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Evaluation of muscle capillary basement membrane in inflammatory myopathy

A morphometric ultrastructural study

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Abstract The capillary basement membranes from 16 skeletal muscle biopsies from patients with a clinical and histological diagnosis of inflammatory myopathy and from six controls were analysed ultrastructurally and morphometrically. Resin sections from 244 endomysial capillaries were examined by light microscope, and the results were correlated with findings seen in electron micrographs of these capillaries. The ultrastructural morphometric measurements and the statistical analysis showed that the capillary basement membrane was thick and multilaminated in 87% specimens affected by inflammatory myopathy. No thick or multilaminated basement membrane was observed in controls. In inflammatory myopathy the endomysial space next to the capillaries contained an increased amount of collagen fibrils and showed signs of a chronic reparative process. It is suggested that the thick multilaminated basement membrane in inflammatory myopathy represents an advanced stage of vascular regeneration.

Key words Capillary basement membrane · Inflammatory myopathy · Ultrastructure

Introduction

Capillary involvement in the skeletal muscles is seen in the majority of patients with inflammatory myopathy (IM) when the tissue is examined with the electron microscope. The vascular abnormalities have been reported many times [1–5; 11–12]. The most significant change is evident at the level of the basement membrane (BM). Jerusalem et al. [5] mentioned thickening and reduplication of capillary BM in scleroderma, dermatomyositis, polymyositis and lupus erythematosus, but in that study only the innermost layer of the BM was measured.

Exact measurement of the BM thickness is of prime importance but different results are achieved with different methods of measurement [14]. In the present study the entire thickness of the BM with all its multiple layers was measured morphometrically and the results were subjected to statistical analysis.

Our aim was to stress the regional circumstances, which are presumably related to a wide capillary BM in IM. This was done by correlating the ultrastructural findings with resin histology findings.

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Materials and methods

The study material comprised a total of 22 skeletal muscle biopsies, taken from 16 patients diagnosed with IM and 6–62 years and 6 controls aged 9–61 years. The group of IM contained 4 patients with polymyositis and 3 with both polymyositis and dermatomyositis, and 9 who had polymyositis associated with collagen-vascular disease.

In each patient the diagnosis was based on clinical observations, elevated serum enzymes and light and electron microscopic results. The control samples revealed no pathologic changes on light, enzymatic and electron microscopic examination. All the samples were obtained from the gastrocnemius or the quadriceps muscle during routine biopsy procedures initiated at the clinician's request for investigation of suspected muscle conditions.

Patient or parental consent was obtained for each biopsy. All the material was processed in a similar manner. For electron microscopy the specimens were cut immediately into small pieces and fixed with cold 3% glutaraldehyde in cacodylate buffer (pH 7.3) for 1 h, post-fixed with 2% osmium tetroxide, dehydrated in alcohol and embedded in Epon. At least three blocks were cut from each muscle. Transversally cut 1-µm-thick sections were stained with 1% toluidine blue and examined with the light microscope. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss 9S electron microscope. At least 10 capillaries were photographed from each muscle. All electron micrographs were obtained at a single tap setting and then enlarged ×19 000.

The morphometric analysis was performed on a semiautomatic image analyzer Zeiss Morphomat 35 (Carl Zeiss, Oberkochen, Germany). The measurements of the total and inner surface area of cross-sectioned endomysial capillaries and the basement mem-

brane thickness were taken from negatives enlarged ×4900. In each capillary the whole width of the uni- or multilaminated BM was measured at 6 constant points of similar coordinates.

Comparison of continuous and demographic variables between the diseased group and the control was performed by Student's *T*-test for independent variables. The equality of variances was tested using Levene's test. Categorical variables were compared using Fisher's exact test Two-tailed *P*-values of 0.05 or lower were considered to be statisitically significant.

Results

The present study comprised a total of 224 endomysial capillaries of skeletal muscles. One hundred and sixty-six were from the IM group and 58, from control muscles. All the capillaries were composed of continous endothelium that showed no more than three intercellular junctions and one to two pericytes. The luminal diameter varied from 2 µm to 4.2 µm.

The inner vascular surface appeared larger in the diseased muscles than in controls. The results are shown in Table 1.

In the presence of IM resin sections showed marked variation in the size and shape of muscle fibres. In some areas the muscle fibres showed homogenization and loss of striation. In all cases there was endomysial proliferation of collagen fibrils. In 80% of sections the endomysial space next to the capillaries showed chronic inflammatory changes. Examination of the photographs of the thin sections taken from the damaged and nondamaged areas permitted us to correlate the muscle and endomysial changes with BM changes. The control muscles showed no abnormalities. No alterations were noted in the endomysial space or at capillary level.

Table 1 Morphometric analysis of basement membrane thickness in inflammatory myopathies (*S* significant, *NS* not significant)

	Diseased muscle (*n*=16)	Controls (*n*=6)	*P*-values
Age	25.3±15.3	25.16±21	0.9 NS
Sex	Male 6 Female 10	Male 4 Female 2	0.2 NS
Basement membrane thickness (median)	Mean SD 0.48±0.04 µm	Mean SD 0.3±0.05 µm	0.04 S
Inner vascular surface (median)	22.6±1.4 µm^2	16±1.4 µm^2	0.016
Coefficient of variation of the inner vascular surface (median)	0.4	0.57	0.04

In IM, the capillary BM appeared as a continuous thick stratified layer closely apposed to the outermost endothelial membrane. The multilaminated layers were either at a slight distance from each other, sometimes incorporating pericytes (Fig. 1), or packed together forming one or two layers (Fig. 2). The capillary multilaminated BM was present in 87% of cases (Fig. 3) and was significantly thicker than in controls, averaging 0.48 µm±0.04 (Table 1). Correlation of these capillaries with

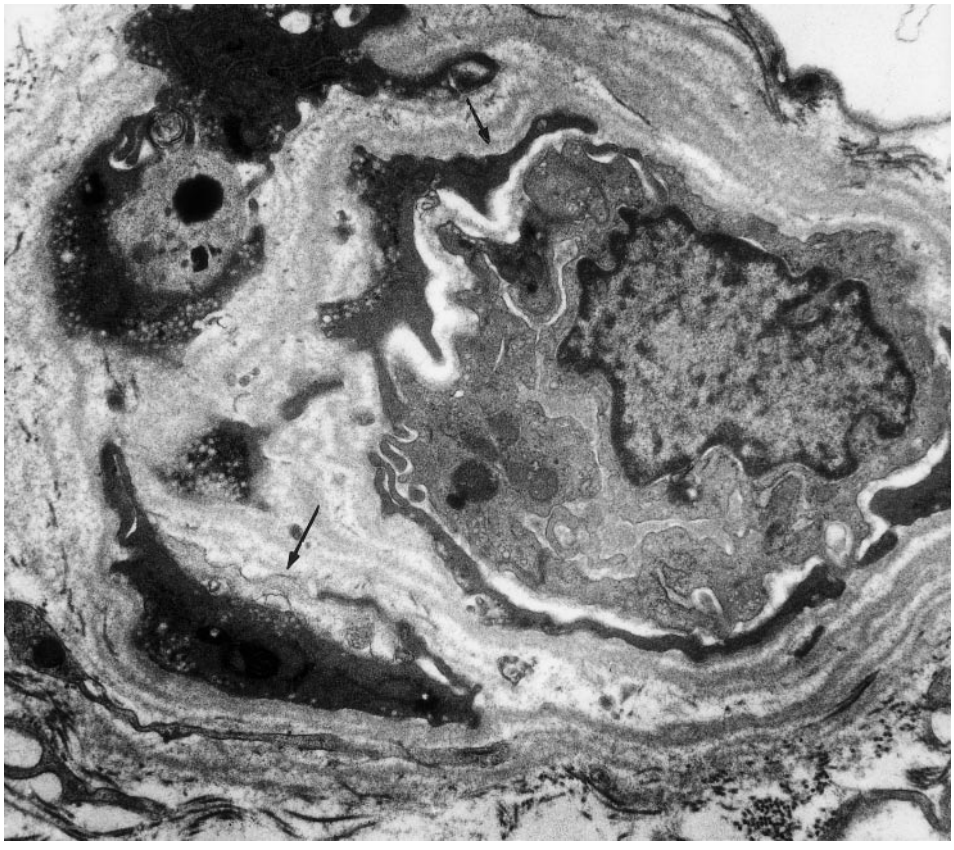

Fig. 1 Electron micrograph of capillary with collapsed lumen from the gastrocnemius muscle of a 32-year-old with inflammatory myopathy. The multiple layers of basement membrane are slightly apart from each other and are enclosing several pericytes (*arrow*). ×9400

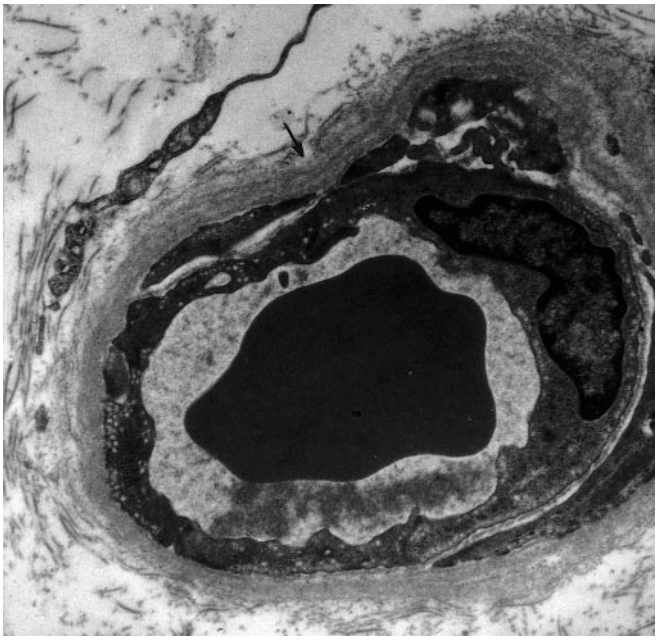

Fig. 2 Case of inflammatory myopathy (58 years). The capillary lumen contains an erythrocyte. The thick basement membrane is composed of several susperimposed layers (*arrow*). Collagen fibrils adhere to the outer side of the basement membrane. ×9800

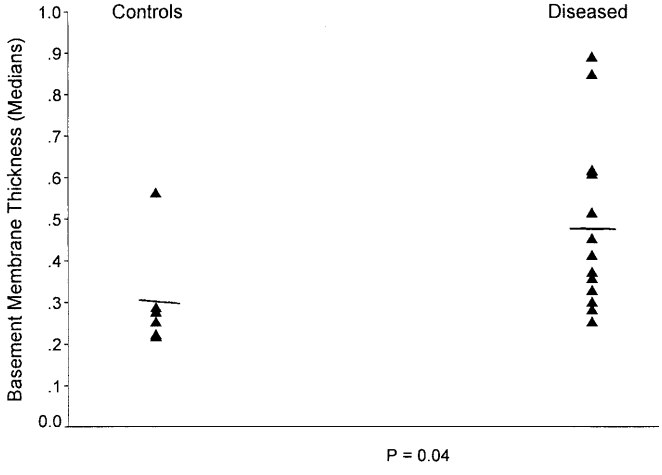

Fig. 3 Graph illustrating basement membrane thickness in diseased muscle and in control muscle

the areas they belong to made evident that the thick multilaminated BM was related to an increased amount of endomysial collagen fibrils and to a regional chronic inflammatory reparative response. Lymphocytes, fibroblasts and few mononuclear cells were randomly dispersed. In many muscle fibres the sarcomere pattern was disorganized and presented degenerative changes. The sarcolemma was well preserved. In several cases the degenerative changes were mild.

In 2 cases (out of 16) the capillary BM was not increased in width and multilamination occurred only in 1 out of 10 capillaries. The resin histology and the electron micrographs showed no inflammatory cells and no muscle damage in these specific areas.

In controls, the capillaries were invested by a continuous and homogenous BM tightly apposed to the endothelial membrane. The BM was significantly thinner than in the IM group of (Table 1). No changes were encountered in the endomysial space or in the muscle fibres of these specimens.

Discussion

In this study we focused on the capillary BM and on the correlative regional factors. Semithin sections from resin-embedded specimens allowed close supervision of the changes undergoing in specific areas, and the findings were then correlated with electron micrographs taken from consecutive thin sections. No precise morphometric measurements of the whole width of the BM in IM have been reported to date, though data on the inner layer of BM have been presented [9]. When the pathologist records a thick capillary BM by light microscopy, it should be borne in mind that in most cases the membrane concerned is a multilaminated BM.

In a recent review of the pathological diagnosis of specific inflammatory myopathies, the usefulness of semithin resin sections and of electron microscopy in diagnosis was pointed out [3]. The changes in the microvasculature of skeletal muscles are important with reference not only to neuromuscular disorders, but also to chronic heart failure [11, 12]. According to Vracko [19] and Vracko and Benditt [20], the BM is involved in a number of biological processes, including tissue regeneration and repair.

In muscle disease a thick capillary BM is frequently encountered. It is well known that the capillary BM grows thicker with age [10], in IM [9], in arteriosclerosis obliterans [14] and in diabetes [17, 18], to name only the most common conditions. This study indicates that in IM the thick BM derives from superimposed multiple layers, which combine to give a mean thickness of 0.48 µm±0.04. Such a thick multilaminated BM was seen in 87% of our cases and was significantly thicker than the BMs in controls. Jerusalem et al. [9] observed replication of BM in muscle capillaries in 24–74% of capillaries in IM and no BM reduplication in controls. We also found no BM multilamination in capillaries of our control specimens.

It is of interest that the thick multilaminated BM in our specimens occurs mainly in areas affected by endomysial chronic inflammatory changes and muscle abnormalities. It is reasonable to believe that the endomysial process is related to the myopathic damage. We suppose that in the diseased muscle the process of vascular regeneration is more active than in normal muscle and that the thick multilaminated BM in our study represents an advanced stage of vascular regeneration. This is in keeping with studies reported by others [2, 9, 13, 20]. No replication of BM and no inflammatory infiltrates were

found in cases of dermatomyositis that showed no muscle damage [5].

Immune mediated events in IM have been reported by many [4, 6, 8]. It is believed that in childhood-type dermatomyositis the immune process is directed against the intramuscular microvasculature [21] and that the deposit of complement on the capillaries is the earliest lesion and precedes inflammation or muscle fibre necrosis [6]. However, in polymyositis the pathogenesis of muscle fibre destruction points to an antigen-directed cytotoxicity mediated by cytotoxic T cells [1, 7].

It should be mentioned that this report includes only 3 cases of dermatomyositis with polymyositis among 16 patients; the rest had polymyositis alone or associated with collagen-vascular disease. In adults, in the group with idiopathic IM polymyositis is the most frequently occurring IM [8]. Polymyositis is reported to be more prevalent in female subjects [8] and this agrees with our data.

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