known virus groups, arena viruses most closely resemble the particles observed (Murphy et al, 1970). They are round or oval, 60-280 nm in diameter, and may have a surface and 20 nm electron dense inclusions. They have a limited geographic distribution and an ecological association with a rodent host, and an immunologically mediated mechanism of pathogenesis. After the initial infection, a carrier state exists in which high antibody titres are present for life. On electron microscopy, virions may be found in only 5% of infected tissue culture cells. These features make arena viruses ideal candidates for the primary role in tropical myositis.

Tropical myositis is a cause of considerable temporary disability to the young adults in several countries. Further studies should be undertaken to detect a change in viral antibody titres during the course of the disease. The possible synergistic role of helminth larvae and metal or vitamin deficiencies must also be elucidated.

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