Pathophysiology of inflammatory and autoimmune myopathies

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In this issue

Inflammatory or necrotizing myopathies, myositis and other acquired myopathies, new insight in 2011
Benveniste O et al., Paris, France
Observations on the classification of the inflammatory myopathies
Hilton-Jones D, Oxford, United Kingdom
Pathogenic aspects of dermatomyositis, polymyositis and overlap myositis
Gherardi RK, Créteil, France
Sporadic inclusion-body myositis: conformational multifactorial aging-related degenerative muscle disease associated with proteasomal and lysosomal inhibition, endoplasmic reticulum stress, and accumulation of amyloid-β42 oligomers and phosphorylated tau
Askanas V et al., Los Angeles, USA
Pathophysiology of inflammatory and autoimmune myopathies
Dalakas MC, Athens, Greece
Myositis or dystrophy? Traps and pitfalls
Benveniste O et al., Paris, France
Therapy of polymyositis and dermatomyositis
Marie I, Rouen, France

Summary

The main subtypes of inflammatory myopathies include dermatomyositis (DM), polymyositis (PM), necrotizing autoimmune myositis (NAM) and sporadic inclusion-body myositis (sIBM). This review provides an update on the main clinical characteristics unique to each subset, including fundamental aspects on muscle pathology helpful to assure accurate diagnosis, underlying immunopathomechanisms and therapeutic strategies. DM is a complement-mediated microangiopathy leading to destruction of capillaries, distal hypoperfusion and inflammatory cell stress on the perifascicular regions. NAM is an increasingly recognized subacute myopathy triggered by statins, viral infections, cancer or autoimmunity with macrophages as the final effector cells mediating fiber injury. PM and IBM are characterized by cytotoxic CD8-positive T cells which clonally expand in situ and invade MHC-I-expressing muscle fibers. In IBM, in addition to autoimmunity, there is vacuolization and intracellular accumulation of degenerative and stressor molecules. Pro-inflammatory mediators, such as gamma interferon and interleukin IL1-β, seem to enhance the accumulation of stressor and amyloid-related misfolded proteins. Current therapies using various immunosuppressive and immunomodulating drugs are discussed for PM, DM and NAM, and the principles for effective treatment strategies in IBM are outlined.

Based on distinct clinical, immunopathological and histological criteria, the most common autoimmune inflammatory myopathies seen in practice are polymyositis (PM), dermatomyositis (DM), necrotizing autoimmune myositis (NAM) and inclusion-body myositis (IBM) [1–3]. This classification also reflects response to therapies as DM responds better than PM, PM better than NAM, and all three better than IBM. These disorders have primarily an autoimmune pathogenesis, mediated either by cytotoxic T cells, as in PM and IBM, by a complement-mediated microangiopathy as in DM, or by macrophages and possibly autoantibodies as in NAM [1–7]. In IBM, the autoimmune and inflammatory features coexist with degenerative, highlighted by vacuolization
and deposition of stressor and amyloid-related molecules within the muscle fibers [8–10]. Progress in immunopathology has helped us to better define these disorders and reach the correct diagnosis utilizing immunocytochemistry as a tool to distinguish IBM from PM, PM from NAM, and PM or NAM from the “inflammatory dystrophies” [1–5].

This review addresses the main immunopathologic features of inflammatory myopathies as related to disease mechanisms and response to therapies.

Clinicopathological correlations and diagnostic principles

All forms of inflammatory myopathies have in common a myopathy characterized by proximal and often symmetric muscle weakness that develops subacutely, over weeks to months as in PM and DM, or insidiously over months to years, as in IBM [4–7]; in NAM, however, the onset may be acute over days or weeks [1–3]. In contrast to all the others, the weakness in IBM affects also the distal muscles and can be asymmetric accompanied by muscle wasting from the outset. DM is a distinct clinical entity identified by a characteristic heliotrope rash on the upper eyelids, face or upper trunk that accompanies, or precedes, the evolving muscle weakness [1–7]. PM does not have any distinct features, and mimics other acquired or even hereditary myopathies, necessitating the need to exclude all conditions that cause a subacute myopathy. PM is uncommon and overdiagnosed; it should be a diagnosis of exclusion and considered in adults who do not have a rash, involvement of the extraocular and facial muscles (mild facial weakness is commonly seen in IBM and if present should be a sign against PM), family history of a neuromuscular disease, history of exposure to myotoxic drugs or toxins, endocrinopathy, neurogenic disease, an inflammatory dystrophy, or IBM [1–7,11]. The most common disorders misdiagnosed as PM are IBM, inflammatory dystrophies, and NAM [1–5]. IBM is the most common acquired myopathy in men above 50 years old [1–10].

Although it is commonly suspected when a patient with presumed PM does not respond to therapy, involvement of distal muscles, especially foot extensors and deep finger flexors, in almost all the cases, may be a clue to the early clinical diagnosis [1–10]. Dysphagia and facial muscle weakness are common, occurring in the majority of patients.

The clinically suspected diagnosis of PM, DM, NAM or IBM is aided by a varying degree of serum CK elevation and myopathic findings on needle electromyography, and confirmed by the muscle biopsy which, in spite of some limitations, is the definitive diagnostic test. Inflammation is the histologic hallmark for all the inflammatory myopathy subsets; however the site of inflammation, and the type of cells involved along with some additional features are characteristic for each subtype [1–11]. In PM and IBM the main inflammatory cells consist of T cells which are located endomysially within the muscle fascicles, and surround healthy muscle fibers expressing the MHC-I class antigen; these cells invade the muscle fibers resulting in phagocytosis and necrosis [1–7]. The MHC-1 upregulation is ubiquitously expressed on the sarcolemma even in fibers not invaded by CD8+ cells, as discussed below, and repeatedly emphasized before [1–5,7,10,11]. Accordingly, the CD8/MHC-1 complex is specific for PM and IBM and fundamental for confirming the diagnosis and excluding disorders with secondary inflammation as seen in inflammatory dystrophies [1–5]. It is a classic example of how an observation derived from the underlying nature of T cell immunopathology, as discussed below, has become a tool of diagnostic value useful to distinguish immune from non-immune myopathies [1–5]. In IBM, in addition to the inflammation, there are COX-negative fibers, rimmed vacuoles and tiny congophilic amyloid deposits within or next to the vacuoles [1–11].

In DM, the endomysial inflammation is predominantly perivascular or in the interfascicular septae and around, rather than within, the muscle fascicles [6,7]. The muscles fibers undergo necrosis, degeneration, and phagocytosis, often in groups involving a portion of a muscle fasciculus in a wedge-like shape or at the periphery of the fascicle, due to microinfarcts within the muscle [1–7], resulting in perifascicular atrophy. The presence of perifascicular atrophy even in the absence of inflammation, should raise the suspicion of DM.

In NAM, there is a characteristic absence of T cells while the MHC-I expression is spotty and mostly on the necrotic fibers; the main inflammatory cells are macrophages invading necrotic fibers [1]. NAM is a truly necrotizing myopathy mediated by macrophages and possibly antibodies, as discussed later.

Immunopathophysiology

Dermatomyositis

The primary antigenic target in DM is the vascular endothelium of the endomysial capillaries and to a lesser extent of larger

Glossary

ADC0 antibody-dependent cell-mediated cytotoxicity
APP amyloid precursor protein
DC dendritic cells
DM dermatomyositis
MHC major histocompatibility
NAM necrotizing autoimmune myositis
HTLV-1 human T cell lymphotropic virus
IBM inclusion body myositis
ICOS inducible costimulator
ICOS-L inducible-costimulator-ligand
PM polymyositis
SRP signal recognition particles
TCR T cell receptors
blood vessels [6,12,13]. The disease begins when putative antibodies directed against endothelial cells activate complement C3 that subsequently forms C3b and C4b fragments and lead to the formation of C5b-9 (MAC), the lytic component of the complement pathway [12–15]. MAC, C3b and C4b are detected early in the patients’ serum [14] and deposited on capillaries before inflammatory or structural changes are seen in the muscle. Sequentially, the complement-mediated alterations begin with swollen endothelial cells followed by vacuolization and necrosis of capillaries, perivascular inflammation, and ischemic muscle fiber damage [1–7]. The characteristic perifascicular atrophy is probably a reflection of the relative hypoperfusion in the perifascicular zones [1–7]. Finally, there is marked reduction in the number of capillaries per muscle fiber with compensatory dilatation of the lumen of the remaining capillaries [1–7,12–15]. The release of cytokines and chemokines related to complement activation, upregulate VCAM-1 and ICAM-1 on the endothelial cells and facilitate their exit through the blood vessel wall to the perimysial and endomysial spaces [16–20].

Immunophenotypic analysis of the lymphocytic infiltrates demonstrates B cells and CD4+ cells in the perimysial and perivascular regions [6] and plasmacytoid dendritic cells in the perifascicular regions [21] supporting the view that a humoral-mediated mechanism plays the major role in the disease. The perifascicular regions in DM contain many regenerating and degenerating fibers as they are in a stage of continuous remodeling [1–3]. As a result, they stain with alkaline phosphatase, desmin, NCAM, with the autoantibody against chromatin remodeler Mi-2, as shown recently [22], and with a variety of antibodies against immune or stressor molecules, including TGF-β, MHC-I, αβ-crystallin, cathepsins, amyloid precursor protein, STAT-1 (triggered by interferon-γ) [23], or myxovirus resistance Mxα protein (triggered by α/β-interferon) [21]. The theory that the perifascicular myofibers may be primarily injured by chronic overproduction of α/β-interferon-inducible proteins such as MxA [21] cannot explain any of the aforementioned observations, including the early complement activation and deposition on the capillaries that precedes perifascicular atrophy or the reduced number of capillaries throughout the fascicle that also occurs early in the disease process. Most importantly, the perifascicular fibers do not only recognize α/β-interferon-inducible proteins but many markers of regeneration, cell stress and tissue remodeling. Further, α/β-interferon-inducible genes lack specificity or uniqueness for DM because they are also overexpressed in PM [24] and other connective-tissue diseases. At the tissue level, there is also no evidence that α/β-interferon molecules exert toxicity to human muscle fibers in vitro, as has been shown for gamma interferon.

Genes for adhesion molecules, cytokines, chemokines and those associated with ischemia and degeneration are upregulated in the muscles of DM patients [25,26]. Among them, a biologically relevant protein, discovered by gene arrays, is the KAL-1 adhesion molecule because it is significantly downregulated in the DM patients who improved after therapy [26]. Further, the KAL-1 is upregulated in vitro by TGF-β and may have a role in inducing fibrosis [26,27]. The upregulation of α/β and γ interferon-inducible genes was proposed as a sign of a virus-driven autoimmune dysregulation [25], no viruses however, have been amplified from these patients’ muscles [28,29].

**Polymyositis and sIBM**

In PM and IBM there is evidence of an antigen-directed cytotoxicity mediated by CD8+ T cells invading MHC-I-antigen expressing muscle fibers [1–7,30,31]. The immune components associated with this process are identical in both PM and sIBM, in spite of poor response to immunotherapies of the latter. The immunopathologic nature of these disorders has been based on a series of landmark observations over the last 30 years.

The immunopathologic nature of these disorders has been based on a series of landmark observations over the last 30 years. The first breakthrough observation in immunopathology of PM and IBM was made by Engel and Arahata in a series of classic papers which showed that in PM and IBM it is the CD8+ cells that surround healthy, non-necrotic muscle fibers that eventually invade [32–35]. By immunoelectron-microscopy, the CD8+ cells and macrophages send spike-like processes into non-necrotic muscle fibers, which traverse the basal lamina and focally displace or compress the muscle fibers [32–35]. The autoimmune CD8+ T cells contain perforin and granzyme granules, which are vectorially directed towards the surface of the muscle fiber inducing necrosis upon release [36,37]. These cells are also cytotoxic in vitro when exposed to autologous myotubes [38] confirming that the major cytotoxic effector mechanism in PM and IBM is the perforin pathway. In contrast, the Fas-Fas-L-dependent apoptotic process is not functionally involved [39,40]. The T cells invading the muscle fibers are also activated expressing ICAM-I, MHC-I, CD45RO, and inducible costimulator (ICOS) on their surface [1–7,10,13,30]. In IBM, the T cell invasion of non-necrotic fibers is found early and in higher frequency than the Congo-red-positive fibers [41], suggesting that inflammation precedes the accumulation of amyloid and stressor molecules, an important observation in understanding what comes first in IBM, the inflammation or the amyloid (see discussion later).

The second most important observation in the immunopathogenesis of PM and IBM was the identification of the CD8/MHC-I lesion. Muscle fibers normally do not express detectable amounts of major histocompatibility (MHC) class I or II antigens. In PM and IBM however, widespread overexpression of MHC is an early event that can be seen even in areas remote from the inflammation [42,43]. Because the MHC-I is induced in vitro by
cytokines and chemokines such as IFN-γ or TNF-α [44–46], it is likely that the upregulation of MHC-I is related to continuous overexpression of cytokines and chemokines which are strongly upregulated in these patients’ muscles [16,17,20,47,48]. In other chronic myopathies, including the inflammatory dystrophies, and in contrast to PM and sIBM, the muscle fibers do not express the MHC-I antigen (or the costimulatory molecules described below) in an ubiquitous and consistent pattern, while the few T cells found in the proximity to the muscle fibers do not release cytotoxic granules, as in PM and IBM [49]. The CD8/ MHC-I lesion, therefore, is characteristic for IBM and PM [1–5,10,11] and is recommended as a diagnostic tool, considering the simplicity of immunocytochemical staining, to distinguish PM and IBM from inflammatory dystrophies and non-immune myopathies.

The third important observation in the immunopathology of PM and IBM was the demonstration that the CD8+ cytotoxic T cells are antigen-driven forming immunological synapses with the MHC-I-expressing muscle fibers [15,30,31]. The fundamental component of the synapse is the rearrangement of the T cell receptor genes. T cells recognize an antigen via the T Cell Receptors (TCR), a heterodimer of two α and β chains; the part of the TCR that recognizes an antigen is the CDR3 region, which is encoded by specific genes [1–3]. In patients with PM and IBM, but not in those with DM or dystrophies, only certain T cells of specific TCR families are recruited to the muscle from the circulation [50–53]. Cloning and sequencing of the amplified endomysial or autoinvasive TCR gene families has demonstrated a restricted use of certain gene families with conserved amino acid sequence in the CDR3 region suggesting that these cells seem to be specifically selected by antigens and clonally expand in situ probably driven by local muscle antigen(s) [50–52]. This conclusion has been further confirmed by combining spectratyping with molecular laser-assisted microdissection and by demonstrating that the clonally expanded TCR families persist over time even in different muscles [54–58]. In an important case of PM, γ/δ T cells of a single clone were the primary cytotoxic effectors that recognized genuine muscle antigens [59–61].

A prerequisite for the immunological synapse is the presence of the B7 family of costimulatory molecules [B7-1, B7-2, BB or inducible-costimulator-ligand (ICOS-L)] on the antigen presenting cells and their respective counterreceptors CD28, CTLA-4, or ICOS on the autoinvasive CD8+ T cells. In PM and IBM, the muscle fibers have the potential to present antigenic peptides to CD8+ cells via the MHC-I molecule, thereby serving as antigen-presenting cells, because they possess BB1 and ICOS-L while the autoinvasive CD8+ T cells to which they bind, express CD28, CTLA-4 or ICOS [37,62,63]. Because both BB1 and ICOS-L are functional molecules induced by interferon-γ or TNF-γ on human myoblasts [62], the BB1/CD28 and ICOS/ICOS-L interactions in PM and sIBM denote participation in antigen presentation, clonal expansion and co-stimulation of T cells. Theoretically, the upregulation of these molecules offers the potential for therapeutic manipulations because of the availability of humanized monoclonal antibodies against CD28, CTLA4 or CD40L.

Another prerequisite for the function of the immunological synapse is the presence of cytokines, cytokine signaling, chemokines and adhesion molecules because they enhance T-cell activation, induce costimulatory molecules and facilitate cell adhesion. Indeed, various cytokines, including interleukins (IL-1, IL-2, IL-6 and IL-10), TNF-α, INF-γ, signal transducer and activation of transcription (STAT), transforming growth factor β (TGF-β), and chemokines such as MCP-1, MIP-1α, IP-10, Mig and its receptor CXCR3, are variably overexpressed on the muscle fibers and on some autoinvasive CD8+ cells [16,17,19,20,46–48]. Various adhesion and extracellular matrix molecules such as VCAM, ICAM, thrombospondins and the metalloproteinases MMP-9 and MMP-2 are also upregulated in the tissues of patients with PM and IBM [64,65] playing a role in T cell adhesion, transmigration and cytotoxicity. Of interest, the muscle, in vivo and in vitro, appears to have the potential to secrete pro-inflammatory cytokines upon cytokine stimulation, such as INF-γ, in an auto-amplificatory mechanism that may facilitate the recruitment of activated T cells to the muscle contributing to the self-sustaining nature of endomyosial inflammation and disease chronicity [16,17,20,46–48,66]. The series of T-cell-mediated autoimmunity in PM and IBM discussed above, is depicted in figure 1, as reported before [10]. Other immune cells also support the autoimmune process in PM and sIBM.

Myeloid dendritic cells (DC), potent cells in antigen presentation, as well as plasma cells and clonally expanded B cells are abundantly found within the endomyosial infiltrates in all inflammatory myopathies including PM, DM and IBM [67,68]. Whether they have a bystander role in muscle fiber injury or are involved in local antigen presentation to T and B cells or in in situ production of autoantibodies, remains unclear. Such cells, which may form germinal centers, are frequently observed in the targeted tissues in several autoimmune disorders such as the synovium on rheumatoid arthritis or the brain of MS patients, and denote that complex effector mechanisms involving T and B cells play concurrently an active role in the autoimmune process.

**Necrotizing autoimmune myositis**

These patients present with high CK, in the thousands, moderate to severe muscle weakness of acute or subacute onset and with histological features of muscle fiber necrosis mediated by macrophages as the main effector cell. There are no T cell infiltrates or MHC-I expression as seen in PM and IBM. A number of patients have deposition of complement on blood vessels [69,70]. Antibodies against signal recognition
particles (SRP) [71] or 100–200 KD proteins [70] have been identified. The cause of NAM is multifactorial. Some patients have cancer or an active viral infection (i.e. HIV); others have been exposed to statins which can induce both a toxic as well as an autoimmune necrotizing myositis, with upregulation of MHC-1 in some patients, that responds to immunotherapy [72,73]; others may have a smoldering underlying autoimmune process; and still others have no other disease or apparent exposure to exogenous agents. It is likely that NAM is an antibody-mediated disease, as suggested by the presence of specific antibodies and complement deposits; the recruitment of macrophages may represent an antibody-dependent cell-mediated cytotoxicity (ADCC) process [1–3]. More studies are needed to understand the pathogenesis and course of this largely overlooked disorder because it is potentially treatable.

**Figure 1**
The putative molecules involved in the transmigration of T cells to the muscle and the dynamic process involved in antigen recognition in patients with PM and sIBM. LFA-1/ICAM-1 binding and TCR scanning for antigen initiates the formation of an immunological synapse between MHC-I and TCR. Stimulation is supported and enhanced by the engagement of costimulatory molecules B71, ICOS and CD40 on the muscle fibers and their ligands CD28, CTLA-4, ICOS-L and CD40L on the autoinvasive T cells. Metalloproteinases facilitate the migration of T cells and their attachment to the muscle surface. Muscle fiber necrosis occurs via the perforin granules released by the autoaggressive T cells. A direct myocytotoxic effect exerted by the released IFN-γ, IL-1 or TNF-α may also play a role. Death of the muscle fiber is mediated by a form of necrosis rather than apoptosis [10]
The use of immunopathology as a diagnostic tool in difficult cases of polymyositis, necrotizing autoimmune myositis and inclusion-body myositis

As discussed, in PM and IBM, the inflammation is in multiple foci within the endomysial parenchyma and consists predominantly of CD8+ T cells that invade healthy muscle fibers expressing the MHC-I antigen which is ubiquitously upregulated on the surface of most fibers. The MHC/DC8 complex is characteristic of PM and IBM because it is not seen in NAM, where CD8 positive cells are absent, or in inflammatory dystrophies where MHC-I is expressed only focally while the few CD8+ cells are rarely autoinvasive. Staining for macrophages is extremely helpful to identify the main cell involved in NAM and distinguish this entity from PM. Immunocytochemistry for the various known dystrophic proteins is also essential to exclude some dystrophies that may present as NAM. In IBM, in addition to inflammation, there are rimmed vacuoles and tiny amyloid deposits in a variable number of fibers, usually in or near the vacuoles, identified with Congo-red or Crystal violet stains, but easily visualized with Texas-red fluorescent optics [74]. The combination of endomyals inflammation with red-rimmed vacuoles, the ubiquitous MHC-I expression with CD8+ cells along with the presence of COX-negative fibers and congophilic deposits, are diagnostic of IBM; in these cases, an additional marker or electronmicroscopy is not needed [1–7,10]. Diagnostic concerns arise however in patients who have the distinct clinical phenotype of IBM but their biopsy does not show vacuoles but only inflammation characterized by MHC-I expression and CD8+ cells. Such cases labeled “PM/IBM”, “probable IBM” or “clinical IBM” comprise up to 15% of IBM patients [75] and dictate close clinicopathologic correlations to avoid the misdiagnosis of PM; a careful view of these biopsies however shows a large number of COX-negative fibers and signs of chronicity (large fibers, splitting, increased connective tissue) that denote probable IBM [1,10]. A repeat biopsy from another site is often helpful to unravel the presence of vacuoles and Congo red-positive deposits. The several histobiomarkers such as SMI31 or TD43 that identify phosphorylated tau, have not been tested in cases of probable IBM where no vacuoles or amyloid are present. The same is true for the new and more promising marker against the transported protein p62/SQSTM1 [76]. Two other distal myopathies that may be confused with sIBM, are the sporadic form of “myofibrillar myopathy” [77] and the hereditary IBM due to GNE mutations [78]. The biopsy in these cases lacks markers of immune inflammation (MHC/CD8) but demonstrates vacuoles with all the stressor-related molecules seen in sporadic IBM. These cases require a high degree of suspicion, careful search for desmin and other myofibrillar deposits, clinicopathologic correlations and molecular genetic studies.

Viral triggers

Although several viruses have been implicated in chronic and acute myositis [79], sensitive PCR studies have repeatedly failed to confirm the presence of such viruses in these patients’ muscle [25,79]. Whether viruses have the potential to replicate within the muscle or “hit and run”, remains unclear. muscles of patients with PM, DM, NAM and IBM. The best evidence of a viral connection is with the retroviruses. Monkeys infected with the simian immunodeficiency virus and humans infected with HIV and human T cell lymphotropic virus (HTLV-1) develop PM or IBM [79–83]. In HIV or HTLV-1 infected individuals, an inflammatory myopathy may occur as the initial manifestation of the infection or later in the disease course [79–88]. In these patients, IBM has been observed in a large number of patients before the age of 50, but several years after the first manifestations of the retroviral infection, suggesting that this association is not fortuitous [1–3,84–88]. In these patients’ muscles, the predominant cells are CD8+ cytotoxic T cells which, along with macrophages, invade or surround MHC-I-antigen—expressing non-necrotic muscle fibers [83–88]. Viral antigens are not detected within the muscle fibers but only in occasional endomysial macrophages. Molecular immunology studies using tetramers have shown that among the autoinvasive T cells there are retrovirally-specific CD8+ cells that clonally expand in situ [86–88]. Collectively, in retrovially-associated IBM there is no evidence of viral replication within the muscle, but the chronic infection triggers an in situ persistent inflammatory response, which, via infected macrophages and viral-specific T cells, may change the local milieu leading finally to IBM. A number of NAM cases had followed a viral illness, but no systematic analysis has been carried out to confirm an association. Cases of rhabdomyolyis have been noted during seroconversion of HIV and, although the HIV infection can trigger NAM, the mechanism by which retroviruses induce muscle fiber necrosis remains unclear.

Interrelationship between inflammation and amyloid or stressor-related molecules in sIBM muscles

IBM is a complex disorder because the immunopathologic events described above, are accompanied by a series of degenerative processes exemplified by the presence of rimmed vacuoles (almost always in fibers not invaded by T cells), and intracellular deposition of misfolded and stressor molecules, similar to those seen in neurodegenerative diseases. These proteins, specifically discussed in this volume by Askanas et al., are β-amyloid, amyloid precursor protein (APP), alpha-chymotrypsin, phosphorylated tau, best detected with antibodies to SMI-31 or the transporter protein p62/SQSTM1, ubiquitin, apolipoprotein E, prion protein, a number of nuclear-related proteins such as TDP-43 and VCP, and others [8,74,76]. These
proteins may accumulate in the cytoplasm of muscle fibers due to malfunctioning of the proteasome machinery, direct toxicity of monomers or oligomers of aberrant proteins and generation of cell stress, as described by Askanas et al. [1–3]. Many of the noted accumulations however do not appear specific for IBM because they are also found in other vacuolar myopathies especially myofibrillar, hereditary IBM or even in chronic neurogenic conditions such as the postpolio syndrome [89–96]. Autophagic processing, which is relevant to degradation of intracellular proteins, may also play a role since the vacuoles have autophagic properties [97] involved in processing of APP/β-amyloid. Since lysosomal processing of peptides can lead to antigen presentation via MHC-2 [98], macroautophagy may present a direct association to inflammatory mechanisms; whether they are relevant to antigen presentation of immune cells that attack the muscle fibers, remains unclear.

Currently, there is no unique molecule to serve as a specific IBM biomarker [1–3]; the recently reported immunoreactivity for p62 [76], is more specific than SMI-31 and quite promising, but more testing is needed in other vacuolar and myofibrillar myopathies to assess specificity for IBM. What appears unique to IBM however, compared to other chronic vacuolar myopathies, is the concomitant accumulation of these molecules with the inflammatory response and the overexpression of pro-inflammatory mediators and MHC-I on all fibers, vacuolated or not. The unique coexistence of the two processes and the original observation of co-localization of APP with cytokines on IBM muscle fibers [99], has led our laboratory to explore an interrelationship between inflammation and degeneration to understand what drives each process, regardless of whether the primary event is inflammatory or protein dysregulation. Based on gene arrays and quantitative immunocytochemistry, the main inflammatory molecules CCL-3, CCL-4, CXCL-9, IFN-γ and IL-1β are expressed to a higher degree in sIBM muscle compared to PM or DM [26,66]. In sIBM, but not in PM or DM, a significant correlation was also found between the mRNA expression of APP, as a key relevant degenerative marker, with the inflammatory mediators IFN-γ and CXCL-9 which also co-localize with APP/β-amyloid proteins [2,3,66]. Further, exposure of muscle cells to pro-inflammatory cytokines IL-1β and IFN-γ induces an overexpression of APP with subsequent accumulation of protein aggregates. As hypothesized, it is possible that a continuous stimulation of inflammatory factors may, after a long period, induce a higher basal expression of APP and an increased sensitivity to de novo pro-inflammatory cytokines that triggers a self-perpetuating cycle [66,100]. The association between inflammation and accumulation of β-amyloid in skeletal muscle has recently been shown in a mouse model of sIBM, where LPS-induced inflammation enhanced the accumulation of proteins such as tau and β-amyloid [101]. A similar effect was noted with αβ-crystallin, a heat-shock protein, which can chaperone proteins in skeletal muscle and is associated with cell stress or β-amyloid clearance. αβ-crystallin had been immunolocalized to healthy-appearing muscle fibers in IBM muscle almost a decade ago [102]. In a current quantitative assessment of multi-labeling and serial immunohistochemistry, a positive correlation between αβ-crystallin and β-amyloid-associated markers was found in sIBM muscles [103]. The normal-appearing muscle fibers that were positive for αβ-crystallin were often double-positive for APP, while APP/β-amyloid co-localized with markers of cell-stress and regeneration/degeneration. It appears therefore that, αβ-crystallin may be an early event associated with a stress-response that precedes accumulation of β-amyloid. This view was further supported by in vitro studies which demonstrated that accumulation of β-amyloid, upon pro-inflammatory cell-stress, was preceded by upregulation of APP and αβ-crystallin [103]. The noted cross-talk between inflammation and degeneration, although does not provide any information on what is the primary event, argues that inflammation may enhance the protein misfolding process. Such an association offers the basis to design targeted therapeutic strategies against the inflammatory component in an effort to arrest deposition of potentially noxious misfolded proteins within the muscle fibers.

### Therapies in dermatomyositis, polymyositis and necrotizing autoimmune myositis

Because the target antigen(s) are unknown, the presently employed immunosuppressive therapies in PM, DM and NAM are not selectively targeting the autoreactive T cells, the complement-mediated process on intramuscular blood vessels, or the putative antibodies. Instead, they are inducing a non-specific immunosuppression or immunomodulation [104]. Furthermore, many of these therapies are empirical [105]. Based on experience, the majority of patients with PM and DM respond to corticosteroids [104–106]. IVIg tested in a controlled study is effective in DM as a second, and at times, first-line therapy [107]. IVIg appears also effective in PM and NAM based on experience. Immunosuppressants are used as steroid-sparing agents but their efficacy remains unclear [105]. New agents in the form of monoclonal antibodies or fusion proteins that target cytokines, adhesion molecules, T cell transduction or transmigation molecules and B cells or their activation factors, are emerging as promising immunotherapeutic drugs [104]. Among them, rituximab, a B cell-depleting agent, has been helpful in some cases of DM, PM but also in patients with NAM [105,108]. A controlled study has now been completed and the results are currently analysed.

### Challenges and promises in the therapy of inclusion-body myositis

In contrast to PM and DM, there is currently no effective treatment for sIBM. Prednisone, cyclosporine, azathioprine,
methotrexate, total body irradiation and IFN-β have generally failed justifying the contention that it could be more of a degenerative disease rather than autoimmune [1–3,105]. A number of patients with IBM however may respond to common immunotherapeutic agents early on, partially and for a period of time [1–3,106]; up to 25% of patients in a controlled study have also responded transiently to IVIg [109]. These benefits are arguably limited and short-lived and do not change the relentless disease progression, but suggest that a more specific immunotherapy may prove encouraging. Such transient benefits are similar to those seen in various other autoimmune diseases, where immune and degenerative features coexist from the outset. For example, patients with primary progressive multiple sclerosis behave to some degree like patients with IBM because some of them may partially respond early in the disease, but then follow a steadily progressive course resistant to most therapies [1].

The lack of treatment efficacy in sIBM may be due to a number of reasons, as recently discussed [1–3]: therapy is always initiated late when the degenerative cascade has already begun, due to insidious nature of disease and very slow progression. It is striking that even patients with minimal clinical weakness who seek medical attention relatively early, already exhibit muscle atrophy and advanced histological changes. The observation that αB-crystallin is, along with pro-inflammatory markers, an early event associated with cell stress-response that precedes accumulation of β-amyloid, supports the view that anti-inflammatory therapy not only can arrest progression if initiated early, but may even lead to complete remission, as recently shown by Layzer et al. [110]. This case—although rare—is remarkable because early treatment resulted in complete resolution of IBM pathology. We have seen a very similar case which, along with the previous one, strengthens the concept of potential reversibility if the disease can be treated early; and the noted interrelationship between inflammatory mediators and degeneration suggests that successful suppression of endomyial inflammation may have an effect on some degeneration-associated molecules with resulting short-term clinical stability. On this basis, Alemtuzumab (Campath), a T-cell-depleting monoclonal antibody was used in a small study [111]. This proof-of-principle and uncontrolled study [111]. In spite of these limitations, this was a novel study which showed that depletion of T cells from the periphery also caused reduction of T cells in the muscle with suppression of some degeneration-associated molecules resulting in a 6-month disease stability period [111]. This study highlights that new anti-inflammatory therapies, if proven safe for long-term therapy, may have an effect not only on inflammatory mediators but also in halting some elements of degeneration [1–3]. It is a new way of thinking about therapeutic interventions that target inflammation to suppress the stressor and degeneration-associated molecules. The ongoing trial of IVIg, an arguably effective immunomodulating drug, in patients with Alzheimer’s disease might provide credence to this concept. In Alzheimer’s disease, the same “degenerative” molecules as those seen in IBM muscle, accumulate in abundance in the patients’ brains. The trial is based on the observation that IVIg does not only suppress inflammation but also beta-amyloid and all the inflammatory mediators that enhance the accumulation of misfolded proteins [112].

Conflicts of interests statement: none

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