The Role of Cytotoxic Effector Molecules and Cytokines in Inflammatory Myopathies

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Abstract

The inflammatory myopathies include dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM). In DM, muscle fiber injury is secondary to an antibody- or immune-complex-mediated immune response against a vascular-endothelial component. In PM and IBM, initially non-necrotic muscle fibers are invaded and eventually destroyed by $CD8^+$ T cells and macrophages. The autoaggressive T cells have the phenotype of activated ($HLA\text{-}DR^+$) memory ($CD45RO^+$) cells. T cell receptor (TCR) analyses revealed that the autoaggressive T cells are oligoclonal. In inflammatory lesions, muscle fibers express a number of cytoplasmic and surface molecules that are not detectable in normal muscle fibers. These molecules, which include HLA-class I antigens, heat-shock proteins, adhesion molecules and Fas, are probably induced by locally secreted cytokines. Although many of the muscle fibers invaded by $CD8^+$ T cells express the Fas "death receptor", signs of apoptosis are absent. However, the autoaggressive $CD8^+$ T cells possess perforin-containing granules, which they orient towards the contact zone with the target muscle fiber. This is consistent with a perforin- and secretion-dependent mechanism of muscle fiber injury.

Key words: autoimmunity, cytokines, dermatomyositis, polymyositis.

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The (idiopathic) inflammatory myopathies include dermatomyositis (DM), different forms of polymyositis (PM) and inclusion-body myositis (IBM) (reviewed in refs. [17, 22, 37, 54]). Clinically, DM can be distinguished from PM and IBM by characteristic skin manifestations. A microscopic feature highly characteristic of DM is perifascicular atrophy, which is due to the degeneration of muscle fibers at the periphery of muscle fascicles secondary to microvascular damage. Quantitative morphological analyses suggest that depletion of capillaries is one of the earliest changes in DM. Immunofluorescence studies revealed the deposition of complement membrane complexes in or around microvascular endothelium in a significant proportion of capillaries [56]. These observations support the concept that an antibody- or immune-complex-mediated response against a vascular-endothelial component is a primary pathogenetic mechanism in DM.

In contrast, in PM and IBM there is a conspicuous endomysial inflammatory exudate containing large numbers of $CD8^+$ T cells and only sparse B cells, along with collections of cytotoxic T cells (CTLs) and macrophages that surround and focally invade non-necrotic muscle fibers. Immunoelectron microscopy demonstrated that $CD8^+$ T cells and macrophages traverse the basal lamina, focally replace, displace or compresss the fiber, and ultimately replace entire segments of muscle fiber [1]. All of the invaded fibers and some noninvaded fibers, express increased amounts of HLA-class I, but not class II molecules (reviewed in refs. [22, 37]). By contrast, normal muscle fibers do not express detectable amounts of HLA class I or class II antigens. Taken together, these observations are consistent with an HLA class I-restricted CTL-mediated response against antigen(s) expressed in muscle fibers in PM and IBM. Consistent with this hypothesis, $CD8^+$ T cells expanded from muscle of patients with different inflammatory myopathies may show low but significant cytotoxicity against autologous cultured myotubes [35].

This article focuses on the role of cytotoxic effector molecules and cytokines in inflammatory myopathies. Specific topics include the phenotype and activation

state of the inflammatory T cells, expression of cytokines and adhesion molecules in inflammatory lesions, T cell receptor (TCR) repertoire of autoaggressive T cells, and mechanisms of muscle fiber injury.

Histological Features

In PM the endomysial inflammatory infiltrate is typically dominated by $CD8^+$ T lymphocytes, which surround, invade and eventually destroy muscle fibers. In a rare subtype of PM the infiltrate consists of gamma-delta T-lymphocytes [38]. In contrast to noninflamed muscle, the invaded muscle fibers express HLA class I molecules. This is a prerequisite for the immunological interaction with $CD8^+$ T cells. The different stages of CTL-mediated myocytotoxicity were analysed by immunoelectron microscopy [1]. Initially, $CD8^+$ cells and macrophages abut on and send spikelike processes into nonnecrotic muscle fibers. Subsequently, an increasing number of $CD8^+$ cells and macrophages traverse the basal lamina and focally replace the fiber.

DM is characterized by perivascular and perifascicular infiltrates consisting predominantly of B-lymphocytes, macrophages and $CD4^+$ T lymphocytes. Immunhistochemistry shows immune complexes and C5b9-complement (membrane attack complex) on small blood vessels, suggesting a humoral immune effector mechanism [56]. The immune processes affecting the muscle microvasculature lead to a reactive proliferation of endothelial cells and a reduction of muscle capillaries. Electronmicroscopy demonstrates tubulovesicular inclusions in endothelial cells. Capillary changes are thought to be the cause of the characteristic perifascicular muscle fiber atrophy in DM. Perifascicular atrophy is diagnostic for DM, even in the absence of an inflammatory infiltrate. As in PM, the molecular target of the autoimmune reaction in DM has not yet been defined.

In IBM eosinophilic inclusions are found in the cytoplasm and nuclei. Irregular ”rimmed vacuoles” are present in 2 - 70 % of the muscle fibers [16]. In sporadic IBM, endomysial infiltrates dominated by $CD8^+$ T lymphocytes resemble those seen in PM. In familial IBM inflammatory changes are absent (”familial inclusion body myopathy”). The inclusions represent an accumulation of proteins, some of which contain proteins also found in Alzheimer’s disease [2, 3, 31]. Electronmicroscopically the inclusions appear as 15-21 nm helical filaments and 6-10 nm amyloid-like fibrils.

Expression of Cytokines and Adhesion Molecules

Using reverse-transcriptase PCR, Lundberg et al. [50] found moderate to strong expression of IL-4 in PM, whereas IL-4 expression was low or absent in IBM and DM. GM-CSF and TGF-ß were expressed in most inflammatory myopathy cases but not in noninflammatory controls. Confalonieri et al. [14] reported that TGFß1 is expressed significantly more strongly in DM than in PM and controls. By immunohistochemistry, TGFß1 was localised mainly in connective tissue, indicating a possible relationship with connective tissue proliferation. Using immunohistochemical techniques, Lundberg et al. [51] found prominent expression of IL-1α, IL-1ß and TGFß isoforms in PM, IBM, and DM, but not in normal control muscle. IL-1α was expressed in endothelial cells and inflammatory cells, whereas IL1-ß was expressed only in inflammatory cells, not in blood vessel walls. TGFß1 and TGFß3 were expressed mainly in inflammatory cells and muscle fiber membranes. TGFß2 was found in endothelial cells and inflammatory cells. Tews and Goebel [67] detected IL-1α and -ß, IL-2, IL-4, TNF-α and -ß, and interferon-γ in a proportion of inflammatory cells, and IL-1α and -ß, IL-2 and TNF-α also in muscle fibers. Tateyama et al. [65] identified TNF-α in inflammatory cells in several PM cases.

Cell adhesion molecules, many of which are inducible by cytokines, participate in target-effector cell interactions in cell-mediated cytotoxicity and in leukodiapedesis in inflammatory diseases. De Bleecker and Engel [18] demonstrated that ICAM-1 is strongly induced on the surfaces of nonnecrotic muscle fibers where they are invaded by autoaggressive cells, suggesting that it serves as an important ligand for these cells. In DM, ICAM-1 was strongly expressed on endothelial cells of perimysial arterioles and venules and on some perifascicular capillaries. In the other myopathies, vascular ICAM-1 expression was restricted to endothelia of capillaries surrounded by inflammatory cells, suggesting that this ligand is differentially activated in DM [18]. Additional studies of adhesion molecule expression in inflammatory myopathies have been reported [4, 13, 66].

It is likely that myoblasts (and perhaps also mature muscle fibers) can be a source of various cytokines, both *in vitro* and *in vivo*. The complete spectrum of cytokines that can be produced by human myoblasts has not yet been established. One example of a cytokine that can be induced in myoblasts is interleukin-6, as demonstrated by Bartoccioni and colleagues [26, 47].

In spite of some discrepancies between the different studies, which are probably explained by methodological problems, it is safe to conclude that in the inflammatory myopathies, many inflammatory cells, muscle fibers and endothelial cells express a complex array of different cytokines. The local production of cytokines is likely to induce several cell interaction and adhesion molecules on these tissue elements.

Phenotype and Activation State of Inflammatory T Cells

In both IBM and PM, approximately one-third of all autoinvasive cells and about one-half of the $CD8^+$

autoinvasive T cells are HLA-DR$^+$, suggesting that they are activated [21]. The vast majority of the inflammatory CD4$^+$ and CD8$^+$ T cells display the phenotype of memory T cells, that is, they express the RO isoform of the leukocyte common antigen CD45 [19]. The intensity of the CD45RO signal was similar in all CD8$^+$ T cells regardless of their position relative to the invaded muscle fiber surface. A similar expression pattern was noted for the leukocyte function associated antigen-(LFA-) 1 [18, 39]. LFA-1 (CD11a/CD18) is a ß2 integrin that has a key role in mediating leukocyte adhesion to endothelium and T cell adhesion to target cells. ICAM-1, a ligand of LFA-1, was upregulated especially on T cells in the vicinity of invaded muscle fibers, suggesting that the expression of CD45RO and ICAM-1 is differentially regulated [18]. Indeed, LFA-1 is mainly constitutively expressed, whereas ICAM-1 is widely inducible on B and T cells. Taken together, these results establish that the autoaggressive (autoinvasive) T cells in the inflammatory lesions of PM and IBM muscle represent activated CD8$^+$ memory T cells.

Receptor Repertoire of Autoaggressive T Cells

The characteristic lesion of PM and IBM has several features that make it an ideal paradigm to study CD8$^+$ T cell-mediated immunopathology. The muscle fiber target cells can be readily distinguished from the effector T cells. Further, different populations of inflammatory T cells can be discerned: one population, which deeply invades muscle fibers (the autoaggressive or autoinvasive T cells), and another, which remains in interstitial areas and therefore seems to represent regulatory or bystander cells (the interstitial T cells).

Bender et al. [7] combined two independent PCR techniques with immunohistochemistry to characterize the TCR repertoire of inflammatory cells in muscle of a patient with typical PM. PCR revealed a preferential usage of TCR Vα33.1, Vß13.1 and Vß5.1. Six of 6 TCR Vα33.1$^+$ cDNA clones and 5 of 7 Vß13.1$^+$ clones had identical nucleotide sequences. In contrast, the Vß5.1$^+$ TCR were more heterogeneous. No TCR sequences could be amplified from noninflammatory control muscle. Furthermore, none of the TCR sequences found in PM muscle could be detected in blood from the same patient or from a normal control subject. Immunohistochemistry using monoclonal antibodies (mAb) specific for Vß5.1 or Vß13.1 confirmed that Vß5.1 and Vß13.1 were overrepresented in the muscle lesions. Thirty-two percent of all CD8$^+$ T cells were Vß13.1$^+$, and 16% were Vß5.1$^+$. However, approximately 60% of the CD8$^+$ T cells that invaded muscle fibers were Vß13.1$^+$, whereas less than 10% were Vß5.1$^+$.

This analysis of the TCR repertoire expressed in PM muscle [7] demonstrates two types of CD8$^+$ inflammatory T cells. One type of T cells is mainly located in interstitial areas. These cells are heterogenous and presumably include nonspecifically recruited bystanders. Another type of CD8$^+$ T cells invades the muscle fibers. These cells are clonally expanded and presumably specific for the yet unknown auto-antigen(s). These findings are consistent with the results of previous studies, which did not combine sequence with histological analysis of TCR Vß expression [46, 53, 59]. Differences in TCR usage in the different studies presumably reflect differences between individual patients, e.g. different HLA types.

A possible clue to the nature of the suspected autoantigen(s) was provided by the discovery of a rare variant of PM. In this variant, CD3$^+$CD4$^-$CD8$^-$TCRγδ$^+$ T cells surrounded and invaded non-necrotic muscle fibers in the same way as CD3$^+$CD8$^+$TCRαβ$^+$ T cells in the more common forms of PM [38]. The autoaggressive myocytotoxic γδ T cells were essentially monoclonal, and expressed an unusual Vγ3Jγ1Cγ1-Vδ2Jδ3Cδ disulfide-linked TCR [61]. In γδ T-cell-mediated PM, all muscle fibers expressed MHC class I antigen and showed intense reactivity with a monoclonal antibody specific for the 65 kDa heat shock protein (hsp) [36]. One possible implication of the striking co-localization of γδ T-cells with the 65 kDa hsp is that the autoinvasive γδ T-cells recognise hsp determinants on muscle fibers. Therefore, hsp may be considered as a candidate autoantigen in some inflammatory myopathies.

A number of studies have addressed the T cell repertoire expressed in IBM muscle, using immunohistochemistry [46] or PCR [25, 58]. Lindberg et al. [46] compared the expression of TCR V genes in IBM, PM and DM, using 10 different TCR Vβ-specific monoclonal antibodies. The most abundant TCR Vß elements detected with these mAbs were Vβ3 and Vβ19. TCR sequences were not reported [46]. Using PCR with TCR V family-specific primers, O´Hanlon et al. [58] analysed the TCR repertoire in muscle biopsy specimens from 13 IBM patients. On average, 6 to 7 TCR Vβ families were detected per specimen. Vβ3 and Vβ6 were detected more frequently than the other Vβ families. Sequence analysis of the expressed Vβ3 and Vβ6 receptors was done in 3 patients. In one patient, both the Vβ3 and Vβ6 sequences were heterogeneous. This raised the possibility of a superantigen effect [58]. However, in the two other patients, 5 of the 10 sequenced Vβ3 cDNA clones were identical [58]. This would be more consistent with clonally dominant T cells recognizing a defined antigen. An additional argument against a superantigen effect is that superantigens typically activate CD4$^+$ T cells by bridging the TCR to HLA class II molecules expressed on other cells [24, 43]. However, muscle fibers do not normally express detectable levels of HLA class II even in an inflammatory environment [20, 42] and the autoinvasive T cells in muscle are CD8$^+$ rather than CD4$^+$ [37]. Using RT-PCR, Fyhr et al. [25]

found a limited repertoire of TCRs expressed in muscle of six analysed patients. TCR Vß3, 5.2, 8, 12, 14, and 22 were each expressed in at least 3 cases. No TCR sequences were reported, and PCR was not combined with immunohistochemistry. Finally, using a combination of PCR and immunohistochemistry, we found a high degree of clonal restriction of TCR Vß families expressed by autoinvasive $CD8^+$ T cell clones in IBM [6]. In conclusion, the presently available data indicate that the TCR repertoire expressed in muscle is similar in IBM and PM: the autoaggressive T cells are oligoclonal, suggesting that they recognise a limited number of HLA-class-I-associated antigenic peptides.

Apart from inflammatory changes, microscopic findings in IBM include rimmed vacuoles, congophilic amyloid deposits typically near or within the vacuoles and occasionally in nuclei, necrotic and regenerating fibers, small groups of atrophic fibers, and mitochondrial abnormalities [3, 11, 31, 57]. The relative significance of these alterations and their possible relation to the inflammatory changes are presently debated [31]. That nonnecrotic muscle fibers invaded by T cells are severalfold more frequent than fibers displaying other pathologic alterations [62] suggests that, despite refractoriness to immunotherapy, immune mechanisms play an important role in the pathogenesis of IBM.

This notion is further supported by the strong association of IBM with the MHC antigens HLA-DR3, DR52 and B8 [27] and the known association with other autoimmune diseases (for reviews of IBM, see [3, 11, 17, 28, 31, 57]).

Cytotoxic Mechanisms

The precise mechanism by which the invading $CD8^+$ T cells kill muscle fibers in PM and IBM are still unknown. There is evidence that a perforin- and secretion-dependent mechanism contributes to the muscle fiber injury. Perforin has been localised in inflammatory T cells by immunohistochemistry [29, 60] and by *in situ* hybridisation [12]. In PM but not in DM, the autoinvasive T cells orient their perforin-containing cytotoxic granules towards the target muscle fiber [29], providing suggestive evidence that secretion of this cytotoxic effector molecule contributes to muscle fiber injury.

Perforin is not the only potentially cytotoxic molecule expressed in inflammatory myopathies. For example, muscle fibers in myositis display distinct up-regulation both of inducible and neuronal nitric oxide synthase (NOS) [69]. It may be speculated that the enhanced expression of NOS with production of nitric oxide contributes to oxidative stress, mediating muscle fiber damage.

In addition to perforin-dependent killing, cytotoxic T cells can kill by a nonsecretory, ligand-mediated mechanism. This second killing mechanism requires the interaction between Fas (expressed on the target cell) and Fas-ligand (expressed on the T cell). Fas-mediated cytotoxicity is thought to induce programmed cell death ("apoptosis") rather than "necrosis", although it is not always possible to relate the different modes and mechanisms of cell death to specific morphological features and triggering events. The special properties of muscle fibers as giant syncytial cells with hundreds of nuclei further complicate the classification of any morphological changes as "necrosis" or "apoptosis" (see “conclusions” at the end of this article) .

In several studies, different groups of investigators found no evidence that apoptosis is a mechanism of muscle fiber injury in human inflammatory myopathies [5, 64, 69] or dystrophies [40]. On the other hand, in PM, DM, and IBM many muscle fibers express Fas [5]. What could explain the discrepancy between the expression of the Fas “death receptor” on muscle fibers and the absence of signs of apoptosis? One possibility is that the Fas pathway is not activated because of absence or insufficient expression of Fas-ligand (Fas-L) in the T cells that contact or invade muscle fibers. However, this is unlikely. Using a polyclonal rabbit anti-Fas-L antiserum, we found that Fas-L immunoreactivity co-localized with $CD3^+$ inflammatory cells (unpublished observation). Another, more likely possibility is that muscle fibers are intrinsically resistant to Fas-mediated classical apoptosis, at least *in vivo*. Resistance could be related to the peculiar properties of syncytial muscle fibers discussed above, or the expression of specific inhibitory factors such as Bcl-2, or both. Indeed, the majority of Fas^+ fibers co-express Bcl-2 [5]. Bcl-2 and another anti-apoptotic protein, Bcl-x, have also been localised in atrophic muscle fibers in late-onset spinal muscular atrophy [68]. Bcl-2 protects against Fas-based but not perforin-based T-cell-mediated cytolysis [44]. The exact mechanisms of Bcl-2-mediated protection need yet to be defined, but it has been proposed that Bcl-2 binds and inactivates various apoptosis-inducing factors including Bax [23].

Fas expression was observed not only in muscle fibres but also in inflammatory cells in PM, IBM, DM and Duchenne muscular dystrophy [5]. Immunologically naive peripheral T cells are known to express little or no Fas on their surface, whereas previously activated memory T cells express relatively high amounts of cell-surface Fas (reviewed in [52]). Interestingly, Fas expression in lymphocytes can have different functional consequences. In freshly isolated T cells, ligation of Fas with anti-Fas mAb leads to enhanced proliferation, increased expression of activation markers and production of cytokines such as interleukin-2, interferon-γ and TNF-α [52]. By contrast, chronically activated T cells are susceptible to Fas-mediated apoptosis [10,52]. It is therefore thought that Fas is an

important factor in the homeostatic regulation of immune responses: Fas-mediated costimulation seems to contribute to clonal expansion and effector function of T cells during the early stage of an immune response. Later, Fas-mediated apoptosis helps to eliminate chronically activated T cells [52]. It appears that although a proportion of inflammatory cells do express Fas, like muscle fibers they are protected from apoptosis by Bcl-2 or other anti-apoptotic molecules.

In summary, the observation that the autoinvasive T cells express and orient perforin towards target muscle fibers is consistent with a secretion- and perforin-dependent cytotoxic mechanism. On the other hand, although many muscle fibers express Fas, the nuclear changes of apoptosis are essentially absent in the inflammatory myopathies. Resistance to Fas-mediated injury could be related to the expression of specific protective factors. Indeed, in PM and IBM, the majority of Fas-positive muscle fibers coexpress Bcl-2, a protein known to protect from apoptosis.

Immunological Properties of Cultured Muscle Cells

Tissue culture systems are a powerful tool for the study of the functional aspects of cell-mediated myocytotoxicity. Human myogenic stem cells (myoblasts) can be isolated and purified from muscle biopsy specimens and expanded in culture [30]. In contrast to fibroblasts, myoblasts express the cytoskeletal protein desmin and the neural cell adhesion molecule N-CAM (CD56/Leu 19/NKH-1) [37]. Myoblasts constitutively express HLA class I antigens and a low level of lymphocyte function-associated (LFA-) molecule 3 (LFA-3, CD58). Tumor necrosis factor (TNF)-α, a cytokine secreted by macrophages, T cells and NK cells induces myoblasts to express the intercellular adhesion molecule-1 (ICAM-1, CD54) [30]. Gamma-interferon, a cytokine secreted by T cells and natural killer cells, induces myoblasts to express HLA-DR and ICAM-1 [30, 33, 55]. HLA-DP and HLA-DQ are also inducible by gamma-interferon but the kinetics of induction and the levels of expression vary with the different HLA class II molecules [30]. Both TNF-α and IFN-γ are expressed in inflammatory myopathies together with other proinflammatory cytokines. Further, various cell adhesion molecules have been detected in muscle, suggesting that the *in vitro* models adequately reflect the situation *in vivo*.

Interaction of Myoblasts and $CD8^+$ Cytotoxic T Cells

Cultured myotubes and myoblasts express HLA class I molecules. This qualifies them as potential targets of $CD8^+$ CTL. Lysis of myotubes by CTL was shown in different experimental situations. On the one hand myotubes were lysed by allogeneic $CD8^+$ CTL lines raised against the allogeneic HLA antigens expressed by the myotubes [34]. Autologous control myotubes were not lysed. Lysis involved the recognition of allogeneic class I HLA antigens since it was completely blocked by a monoclonal antibody against a monomorphic determinant of HLA class I [34]. Furthermore, myotubes were lysed by autologous polyclonal $CD8^+$ T cell lines directly expanded from muscle of patients with different inflammatory myopathies [35]. The results obtained in this model system clearly establish that cultured myotubes are fully susceptible to HLA-class I restricted lysis by $CD8^+$ CTL. The autoreactive myocytotoxicity is consistent with the hypothesis that some of the CTL isolated from muscle recognize the same antigen on myotubes *in vitro* that they recognize on muscle fibers *in vivo*.

Interaction of Myoblasts and $CD4^+$ T Cells: Myoblasts as Antigen Presenting Cells

Antigen presentation to $CD8^+$ T cells requires the expression of HLA class I antigen on the antigen presenting cell. Antigen presentation to $CD4^+$ T cells depends on the constitutive or induced expression of HLA class II on the presenting cell. Since myoblasts can be induced to express HLA class II by gamma-interferon, we tested the ability of highly purified human myoblasts to present various protein antigens to autologous $CD4^+$ T cell lines specific for tuberculin, tetanus toxoid or myelin basic protein [30]. Noninduced myoblasts or myoblasts treated with TNF-α alone could not present any of these antigens to T cells. However, gamma-interferon-treated myoblasts induced antigen specific T cell proliferation and were killed by the T cells only in the presence of the relevant antigen [30]. Antigen specific lysis was reduced to background level by adding the anti-HLA-DR monoclonal antibody L-243. These results suggest that HLA class II-positive human myoblasts can act as facultative local antigen presenting cells in muscle by providing the signals necessary to trigger both antigen specific lysis and T cell proliferation. It is difficult to demonstrate HLA-DR by immunohistochemistry on the surface of human muscle fibres in inflammatory lesions, but this does not necessarily imply that HLA-DR is absent [47].

An interesting question is whether myoblasts express co-stimulatory molecules like B7.1 (CD80) and B7.2 (CD86). The co-stimulatory molecules B7.1 (CD80) and B7.2 (CD86) are members of the immunoglobulin superfamily [9, 41, 45, 63, 70]. They are expressed mainly on professional antigen-presenting cells and T cells. Although their precise function(s) and relationship with the ligands CD28 and CTLA-4 is still not completely understood, there is universal agreement that the B7 molecules provide critical co-stimulatory signals to T cells and play an essential role in normal and pathological immune reactions [9, 41, 45, 48, 63, 70].

Human myoblasts do not express B7.1 or B7.2 under normal conditions [8, 15]. Interestingly however, they can be induced to express a novel, hitherto undefined functional member of the B7 family of co-stimulatory molecules (L.Behrens et al., unpublished observation).

Conclusions

The results of the studies reviewed here are consistent with the following sequence of pathogenetic events. First, some muscle fibers, which do not constitutively express detectable levels of MHC class I, are induced to express MHC class I and class I-associated (auto)antigen(s). Next, the MHC class I-positive muscle fibers are surrounded by $CD8^+$ T cells, some of which traverse the basal lamina of the muscle fiber and contact the muscle fiber surface. After recognition of "their" antigen, the $CD8^+$ become activated and secrete perforin and perhaps other cytotoxic effector molecules.

In the early stages of muscle fiber invasion, the surface membrane of muscle fibers appears to remain intact at the light microscopic [21] and electronmicroscopic [1] level. Pore-like structures could not be detected in the sarcolemma of muscle fibers attacked by T cells in PM [1]. One possible explanation is that perforin pores/channels on nucleated cells *in vivo* are smaller in size than the pores generated *in vitro* on erythrocytes and other target cells by the addition of purified perforin. Perforin pores containing less than 10-20 monomers would escape detection by electron microscopy [49]. Another explanation for the lack of morphologically visible muscle cell damage is that the surface membrane of the muscle fiber is rapidly repaired at least during the early stages of muscle fiber invasion. Repair could occur, for example, by shedding or endocytosis of pore-damaged membrane (reviewed in ref.[32]).

As pointed out by Arahata and Engel [1], it is interesting to note that the volume of a 25-mm-long and 50-µm-wide muscle fiber is nearly 28,000-fold larger than, for example, that of a spherical 15-µm tumor cell. Perforin pores would allow the influx of calcium. Consistent with this assumption is the observation that invaded muscle fibers show signs of focal myofibrillar degeneration near invading cells [1]. These changes could be a consequence of membrane insertion of perforin and focal protease activation [1]. Another indirect sign of muscle fiber damage is the intense focal regenerative activity noted in areas immediately adjacent to autoinvasive T cells [1].

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Abbreviations

CTL, cytotoxic T lymphocyte; DM, dermatomyositis; HLA, tel. 49 89 7095 4780, fax 49 89 7095 4782, human leukocyte antigen; hsp, heat-shock protein; IBM, inclusion body myositis; ICAM, intercellular adhesion molecule; IL, interleukin; LFA, leukocyte function-associated antigen; NOS, nitric oxide syntase; PCR, polymerase chain reaction; PM, polymyositis; TCR, T cell receptor; TGF, transforming growth factor; TNF, tumour necrosis factor.