Inclusion body myositis: new insights into pathogenesis

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Purpose of review

The pathogenesis of sporadic inclusion body myositis is complex and the disease has a relentless course. Recent observations regarding possible mechanisms of disease may provide targets for therapy.

Recent findings

Evidence is strengthening that specific T-cell and B-cell responses are ongoing in skeletal muscle in sporadic inclusion body myositis and that cytokines and chemokines generated by an autoimmune response are likely to influence antigen presentation by intramuscular dendritic cells and muscle cells, expression of amyloid precursor protein and the endoplasmic reticulum stress response. Early β-amyloid expression and perhaps aberrant expression of protein processing enzymes, such as E3 ligases, seem to be involved in the myopathic process. NF-κB activation by endoplasmic reticulum stress and cytokine action further stimulates amyloid precursor protein production, exacerbates endoplasmic reticulum stress and increases myostatin content in muscle contributing to muscle atrophy.

Summary

Understanding the paradoxes in sporadic inclusion body myositis is important in determining rational therapy for the disease. Amyloid precursor protein is expressed in muscle in other inflammatory muscle diseases but the cellular distribution differs and inclusions do not form so that other metabolic defects seem to be important. Intramuscular immune cells influence muscle function and viability in inclusion body myositis but immunotherapy is ineffective. A useful target for therapy may be restoration of muscle regenerating capacity.

Keywords

autoimmunity, β-amyloid, E3 ligase, inclusion body myositis, myostatin

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Introduction

Sporadic inclusion body myositis (s-IBM), the most common form of inflammatory myopathy in patients over 50 years of age [1], has a slowly progressive course extending over a period of 15–20 years and is poorly responsive to treatment with glucocorticoids or other immune therapies. It is characterized by a selective pattern of muscle weakness and atrophy, with a predilection for the quadriceps femoris muscles in the lower limbs and the finger flexors and extensors in the forearms, which is often asymmetric and more severe on the nondominant side [2]. The serum creatine kinase level is moderately elevated in some cases but can be normal or borderline. Electromyography and muscle imaging with MRI or computed tomography (CT) can be helpful for diagnosis but a muscle biopsy is the definitive diagnostic procedure [1,3].

The pathological hallmark of the disease is an inflammatory infiltrate with invasion of major histocompatibility complex (MHC) class I expressing muscle fibres by CD8+ lymphocytes. Myodegenerative changes, including autophagic 'rimmed' vacuoles, congophilic amyloid inclusions and muscle fibre atrophy, become more prominent as the disease progresses [3]. Characteristic 15–20 nm filamentous cytoplasmic or intranuclear inclusions can be demonstrated by electron microscopy and immunohistochemically using the SMI-31 antibody that labels phosphorylated tau protein. Mitochondrial changes are also a feature and there is segmental loss of cytochrome oxidase activity with accumulation of multiple clonally expanded mtDNA mutations in muscle fibres [4].

Immunohistochemical analysis reveals intracellular accumulation of β-amyloid as a major component of the amyloid deposits. A range of other proteins is associated with the inclusions, the filaments or as part of amorphous protein aggregates in the cytoplasm. Included among these are proteins associated with stress responses as well as kinase enzymes known to be active in phosphorylation of tau, such as casein kinase-1 [5] and

proteins that influence the synthesis and processing of β-amyloid, such as β-secretase (BACE-1).

Pathogenesis

The pathogenesis of s-IBM is unclear. The abundant lymphocytic infiltrate, the frequent cooccurrence with other autoimmune diseases [3] and the strong immunogenetic association [6] have led to the belief that it is an autoimmune muscle disease. This is confounded by the progression of the disease despite reduction in the inflammatory component by immunosuppressive agents [7].

There are at least three possibilities:

(1) A T-cell-mediated autoimmune response leads to muscle damage and myofibre degeneration;
(2) Aberrant intramuscular protein synthesis and processing and subsequent endoplasmic reticular stress cause myofibre degeneration, aberrant antigen presentation and autoimmunity;
(3) The two processes are independent. The myofibre degeneration occurs as a consequence of genetic or external triggers that stimulate aberrant intracellular protein metabolism and a separate autoimmune response occurs.

Clues to the pathogenesis of s-IBM have been sought by exploring the nature of the proteins deposited in affected muscle, the nature and expression profiles of the cellular components of affected muscle, genetic associations with the disease, genetic defects in hereditary forms and the histological, immunological and biochemical abnormalities that manifest in a range of transgenic animal and in-vitro cellular models.

Hereditary inclusion body myopathies

The hereditary inclusion body myopathies (h-IBM) are a heterogeneous group of disorders with autosomal dominant or recessive inheritance that have a number of pathological features in common with s-IBM, such as rimmed vacuoles and cytoplasmic and intranuclear filamentous inclusions, but usually lack the inflammatory component.

The best characterized recessive form, associated with mutations in the epimerase or kinase domains of the *GNE* gene, manifests as a quadriceps-sparing myopathy and includes an allelic form of distal myopathy with rimmed vacuoles (DMRV) seen in Japanese [8,9]. These mutations result in hyposialylation of muscle glycoproteins that is thought to be central to the pathogenesis of the disease [10,11]. Mutations in *GNE* have so far not been found in any cases of typical s-IBM [12].

Dominant forms of h-IBM include a childhood-onset form with congenital joint contractures, ophthalmoplegia, rimmed vacuoles and a mutation in the myosin heavy chain IIa gene [13], and a familial form with associated Paget's disease of bone and frontotemporal dementia (IBMPFD) that has been linked to mutations in the valosin-containing protein (*VCP*) gene on chromosome 9p13–p12 [14,15]. Muscle biopsies show a noninflammatory rimmed vacuolar myopathy with VCP-containing and ubiquitin-containing inclusions and nuclear protein aggregates.

Valosin-containing protein has an important role in clearance of improperly folded proteins via endoplasmic-reticulum-associated degradation (ERAD). Mice transgenic for the mutant form of this gene under the control of a muscle-specific promoter [16•] accumulate ubiquitinated high molecular weight proteins in the sarcoplasm from around 1 month after birth and autophagocytosis, intramuscular vacuolation, congo red positive inclusions, sarcoplasmic membrane perturbation and muscle weakness develop throughout life. These data are consistent with observations in s-IBM suggesting that accumulation of ubiquitinated proteins via overproduction or failure of clearance is toxic in skeletal muscle. The mice do not show evidence of inflammation suggesting that accumulation of ubiquitinated protein and the consequent muscle damage *per se* do not lead to an intramuscular inflammatory response.

Immune aspects of sporadic inclusion body myositis

CD8+ T cells have been considered to be the predominant inflammatory players in s-IBM; however, recent investigations suggest that other cells of the immune system also play a role. Both CD8+ and CD4+ cells have been demonstrated in s-IBM muscle, CD4+ cells being present in both perivascular infiltrates and the endomysium. The cytotoxic protein, granulysin, was expressed by both subsets of T cells [17,18], suggesting that its release may be a factor in inducing muscle damage along with perforin expressed by CD8+ cells invading muscle cells [18].

The potential for immunological interaction between these CD4+ and CD8+ T cells and the muscle fibres is emphasized by the expression of the ligand for inducible costimulatory molecule (ICOS-L) on both affected and healthy-looking muscle cells and the expression of ICOS on both CD8+ and CD4+ T cells [19]. The close intramuscular proximity of CD4+ and CD8+ cells perhaps facilitates helper function. Skeletal muscle cells can also express other members of the costimulatory family of molecules, such as BB-1 [18], and are able to present antigen [20,21]. It is well recognized that MHC class I

antigen expression is abundant on and within affected as well as unaffected myofibres, providing an opportunity for presentation of antigenic peptides to CD8+ T cells. This presumably leads to direct muscle fibre damage and the release of cytokines that can have more remote effects on muscle and other cells. MHC class II antigen was also expressed in areas of inflammation and on degenerating, β-amyloid positive fibres that had both CD4+ and CD8+ T cells associated with them [22]. Further, autophagosomes that are a feature of affected muscle in s-IBM fuse with MHC class II antigen loading compartments, providing the opportunity for presentation of autoantigens to CD4+ T cells [22] in an inflammatory environment. The recent demonstration of myeloid dendritic cells, potent antigen presenting cells, between and penetrating muscle fibres in s-IBM, suggests another potent avenue for intramuscular presentation of autoantigen to T cells [23••].

In the absence of any knowledge of the autoantigenic target, researchers have analysed the T-cell receptor (TCR) V gene repertoire in s-IBM muscles and blood to seek evidence of antigen specificity [3]. Recent studies suggest that clonal recruitment, expansion and subsequent epitope spreading occur. TCR *Vβ* gene usage showed restricted distribution in peripheral blood and autoinvading CD8+ cells were clonally expanded. In follow-up biopsies in the same patients the Vβ usage remained restricted, though there was some change in the spectrum of subfamilies used, suggesting epitope spreading [24•]. The localization of myeloid dendritic cells in muscle in close proximity to the T cells provides support for local recruitment and expansion of autoreactive T cells [23••].

Humoral immunity has previously been overlooked in the pathogenesis of s-IBM; however, B cells and plasma cells are present in s-IBM muscle [25]. Immunoglobulin *VH* gene sequence analysis of mRNA derived from s-IBM muscle suggested clonal expansion of B cells and plasma cells and maturation of B-cell responses within muscle, along similar lines to T cells. These data are consistent with an ongoing local response to an intramuscular autoantigen and they bring B cells into focus in s-IBM [26], though to date, no autoantibodies specific for, or characteristic of, s-IBM have been demonstrated.

The strong association of s-IBM with the MHC suggests aberrant immune control as a possible contributor to disease. Fine mapping of the MHC-associated susceptibility region has focussed on a short area of chromosome 6 that includes the gene encoding the butyrophylin/B7-like molecule, BTNL2 [6], which has been shown to have a negative costimulatory effect on T cells and to inhibit their proliferation and cytokine production in response to stimulation. It is overexpressed in inflammatory sites in the gut perhaps as part of a homeostatic response to modulate T-cell activation [27]. An allele of this gene encoding a truncated form has been associated with sarcoidosis [28]. Because BTNL2 is expressed on dendritic cells and other cells and can be upregulated in response to inflammatory stimuli [27], a poorly or nonfunctional allele may contribute to uncontrolled inflammation. BTLN2 has not been directly implicated in s-IBM but its expression and control in skeletal muscle require clarification, as does its allelic variation in s-IBM, particularly in the context of the 8.1 ancestral MHC haplotype.

In considering the MHC encoded susceptibility to s-IBM a polymorphism in the class II region encoding an antigen presenting MHC molecule remains a candidate given the renewed focus on CD4+ T cells, B cells and dendritic cells in s-IBM. The reported influence of human leukocyte antigen (HLA)-DR3 on the phenotype and clinical course of s-IBM suggests that the MHC influences both the predisposition to disease and disease progression [2].

As might be expected, in s-IBM muscle there is increased mRNA expression of a range of cytokines (often in T cells and macrophages invading nonnecrotic fibres) including IFN-γ, TNF-α and IL-1β and chemokines and chemokine receptors including CXCL-9, CCL-3, CCL-4, CXCL10, CCL-2 and CXCR3 [29,30•]. Intramuscular cytokine expression seems to contribute to amyloid precursor protein (APP) overexpression. Expression of APP correlated with levels of expression of the chemokines and cytokines, IL-1β colocalized with β-amyloid in muscle [30•] and in-vitro experiments showed that IL-1β can stimulate APP expression and subsequent amyloid aggregation in human myotubes. Further, mature human myotubes can produce a range of cytokines and chemokines in response to IL-1, TNF-α and INF-γ [30•]. Interestingly, APP mRNA was also upregulated in polymyositis and dermatomyositis [30•] but its distribution was confined to fibres in areas of intense inflammation (polymyositis) and nonmuscle cells (dermatomyositis) compared with s-IBM in which a large fraction of muscle fibres expressed both APP and other degeneration markers such as neural cell adhesion molecule (N-CAM), desmin and alpha B-crystallin (αBC) [30•].

The interaction between inflammation and the expression of s-IBM-associated proteins was explored in a transgenic mouse model of IBM (APP/PS1), in which there is enhanced expression of β-amyloid in skeletal muscle [31]. Induction of chronic systemic or acute intramuscular inflammation by injection of lipopolysaccharide produced a marked intramuscular mononuclear infiltrate, enriched in CD8+ T cells, increased β-amyloid and APP levels, an increase in phosphorylated tau in

muscle mediated by GSK3β activation and impairment of skeletal muscle function [32••]. IL-1β, IL-6 and TNF-α (particularly together) increased activity of GSK3β and tau phosphorylation in murine C2C12 cells *in vitro* [32••]. These data have been used to support the idea that an inflammatory intramuscular milieu can trigger the overproduction of such proteins and the endoplasmic reticulum stress response. Cytokine activation of NF-κB in muscle and immune cells can then promote further cytokine expression and the genesis of APP and BACE-1.

Aberrant protein accumulation and the stress response

Detailed analysis of the protein components of the inclusions in s-IBM, together with data gleaned from experimental animal models and in-vitro manipulation of muscle cell cultures has provided potential scenarios for the degenerative processes that are characteristic of the disease [33]. A sophisticated system exists by which cells can remove misfolded proteins, and deal with proteins that are expressed at rates greater than can be handled by the endoplasmic reticular folding machinery. Misfolded proteins tend to aggregate, are toxic and can induce apoptotic cell death, unless cleared. These misfolded proteins are retranslocated to the cytoplasm by the ERAD machinery, subjected to ubiquitination by a variety of ligases and then degraded by the proteasome [34].

The endoplasmic reticulum stress response (or unfolded protein response, UPR) occurs when the capacity of this system to remove unfolded proteins is overloaded. This response has four facets: inhibition of protein translation, stimulation of transcription of endoplasmic reticulum chaperone genes, stimulation of transcription of ERAD protein genes and induction of apoptosis. One consequence of this process is the activation of NF-κB [34].

Most hypotheses regarding the pathogenesis of s-IBM include upstream overexpression of APP in skeletal muscle and an inability of the UPR to clear this, and other misfolded proteins, due to proteasomal malfunction. This results in endoplasmic reticulum stress, impaired clearance, accumulation of ubiquinated proteins and upregulation of chaperone proteins such as GRP78 and GRP94, which can be demonstrated associated with β-amyloid aggregates [33]. In mammalian muscle RNF5, an E3 ligase, contributes to ubiquitination of misfolded protein. In s-IBM (and mouse h-IBM) muscle it is overexpressed, localizes to protein aggregates, in particular with β-amyloid (not with phosphorylated tau), and can be demonstrated throughout the cytoplasm of vacuolated as well as normal fibres [35••]. Transgenic mice that overexpress RNF5 in skeletal muscle showed evidence of muscle degeneration, regeneration and blue-rimmed vacuoles containing inclusions and congophilic fibres as well as increased levels of endoplasmic reticulum stress markers, including GRP94, GPI and GRP78. It is of interest that parkin, another E3 ubiquitin ligase, also accumulates in s-IBM skeletal muscle and recent data indicate that it has a protective role against mitochondrial toxins and against the toxicity of β-amyloid [36].

It has been suggested that RNF5 overexpression in s-IBM may be an early event that contributes to impairment of ERAD and the endoplasmic reticulum stress response resulting in protein accumulation and aggregation [35••]. Its accumulation may reflect a cellular response to overaccumulation of β-amyloid. Disordered control (either genetic or biochemical) of genes that control the ubiquitination and proteasomal clearance systems has the potential to induce inclusion body myopathy as illustrated by the mutation in the *p97/VCP* gene in IBMPFD [14,15].

Muscle biopsies from s-IBM show signs of autophagic processes [22,37]. Misfolded proteins are ejected from the endoplasmic reticulum into cytoplasmic proteasomes for degradation. If the capacity of the UPR system to deal with misfolded proteins is exceeded then degradation resistant aggregates form that are transported via microtubules to a ubiquitin-rich structure near the nucleus called the aggresome. This facilitates incorporation of the aggregates into autophagic structures that fuse with lysosomes. In s-IBM β-amyloid was colocated with the essential autophagy protein Atg8/LC3 in autophagosomes of degenerating type II fibres and associated with MHC class I and class II antigen overexpression and T-cell infiltration. These data provide further support for the overload of the ERAD, and aggresomes [38] and the targeting of APP/β-amyloid for lysosomal degradation in s-IBM. The autophagosomes may facilitate antigen processing for MHC class II presentation to autoreactive T cells, providing a possible link between the endoplasmic reticulum stress response and autoimmunity [22].

Some of the upregulated proteins in s-IBM muscle may represent an ineffectual homeostatic response aimed at limiting damage from overproduction of β-amyloid. These include αBC, a stress-related heat shock protein that recognizes and stabilizes proteins with a propensity to form aggregates and precipitate [39,40], and NOGO-B [41], an integral membrane protein that binds to BACE-1 and blocks its activity by blocking access to APP. APP overexpression in cultured muscle stimulated expression of both proteins [40,41]. The effect of αBC is unclear. It may bind to soluble β-amyloid oligomers preventing their aggregation but prolonging their toxic effects or, conversely, might block a toxic moiety of β-amyloid [40].

The control of protein expression in s-IBM requires elucidation. Micro-RNAs are believed to influence gene expression and in s-IBM muscle a series of micro-RNAs were shown to be upregulated. At least one is believed to influence ubiquitin-mediated proteolysis and others influence B-cell function and insulin-signalling pathways [42].

In a transgenic model of MHC class I overexpression in skeletal muscle an inflammatory myopathy, similar in many respects to human myositis, develops providing further support that overload of the protein folding system can lead to intramuscular and autoimmune disorder, though not necessarily intramuscular inclusions [43]. Activated NF-κB was increased in muscle of these mice along with activated (cleaved) caspase-12, a downstream mediator of apoptosis after endoplasmic reticulum stress [44] and overexpression of class I MHC in C2C12 cells, induced the chaperone protein Grp78. Muscle biopsies from patients with polymyositis and dermatomyositis [44] show evidence of membrane and reticular class I MHC in both affected and unaffected muscle cells, activated NF-κB in muscle nuclei and increased expression of a number of genes that are targets for NF-κB as a transcription factor. However, despite the overexpression of MHC class I in these situations the inclusions and protein aggregates of IBM are not evident.

Inhibition of muscle regeneration

Part of the pathogenic process in s-IBM may be inhibition of muscle regrowth and differentiation induced as a consequence of endoplasmic reticulum stress and the activation of NF-κB. Overexpression of class I MHC and activation of the endoplasmic reticulum stress response and NF-κB, apart from promoting inflammation, may also contribute to inhibition of muscle repair leading to atrophy. The transgenic mice described above had progressive muscle wasting [44], while intramuscular inoculation of plasmids expressing MHC class I can inhibit muscle regeneration [45]. Myostatin precursor protein (MPP) and myostatin are increased in s-IBM muscle [46]. It has been proposed that NF-κB activation after induction of endoplasmic reticulum stress by amyloid protein accumulation early in the pathogenic process, results in MPP overexpression. Induction of endoplasmic reticulum stress and NF-κB activation in muscle cultures using agents such as tunicamycin, or after APP overexpression [47•,48•], increases MPP protein levels. In APP transfectants the MPP was associated with 6–10 nm fibrils and floccular material (amyloid and preamyloid) and perinuclearly. The system seems complex because tunicamycin increased MPP mRNA and protein, APP overexpression increased protein only and TNF-α activation of NF-κB does not alter MPP expression [47•]. The binding of APP to MPP may reduce MPP degradation through the proteasomal degradation system so prolonging its half-life [48•]. Whatever the mechanism the increased constitutive myostatin levels in s-IBM may contribute to the progressive muscle fibre atrophy [48•] and approaches to inhibit myostatin action or production are worthy of exploration for therapy [49•].

Other effects may also be operative in inhibiting muscle regeneration and promoting atrophy in s-IBM. Mesangioblasts (pericyte-derived) from s-IBM did not differentiate into myotubes in culture (but did differentiate to smooth muscle or osteoblasts), in contrast to those from other inflammatory myopathies and normal muscle [50]. This defect correlated with an absence of these stem cells in s-IBM muscle connective tissue. Further, a myogenic inhibitory helix–loop–helix factor, B3, was overexpressed in mesangioblasts from s-IBM. The capacity of these cells to differentiate to skeletal muscle was restored by MyoD expression or by silencing the B3 gene with siRNA. These data suggest that factor(s), such as a cytokine or combination of cytokines, in the milieu of s-IBM muscle inhibit mesangioblast to satellite cell transition via activation of the B3 gene and so contribute to muscle atrophy [50]. Of further interest, TNF-α inhibits differentiation of cultured skeletal muscle by downregulation of MyoD, after activation of NF-κB, and by activation of caspases, providing a further link between inflammation and muscle atrophy in s-IBM [51].

Conclusion

T-cell-mediated autoimmunity is a key factor in the pathogenesis of s-IBM and it is likely that soluble mediators such as IL-1β contribute to the endoplasmic reticulum stress and aberrant protein accumulation. The myopathy is unlikely to be solely due to inflammatory mediator interaction with muscle and evidence is mounting to support a role for aberrant expression of enzymes involved in the endoplasmic reticulum stress and ubiquitination responses early in disease. Overexpression of APP and accumulation of ubiquinated proteins *per se* are not sufficient to explain the pathological features of s-IBM so that a genetic predisposition to autoimmunity may be necessary for manifestation of the disease.

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