Microvascular alterations and the role of complement in dermatomyositis

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Different mechanisms have been proposed to explain the pathological basis of perifascicular muscle fibre atrophy in dermatomyositis. These include ischaemia due to immune-mediated microvascular injury, enhanced expression of type 1 interferon-induced gene transcripts in perifascicular capillaries and muscle fibres, and occlusion of larger perimysial blood vessels. Microvascular complement deposition is a feature of dermatomyositis pathology but the trigger for complement activation, the predominant complement pathway involved, or its role in the pathogenesis of the disease, has not been clearly defined. In the first step of this study we examined the density of capillaries and transverse vessels and searched for occlusion or depletion of larger perimysial blood vessels in 10 patients with dermatomyositis. This revealed an invariable association of perifascicular atrophy with capillary and transverse vessel depletion. The capillary and transverse vessel densities in non-atrophic fibre regions were not significantly different from those in muscle specimens of 10 age-matched controls. Next, in the same 10, as well as in 40 additional dermatomyositis patients, we searched for vascular deposits of IgG, IgM, and the C5b-9 complement membrane attack complex. Thirty-one of 50 dermatomyositis specimens contained C5b-9 reactive endomysial microvessels but none of these or other vessels reacted for IgG. Ten of 50 specimens harboured IgM-positive capillaries but only a few of these reacted for C5b-9. Finally, we analysed and compared different pathways of complement activation in dermatomyositis, lupus nephritis, and necrotic muscle fibres in Duchenne dystrophy. In lupus nephritis, C5-b9 deposits co-localized with IgG, IgM, C1q, and C4d, consistent with immune complex dependent activation of the classical complement pathway. In both dermatomyositis and Duchenne dystrophy, C5-b9 deposits co-localized with C1q and C4d and rarely with IgM indicating activation of the classical complement pathway. We conclude that: perifascicular atrophy in dermatomyositis is consistently associated with focal microvascular depletion, and that microvascular membrane attack complex deposits in dermatomyositis result from activation of the classical complement pathway triggered by direct binding of C1q to injured endothelial cells.

Introduction

Dermatomyositis is a multisystem inflammatory disorder that frequently involves skeletal muscle. The hallmark of muscle pathology in dermatomyositis is perifascicular muscle fibre injury resulting in perifascicular atrophy, and different lines of evidence implicate the muscle vasculature in its pathogenesis. Early histopathological studies revealed that some perimysial blood vessels were surrounded and infiltrated with lymphocytes associated with thickening and occasionally obliteration of the vessel walls (Batten, 1912; Banker and Victor, 1966). Subsequent ultrastructural
studies revealed abnormalities in endothelial cells of capillaries, arterioles, and veins (Banker, 1975; De Visser et al., 1989). Later, based on findings in juvenile dermatomyositis, it was suggested that reduction in capillary density and muscle fibre injury were co-extensive (Carpenter et al., 1976). Finally, a detailed morphometric and immunocytochemical study in early dermatomyositis without perifascicular atrophy showed reduced capillary density and C5b-9 complement membrane attack complex (MAC) deposits on a proportion of intrafascicular capillaries (Emslie-Smith and Engel, 1990). A recent study aimed at explaining the pattern of clustered capillary loss in dermatomyositis proposed that the capillary loss was due to upstream lesions at the level of perimysial arcade arteries or penetrating transverse arterioles (Gitiaux et al., 2013).

The MAC deposits on capillaries suggested that dermatomyositis was an antibody-dependent and complement-mediated microangiopathy resulting in ischaemia and perifascicular atrophy (Kissel et al., 1986, 1991; Emslie-Smith and Engel, 1990; Mendell et al., 1996; Goncalves et al., 2002; Benveniste et al., 2004; Jain et al., 2011; Dalakas, 2015). This notion was supported by previous studies that showed IgM and rare IgG deposits on blood vessels in a proportion of dermatomyositis cases (Whitaker and Engel, 1972; Kissel et al., 1986). However, no subsequent studies showed immunoglobulin or immune complex deposits in dermatomyositis, transplacental transfer of the disease by affected mothers to newborns has never been reported, and passive transfer of dermatomyositis with immunoglobulins from patients to animals has not been demonstrated. Furthermore, the trigger and pathway for complement activation and its contribution to the pathogenesis of dermatomyositis were not elucidated.

Subsequently, microarray studies identified high expression of type 1 interferon-inducible gene transcripts in adult (Greenberg et al., 2002; Greenberg, 2007) and childhood (Tezak et al., 2002) dermatomyositis. Further studies revealed that type 1 interferon-producing plasmacytid dendritic cells were localized in perimysium of dermatomyositis muscles and that transcripts of type 1 interferon-induced genes, MX1, ISG15, OAS1 were highly expressed by the perifascicular muscle fibres and capillaries closest to the interferon-secreting perimysial plasmacytid dendritic cells. Similarly, the cutaneous lesions in dermatomyositis first involve the deepest layers of epidermis below which the type 1 interferon producing plasmacytid dendritic cells reside. The above studies attribute perifascicular capillary and muscle fibre injury to intracellular overproduction of one or more of the IFNInducible transcripts (Greenberg, 2009) and do not attribute perifascicular fibre alterations to impaired vasculature (Greenberg, 2010a).

Another recent study of microdissected MHC1 expressing perifascicular dermatomyositis fibres found upregulation of RIG-1 (now known as DDX58) and of a novel antiviral factor DDX60 that promotes RIG-1 signalling. Overexpression of RIG-1 in perifascicular dermatomyositis fibres was confirmed histochemically, and stimulation of human myotubes with a RIG-1 ligand enhanced the secretion of interferon β (Suarez-Calvet et al., 2014).

Although several lines of evidence support oversecretion of interferon 1 as an initial or instigating event in the pathogenesis of dermatomyositis, a recent review of inflammatory muscle diseases postulates that activation of complement component C3 is probably triggered by antibodies against endothelial cells. This would lead to formation of C3b and assembly of MAC, which is deposited on the capillaries (Dalakas, 2015). According to this scheme, cytokines released by activated complement lead to activation of CD4+ T cells, macrophages, B cells, and plasmacytoid dendritic cells. However, as mentioned above, there is no direct evidence for circulating anti–endothelial cell antibodies in dermatomyositis, and the above scheme does not explain selective injury of the perifascicular muscle fibres. The aim of this study was to re-examine the microvascular alterations in dermatomyositis, analyse the topographic distribution of capillaries binding MAC, and investigate the mechanism of complement activation in dermatomyositis.

**Materials and methods**

**Patients**

All human studies were in accord with and approved by the Institutional Review Board of Mayo Clinic. Muscle specimens from 50 patients with clinically and pathologically confirmed dermatomyositis were evaluated for various components of this study. The investigated patients had no clinical or laboratory features of another associated autoimmune disease.

**Clinical data**

The muscle microvasculature was quantitatively analysed in 10 patients with dermatomyositis 3 to 70 years of age (median, 16 years) and in 10 age-matched normal control subjects. Supplementary Table 1 lists the relevant clinical and laboratory findings in the 10 patients. All patients with dermatomyositis had muscle weakness ranging from 2 months to 5 years prior to biopsy; eight patients had a skin rash, one had subcutaneous calcifications, and one had interstitial pulmonary fibrosis. The serum creatine kinase level was elevated in four patients ranging from 400 to 3000 IU/l. Results of serological testing were available in eight patients. Four tested positive for antinuclear antibodies (ANA) but specific autoantibodies including anti-Jo-1 antibodies were absent in all patients. Three patients had been exposed to long-term immunosuppressants prior to the muscle biopsy: Patient 8 had received 5 mg of prednisolone for 3 months; Patient 9 received 20 mg of prednisolone for 5 months, and Patient 10 received 10 mg prednisolone for 4 months and 15 mg methotrexate per week for 3 months.

Muscle specimens from the same 10 patients with dermatomyositis and from 40 additional patients with dermatomyositis 3–84 years of age were qualitatively analysed for deposits of IgG, IgM, and MAC. Supplementary Table 2 lists the relevant clinical and laboratory findings in the additional 40 patients. None had clinical features suggesting overlap myositis, and all
had negative serological test for autoantibodies, including anti-Jo-1 antibodies. The duration of clinical symptoms prior to biopsy ranged from 1 month to 17 years (median, 9.5 months). Muscle weakness was clinically detected in 38 of the 40 patients. The rash of dermatomyositis was present in 31, and lung involvment in two. Serum creatine kinase levels were elevated in 15, ranging from 218 to 3838 IU/l (median, 597 IU/l). Eleven of the 40 patients had received long-term immunosuppressant therapy prior to the muscle biopsy.

**Histological and immunohistochemical studies**

We quantitatively analysed the capillary and precapillary transverse vessel densities in abnormal perifascicular regions in dermatomyositis and control muscle specimens and searched for complement, IgG and IgM deposits on capillaries, capillary remnants, and transverse vessels.

Ten- and 6-μm thick sections were obtained from cryopreserved muscle specimens for bright-field and immunofluorescence microscopy, respectively. Supplementary Table 3 indicates the immunoreagents used in the study. Non-specific binding of the immunoreagents was blocked by preincubation with phosphate-buffered saline, pH 7.4, containing 2% bovine serum albumin (BSA) and 10% donkey, human or goat serum. Control sections for each experiment were treated either with a matching concentration of non-immune immunoglobulin or with phosphate-buffered saline, pH 7.4, containing 2% bovine serum albumin (BSA) and 10% donkey, human or goat serum. Control sections for each experiment were treated either with a matching concentration of non-immune immunoglobulin or were processed without the primary antibody.

**Capillary density estimation and complement deposition in dermatomyositis muscle**

In 10 dermatomyositis and 10 age-matched control specimens we co-localized the C5b9 membrane attack complex (MAC) with vascular endothelial cells using biotin labelled *Ulex europaeus* agglutinin 1 (UEA1) (Emslie-Smith and Engel, 1990) and then counterstained the sections with eosin. In each specimen, three normal fibre regions and three regions displaying perifascicular atrophy were randomly selected to determine the density per mm square of all capillaries, and the total number of C5b9-positive capillaries and capillary remnants. The morphometric analysis was performed using SigmaScan Pro 5 (Systat Software, Inc., San Jose, California).

**Estimation of transverse vessel density and complement deposits in normal and dermatomyositis muscle**

In this part of the study we evaluated the precapillary transverse vessel density in 10 dermatomyositis and their age-matched control specimens using biotin-labelled UEA1 and the ABC immunoperoxidase method and then counterstained the sections with eosin.

As a first step, we used 50 consecutive 10-μm thick sections in one dermatomyositis and one control specimen. We defined two large randomly selected areas in the first section and then demarcated the same two areas in the remaining 49 sections.

Transverse vessels were counted in the demarcated regions in the 50 consecutive sections.

In the second step, we cut 50 consecutive 10-μm thick sections from the 10 dermatomyositis and 10 control specimens. Then, using two preselected areas in the control sections, and two normal areas and two areas showing perifascicular atrophy in the dermatomyositis sections, we counted the transverse vessels in every 10th section as well and the transverse vessels reacting for MAC in every 11th section.

**Perimysial alterations in dermatomyositis specimens**

In all 10 specimens we performed routine histology and immunohistochemistry studies to evaluate inflammatory infiltrates, connective tissue changes, and perimysial MAC deposits and their topographic relationship to regions of perifascicular muscle fibre atrophy.

**Semi-quantitative analysis of plasma cell distribution**

In muscle specimens demonstrating significant inflammation and in all IgM-positive specimens, we searched for the presence of plasma cells by immunolocalizing the CD138 antigen by the ABC immunoperoxidase method.

**Evaluation of immunoglobulin deposits and complement pathway studies**

As the first step, we co-localized MAC with IgG and IgM in separate sections of all 50 dermatomyositis specimens. We detected no IgG-positive capillaries and hence no MAC positive vessels co-localizing IgG. Therefore we used all specimens with IgM-positive capillaries (*n* = 10) and those with significant MAC deposits (*n* = 5) for complement pathway studies. We considered various hypotheses of complement activation in dermatomyositis (also see ‘Discussion’ section). To evaluate the trigger for complement activation and the predominant pathway involved, we co-localized MAC deposits (i) with C1q, the primary pattern recognition molecule for the classical pathway; (ii) with mannose binding lectin, the primary pattern recognition for the lectin pathway; and (iii) with C4d, a reliable marker for the classical pathway activation presence of which also excludes selective activation of the alternative complement pathway. To illustrate immune complex-dependent activation of the classical pathway, we used a renal specimen from a patient with lupus nephritis and from an autopsy of a patient without known renal disease. To investigate the trigger for complement activation in a non-autoimmune setting, we used two Duchenne dystrophy muscle specimens harbouring MAC-positive necrotic fibres.

**Statistical methods**

Two-tailed *t*-test, rank-sum test, and Fishers exact test were used to calculate statistical significance.
Results

Pattern of capillary loss in dermatomyositis

Simple inspection revealed a reduced capillary density in multiple perifascicular atrophic fibre regions in each of 10 dermatomyositis specimens (Figs 1 and 2). The capillary density per mm² in regions without perifascicular atrophy was 297 ± 34 [mean ± standard error (SE)] and 143 ± 26 in regions with perifascicular atrophy (P < 0.0003) (Supplementary Fig. 1 and Supplementary Table 4). The capillary density in muscles of 10 age-matched controls, 324 ± 34 per mm², was not significantly different from that in non-perifascicular atrophy regions of the 10 patients with dermatomyositis. Dermatomyositis Patient 9 with the least decrease in capillary density had been treated with 20 mg of prednisone per day for 5 months before biopsy and had only mild weakness and no serum creatine kinase elevation when biopsied. In contrast, dermatomyositis Patient 6 who was untreated and acutely ill, displayed severe capillary depletion even in fascicles without perifascicular atrophy.

We also searched for differences in the extent of capillary depletion between children (Patients 1–5) and adults (Patients 6–10). Interestingly, in children the median capillary density per mm² in the unaffected fibre regions was higher than the corresponding adult value (365 versus 261; P = 0.008 by the rank sum test) but in the capillary depleted perifascicular atrophy regions the capillary densities for children (170 ± 40) and adults (116 ± 32) were not significantly different. These data suggest that a larger proportion of pre-existing perifascicular atrophy capillaries was lost in the juvenile than adult patients.

Transverse vessels in normal and dermatomyositis muscle

The mean transverse vessel densities in 10 control muscles (15.7 ± 1) and in unaffected regions of 10 dermatomyositis muscles (12.5 ± 1.5) were not significantly different. In perifascicular atrophy regions of the 10 dermatomyositis muscles the transverse vessel density was reduced to 5.3 ± 0.81 (P < 0.001); the mean was 5.2 in juvenile and 5.4 in adult dermatomyositis (Supplementary Table 5 and Supplementary Fig. 2; cf. Fig. 2A–D and Fig. 2E–H). Thus, transverse vessel depletion invariably accompanies capillary loss (Fig. 2). In regions with perifascicular atrophy, occasional transverse vessels were abnormally dilated (Fig. 3B), or displayed occluded segments (Fig. 3C), or reacted for MAC (Fig. 3D).

Frequency of membrane attack complex reacting capillaries and capillary remnants

The density of MAC reacting capillaries and capillary remnants in perifascicular atrophy regions was 9.3 for all patients, 8.8 for the children, and 9.8 for the adults. Three adults also harboured rare MAC reacting capillaries in normal fibre regions (Supplementary Table 4).

Pathological findings in the perifascicular areas

The perifascicular regions harbouring atrophic fibres displayed one or more of the following alterations: internalized nuclei, regeneration, necrosis, rarefaction or vacuolization, focal basophilia, increased oxidative enzyme activity, glycogen accumulation, ring fibres, small nemaline rods, infiltrating mononuclear cells, and endomyositis fibrosis (Fig. 4). Comparison of these alterations between 15 juvenile and 35 adult patients showed that inflammatory cells occurred in a higher proportion of juvenile than adult patients whereas small nemaline rods occurred in a higher proportion of adult than juvenile patients (P < 0.05 by Fisher test). We also compared the perifascicular alterations between 14 treated and 36 untreated patients and found that focal basophilia appeared in a much higher proportion of treated than untreated patients (P < 0.001). No differences were noted in the incidence of MAC-positive or IgM-positive capillaries between juvenile and adult onset, or between treated and untreated patients (Supplementary Tables 6 and 7).

MxA expression in perifascicular areas

To confirm that the perifascicular pathological alterations are associated with increased expression of interferon inducible gene products, we immunolocalized the MxA in nine patients with dermatomyositis. The brown reaction product are associated with increased expression of interferon inducible gene products, we immunolocalized the MxA in nine patients with dermatomyositis. The brown reaction product was concentrated in the perifascicular capillaries and atrophic muscle fibres, in subjacent transverse vessels as well as in some non-atrophic fibres and capillaries in deeper regions of the fascicles (Supplementary Fig. 3).

Perimysial alteration in dermatomyositis muscle specimens

All 10 dermatomyositis specimens displayed increased perimysial connective tissue. Collections of mononuclear cells appeared in the perimysium in eight and in the endomyosium in 3 of 10 patients, but only in one patient did inflammatory cells abut on areas of perifascicular atrophy. In
eight patients few punctate MAC deposits appeared in perimysium (Supplementary Fig. 4A) that frequently were adjacent to perifascicular atrophy regions. Owing to the paucity of the perimysial vessels in patient and control muscles, we were unable to compare their respective densities. None of the perimysial vessels displayed MAC deposits.

**Plasma cells in dermatomyositis muscle**

Seventeen of 50 dermatomyositis muscle specimens with significant inflammatory infiltrates or IgM-positive capillaries were analysed for plasma cells. The plasma cells appeared at perivascular sites in perimysium or were randomly scattered in the endomysium and accounted for <2% of the total mononuclear cells in all specimens (Supplementary Fig. 4B and C).

**Membrane attack complex, IgG, and IgM deposits in dermatomyositis muscle**

In 31 of 50 patients with dermatomyositis, MAC deposits appeared in perifascicular atrophy regions on capillaries (Fig. 5A–F) or more frequently on capillary remnants; in three patients MAC deposits also appeared on preserved capillaries in nonatrophy fibre regions (Supplementary Table 4). Microvascular MAC deposits were also noted in one to four transverse vessels in five patients with dermatomyositis. No IgG deposits appeared on any MAC positive capillaries or capillary remnants.

IgM deposits were present in 10 of 50 dermatomyositis specimens (Fig. 5H and K). In 3 of 10 specimens a few IgM positive capillaries also reacted for MAC (Fig. 5G–I); in the remaining seven specimens IgM positive capillaries failed to react for MAC (Fig. 5J–L).

**Complement pathway analysis in lupus nephritis, Duchenne dystrophy and dermatomyositis**

We next examined complement pathways leading to assembly and binding of MAC to targets in lupus nephritis, necrotic fibres in Duchenne dystrophy, and microvessels in dermatomyositis. In lupus nephritis, MAC reactive glomeruli and tubules (Fig. 6A, D, G and J) co-localized with IgG (Fig. 6B and C), IgM (Fig. 6E and F), C1q (Fig. 6H and I), and C4d (Fig. 6K and L), consistent with immune complex-dependent activation of the classical complement pathway. In the renal autopsied specimens from a control subject without known renal disease, we detected no IgG, IgM, C1q, and C4d deposits but did observe variable amounts of MAC in glomeruli, tubular basement membranes, and vessel walls (Supplementary Fig. 5), as previously noted by other investigators (Hinglais et al., 1986; Bariety et al., 1989; Herlitz et al., 2012).

In Duchenne dystrophy, necrotic fibres identified by routine histology (Fig. 7A), reacted for MAC (Fig. 7B, E, H and K), C1q (Fig. 7I) and C4d (Fig. 7L) but not for IgG (Fig. 7C), consistent with activation of the classical pathway but rare necrotic fibres also reacted for IgM (Fig. 7F).

In dermatomyositis, like in Duchenne dystrophy, the pattern of complement activation was consistent with activation of the classical pathway in that the MAC-reactive capillaries and transverse vessels (Fig. 8A, D and G) did not bind IgG (Fig. 8B), but reacted for C1q (Fig. 8E and F) and C4d (Fig. 8H and I) but few MAC-positive capillaries also reacted for IgM (Fig. 8H and K). In all three tissues investigated, none of the MAC deposits co-localized with mannoside binding lectin indicating that complement activation did not occur through the lectin pathway.

**Discussion**

In this study we confirm that perifascicular atrophy in dermatomyositis is invariably associated with capillary
(Carpenter et al., 1976; Emslie-Smith and Engel, 1990) and transverse vessel (Gitiaux et al., 2013) depletion and demonstrate that the MAC deposits in capillaries result from activation of the classical complement pathway. Whether damage of the transverse vessels precedes or occurs concurrently with the capillary damage cannot be determined from our findings. That focal capillary and transverse vessel depletion cause ischaemia is supported by microinfarcts in muscle in some patients with dermatomyositis. Indirect evidence for focal ischaemia in dermatomyositis comes from upregulation of 4-hydroxy-2-nonenal, a marker of oxidative stress/lipid peroxidation, in blood vessels and muscle fibres in regions of perifascicular atrophy (Gitiaux et al., 2013).

Figure 2 Transverse vessels and capillaries in normal control and dermatomyositis muscle tracked in four non-consecutive 10-μm thick sections 100 μm apart. (A–D) Normal control muscle demonstrates multiple transverse vessels at each level (asterisks). (E–H) Perifascicular regions in dermatomyositis muscles show profound capillary and transverse vessel depletion at each level of sectioning. Asterisks in E indicate atrophic fibres. Scale bars = 50 μm.
Complement activation in dermatomyositis could be caused by four different mechanisms: first, by activation of the classical pathway triggered by binding of IgG or IgM to endothelial antigens or neoepitopes expressed by injured endothelial cells (Greenberg, 2007; Salajegheh et al., 2010); second, by activation of the lectin pathway due to the binding of mannose binding lectin to cytokeratin exposed on ischaemic endothelial cells (Ricklin et al., 2010; Kaveri et al., 2012); third, by activation of the alternative complement pathway due to loss of complement inhibitory proteins from the surface of damaged cells (Diepenhorst et al., 2009; Uchida, 2014); and fourth, by activation of the classical complement pathway in response to tissue injury (Ricklin et al., 2010). The presence of C1q and C4d on most MAC-positive capillaries and capillary remnants is strong evidence for activation of the classical pathway in dermatomyositis. The absence of IgG from all MAC-positive capillaries and the presence of IgM on only a few capillaries in few specimens (also see below) suggest that activation of the classical pathway in dermatomyositis is triggered by the direct binding of C1q to necrotic or damaged capillaries.

The infrequent IgM deposits on capillaries in dermatomyositis and on necrotic fibres in Duchenne dystrophy likely represent natural polyspecific antibodies with innate immunity-like properties found in sera of healthy individuals that play a role in removal of cellular debris (Kaveri et al., 2012; Uchida, 2014). The binding of natural IgMs to neoepitopes expressed on damaged endothelium could result in unregulated complement activation and enhanced tissue injury, as observed in ischaemia-reperfusion injury (Diepenhorst et al., 2009). When this occurs, acute inflammatory cells are chemotactically recruited and microthrombi form at the sites of unregulated complement activation (Diepenhorst et al., 2009; Nankivell and Alexander, 2010; Ricklin and Lambris, 2013) but these features were not observed in relation to the infrequent IgM reactive capillaries in the 50 dermatomyositis specimens examined by us.

Our observations do not shed light on the primary cause of the microvascular injury in dermatomyositis. Previous investigations (Greenberg et al., 2005; Greenberg, 2007, 2010a, b) have attributed the capillary loss and the perifascicular atrophy in dermatomyositis to effects of enhanced expression of interferon-inducible gene products. Interestingly, damaged muscle fibres in tissue culture can also release type 1 interferon and this might act as a positive feedback after initial tissue injury has been initiated by interferon released from plasmacytoid cells (Vaesoontachoon et al., 2013; Suarez-Calvet et al., 2014). The pathogenic role of type 1 interferon in dermatomyositis is in harmony with the evidence that plasmacytoid dendritic cells and type 1 interferons are critically involved in the pathogenesis of systemic lupus erythematosus (Rowland

**Figure 3** Microvascular abnormalities in dermatomyositis. In A–C endothelial cells are visualized with the biotin-UEA1-peroxidase method. (D) Immunostained for MAC. (A) Normal transverse vessel (arrowhead) in a normal fibre region. (B) Abnormally dilated transverse vessel in a perifascicular atrophy area (arrow) with accumulation of debris in the nearby fibre region. (C) Segmental occlusion of a transverse vessel (arrow). (D) MAC deposition in a transverse vessel (arrow) and in scattered capillaries. Scale bars = 50 µm.
et al., 2014; Sisirak et al., 2014), and the pathology in systemic lupus erythematosus myositis closely resembles that of dermatomyositis (Oxenhandler et al., 1982). Why the cytokine damage in dermatomyositis but not in inclusion body myositis or polymyositis, is so focused on the capillaries is a challenging but still unsolved question.

The exact mechanisms by which interferon 1-induced gene products cause capillary injury remains to be
established. A plausible mechanism would be that MHC1 upregulation and interferon 1 inducible gene products in endothelial cells cause endoplasmic reticulum stress and cell injury (Nagaraju et al., 2005; Rayavarapu et al., 2012). The pathological changes in the perifascicular muscle fibres could be due to expression of interferon 1-induced gene products, to ischaemia due to capillary depletion, or a combination of both factors (Franzi et al., 2013). That capillary depletion can precede perifascicular fibre injury would be consistent with the observation that in early dermatomyositis with minimal structural changes in the muscle fibres there is significant focal capillary depletion (Emslie-Smith and Engel, 1990).

Both light and electron microscopic studies indicate the perifascicular atrophy itself is preceded by or associated with structural changes in the muscle fibres such as rarefaction of myofibrillar markings, microvacuoles, focal basophilia, small lakes of glycogen, focal decreases or increases in oxidative enzyme activity, and internal nuclei (illustrated in Fig. 4). A variety of ultrastructural alterations also suggest that perifascicular atrophy is an end result of protracted local injury and incomplete repair. These include focal loss of mitochondria, focal myofibrillar degeneration beginning at the Z disks, and abnormal areas devoid of myofibrils containing glycogen granules, displaced sarcotubular components, small clusters of mitochondria, and debris. Other fibre regions show signs of

**Figure 5** Co-localization of MAC with UEA1 and of MAC with IgM in two patients with dermatomyositis. (A–C and G–I from Patient 1; D–F and J–L from Patient 2). MAC binds only to segments of a few capillaries in the imaged fields (yellow signal in C and F). Patient 1 displays numerous MAC reactive capillaries (G) but only a few of these react for IgM (H and I). In Patient 2, the imaged field displays few IgM reactive capillaries (K) but none of these bind MAC (J). Scale bars = 20 μm in A to F and 50 μm in G to L.
local regeneration and are rich in ribosomes, rough endoplasmic reticulum profiles, primitive myofibrils bearing small nemaline rods, and proliferating transverse tubular and sarcoplasmic reticulum profiles (Carpenter and Karpati, 2001; Engel and Hohlfeld, 2004).

The inflammatory cells in dermatomyositis, composed mostly of CD4+ T cells, CD20+ B cells, and CD25+ plasma cells are concentrated at perimysial sites where they are often perivascular in location (Arahata and Engel, 1984). We also detected plasma cells among perimysial inflammatory cells, but they accounted for <2% of the cells and were often remote from sites of perifascicular atrophy. The possibility remains that these cells are the source of IgM identified in few capillaries in 10 of 50 patients, but if so this is unlikely to be a major cause of the microvascular injury. The significance of the infrequent punctate perimysial MAC deposits remains unclear. Although they could be tombstones of degraded perimysial blood vessels, they were infrequent and did not always abut on regions of perifascicular atrophy.

We conclude that, except for early dermatomyositis, (i) perifascicular atrophy is invariably associated with reduction of capillary and transverse vessel densities; (ii) ischaemia is a contributory factor for perifascicular atrophy; and (iii) the microvascular MAC deposits are caused by the activation of the classical pathway triggered not by binding of IgG but binding of C1q to damaged endothelial cells.

Figure 6 Immunolocalization studies in lupus nephritis indicate antibody-dependent activation of the classical complement pathway. MAC (left column, green) co-localizes with IgG, IgM, C1q and C4d (middle column, red) and merge (right column). Reactivity for mannose binding lectin was absent (not shown). Scale bars = 20 μm.
Figure 7 Immunolocalization studies in Duchenne dystrophy indicate antibody-independent activation of the classical complement pathway. Non-consecutive serial section of a cluster of necrotic fibres shown in trichromatically stained section (A) were immunoreacted for MAC (left column, green), with IgG, IgM, C1q and C4d (middle column, red), and merge (right column). Co-localization of MAC with C1q and C4d, and rarely with IgM and absence of IgG deposits is consistent with activation of the classical complement pathway due to direct binding of C1q to necrotic fibres. Reactivity for mannose binding lectin was absent (not shown). Scale bars = 20 μm.
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Supplementary material
Supplementary material is available at Brain online.

References