Sporadic inclusion-body myositis: Conformational multifactorial ageing-related degenerative muscle disease associated with proteasomal and lysosomal inhibition, endoplasmic reticulum stress, and accumulation of amyloid-β42 oligomers and phosphorylated tau

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Summary

The pathogenesis of sporadic inclusion-body myositis (s-IBM), the most common muscle disease of older persons, is complex and multifactorial. Both the muscle fiber degeneration and the mononuclear-cell inflammation are components of the s-IBM pathology, but how each relates to the pathogenesis remains unsettled. We consider that the intramuscle fiber degenerative component plays the primary and the major pathogenic role leading to muscle fiber destruction and clinical weakness. In this article we review the newest research advances that provide a better understanding of the s-IBM pathogenesis. Cellular abnormalities occurring in s-IBM muscle fibers are discussed, including: several proteins that are accumulated in the form of aggregates within muscle fibers, including amyloid-β42 and its oligomers, and phosphorylated tau in the form of paired helical filaments, and we consider their putative detrimental influence; cellular mechanisms leading to protein misfolding and aggregation, including evidence of their inadequate disposal; pathogenic importance of endoplasmic reticulum stress and the unfolded protein response demonstrated in s-IBM muscle fibers; and decreased deacetylase activity of SIRT1. All these factors are combined with, and perhaps provoked by, an ageing intracellular milieu. Also discussed are the intriguing phenotypic similarities between s-IBM muscle fibers and the brains of Alzheimer and Parkinson’s disease patients, the two most common neurodegenerative diseases associated with ageing. Muscle biopsy diagnostic criteria are also described and illustrated.
General considerations

Sporadic inclusion-body (s-IBM) is pathogenically a complex, multifactorial muscle disease in older persons. The s-IBM muscle biopsy exhibits an unusual and specific pathologic phenotype, which combines multifaceted muscle fiber degeneration with an extracellular T-cell inflammation. s-IBM muscle fiber degeneration is characterized by vacuolization and intramuscle fiber accumulation of ubiquitinated, congoophilic multiple-protein aggregates [1–3]. It is still not known whether mononuclear-cell inflammation precedes muscle fiber degeneration, or the abnormal chemically-modified proteins accumulated within the muscle fibers provoke the inflammatory response [1–7]. We suggest that the multiple post-translationally-modified proteins (see below) accumulated within the s-IBM ageing muscle fibers may be eliciting the T-cell inflammatory reaction. In support of this hypothesis is the fact that some of the older patients with hereditary IBM (h-IBM) caused by missense mutations in the UDP-N-acetylgalactosamine-2 epimerase/N-acetylmannosamine-kinase (GNE) gene have various degrees of lymphocytic inflammation [8–10], even though that form of h-IBM is considered not immune-mediated. Muscle biopsies of h-IBM patients are similar to those of s-IBM having a similar spectrum of accumulated abnormal proteins [11,12]. It is possible that in older h-IBM patients, their “ageing” muscle fiber environment, and perhaps other individual intrinsic muscle fiber abnormalities make some of the accumulated proteins interpreted “foreign” by the immune system, thereby inducing the T-cell lymphocytic inflammation. Interestingly, in muscle biopsies of some patients with Duchenne muscular dystrophy caused by dystrophin mutation there is T-cell-inflammation, which has now been shown to be elicited by revertant dystrophin or mini-dystrophin therapeutically delivered by an rAAV vector, both of which apparently are not recognized as “self” proteins by the Duchenne muscular dystrophy immune system [13,14]. Another argument that inflammation might be secondary in s-IBM is provided by a new mouse alleged “model of s-IBM” based on overexpression of mutated gelsolin D187N [15]. In this model, within the myofibers of aged mouse there was an intramyofiber accumulation of misfolded and congoophilic proteins, including Aβ and gelsolin and, in addition, there was perivascular and endomysial lymphocytic cell infiltration, strongly suggesting that inflammation was secondary to the overexpressed abnormal mutant protein or other proteins accumulated within the muscle fiber. The possibility that in s-IBM the inflammation might be a secondary to the ongoing degeneration and production of abnormal proteins within the muscle fibers can explain why s-IBM patients do not satisfactorily respond to various anti-dysimmune/anti-inflammatory treatments that have been extensively tried [7,16–18].

In our opinion, it is becoming more evident that in s-IBM a unique type of muscle fiber degeneration leads to muscle fiber atrophy, and resulting in muscle fiber degeneration and death, manifest clinically by relentlessly progressive muscle weakness. Additional intriguing phenomena related to s-IBM are the many similarities to the complex neuronal degenerations occurring both in Alzheimer and Parkinson disease brains. The similarities include: abnormal accumulations of many of the same putatively pathogenic proteins; their similar post-translational modifications; and similar defective mechanisms of protein disposal, including inhibition both the 26S proteasome and autophagy [1,2,19–29]. The 26S proteasome and autophagy inhibitions contribute to the observed abnormal protein aggregations, β-pleated sheet amyloid accumulation, and cytoplasmic vacuolization.

Abnormal accumulation of ubiquitinated intracellular proteins—congophilic inclusions is characteristic of the s-IBM phenotype—accordingly s-IBM, similarly to AD and PD, is considered a “conformational disorder”, caused by protein unfolding/misfolding and associated with formation of multiprotein inclusion-bodies (reviewed in [1–4]). As with AD and PD brain, the sequence of detrimental pathologic events comprising cellular degeneration in s-IBM muscle fibers is not well delineated. However, several aspects of the s-IBM intramuscle fiber pathogenic cascade are being uncovered and their causative mechanisms elucidated.

In this review, we describe the most recent research advances directed toward understanding the underlying mechanisms
causing impaired protein degradation, their molecular modifications and abnormal aggregation. Also discussed is putative pathogenic importance of the endoplasmic reticulum stress that we have identified in s-IBM muscle.

Of the multiple proteins accumulated in the form of aggregates within s-IBM muscle fibers (recently reviewed and illustrated in [2]), we have discussed the ones that we consider the most central to the s-IBM pathogenesis—these include amyloid-β-42, phosphorylated tau (p-tau) and α-synuclein. First, brief consideration is given to the diagnostic pathologic criteria of the s-IBM muscle biopsy.

**Diagnostic criteria of s-IBM muscle biopsy**

The most important aspect of s-IBM diagnosis is evaluation of the muscle biopsy. Even though the clinical features of s-IBM are usually characteristic, the s-IBM patients’ biopsy is often misdiagnosed as having polymyositis—especially in the earlier stages of disease. The reason is that various degrees of lymphocytic inflammation (with some macrophages) and the expression on muscle fibers of the MHC-I (major histocompatibility complex-I) and its associated molecules, can exist in muscle biopsies of both disorders. Accordingly, expression of the MHC-I on muscle fibers is not a diagnostic criterion of s-IBM. It is important to avoid misdiagnosing s-IBM patients as polymyositis, since this often results in s-IBM patients being treated chronically and ineffectively with immunosuppression drugs.

**Typical diagnostic abnormalities of the s-IBM muscle biopsy**

**Light-microscopy**

Vacuolated muscle fibers

The characteristic feature of s-IBM on the Engel-trichrome staining [30] (the standard general stain used for fresh-frozen sections of muscle biopsies) are muscle fibers, which may contain one or a few vacuoles in a given cross-section. Most of the vacuoles appear to be autophagic since they contain poorly differentiated bluish-reddish material within the vacuoles or at their periphery, indicating lipoprotein membranous material [30,31]. Definitely “rimmed” vacuoles are rare, and most of s-IBM vacuoles do not have a sharply defined peripheral red rim. Many s-IBM vacuoles appear rather empty, but at higher magnification they often contain greenish-gray-reddish material characteristic of proteinaceous material (figure 1 A–C). Definitely “rimmed” vacuoles are rare, and most of s-IBM vacuoles do not have a sharply defined peripheral red rim. Many s-IBM vacuoles appear rather empty, but at higher magnification they often contain greenish-gray-reddish material characteristic of proteinaceous material (figure 1 A–C), in contrast to empty freezing artifact holes. The number of vacuolated muscle fibers varies not only among s-IBM patients, but also on different transverse-sections of the same muscle biopsy, and in two different muscle biopsies obtained at the same time from the same patient. Accordingly, we consider that evaluating muscle biopsies before and after treatment, as often done in some therapeutic trials, to be uninformative and misleading.

**Intracellular amyloid deposits**

Congo-red staining. Multiple or single foci of β-pleated sheet amyloid, as identified by Congo-red fluorescence visualized through Texas-red filters [32], are evident within about 40–70% of the s-IBM vacuolated muscle fibers in a given transverse section, mostly in their non-vacuolated regions. In addition, a number of “non-vacuolated”, normal-appearing muscle fibers also contain amyloid deposits (figure 2 A–C), but because each muscle fiber is a very long cylinder such amyloid-containing fiber might be vacuolated at a different level. Our fluorescence-enhanced Congo-red technique is the best, most sensitive method for highlighting β-pleated-sheet amyloid inclusions, which sometimes are very small or few (Congo-red visualized in polarized light, is widely used to seek amyloid, but it is much less precise, more difficult to interpret, can miss identifying amyloid deposits, an thus should not be used routinely for s-IBM diagnosis). We have shown in s-IBM that intramuscle fiber amyloid deposits contain Aβ1-42 in the form of plaque-like inclusions, or p-tau in the form of squiggly-paired helical filaments (PHFs) [5,33]. Both Aβ42 and p-tau are known to self-aggregate to form β-pleated-sheet congophilic amyloid in s-IBM muscle fibers and AD brain [2,11]. A number of other proteins accumulated in s-IBM muscle fibers, including normal cellular prion, α-synuclein, and others [2,11,34,35], have also the propensity to self-aggregate into β-pleated-sheet amyloid.
Crystal violet. Crystal violet metachromatic staining can also show the intramyofiber amyloid deposits in s-IBM muscle fibers (figure 2 D, E). While this method is more convenient because it does not require fluorescence microscopy, it is less precise because small amyloid deposits can be difficult to identify.

In our experience, among our patients with other non-IBM vacuolar myopathies, none had had true amyloid deposits as identified by crystal-violet staining. Abnormal muscle fibers in myofibrillar myopathy (as originally reported by DeBleeker et al. [36]) indeed have fluorescence-enhanced congophilic accumulations, but we doubt whether in those patients congophilia represents true amyloid deposits because in none of our four myofibrillar myopathy patients was their congophilic material positive with crystal violet, nor was it giving an orange fluorescence with thioflavin-T as it is typical for ‘real’ amyloid (Askanas et al., unpublished observations).

**Intramuscle fiber clusters of p-tau-containing PHFs**

p62/SQSTM1. p62/SQSTM1, simply ‘p62’ is a shuttle protein transporting polyubiquitinated proteins for both the proteasomal and lysosomal degradation [37,38]. In s-IBM muscle fibers p62 is an integral component of PHFs containing p-tau [39]. For diagnostic purposes, we recommend light-microscopic HRP-immunohistochemical staining of p62, which appears in the form of strongly-immunoreactive, various-sized, mainly squiggly, linear or small rounded aggregates (figure 3 A–C). These are in the non-vacuolated cytoplasm of approximately 80% of the vacuolated muscle fibers, and in about 20–25% of the muscle fibers that appear “non-vacuolated” on a given 10 μm transverse section. By immunoelectronmicroscopy, p62 embraces bundles on PHFs (figure 3 D), SMI-31 monoclonal antibody. Immunostaining with SMI-31 antibody, which identifies squiggly inclusions containing p-tau in s-IBM muscle fibers [40], has been previously recommended by us as diagnostic [41], but currently we prefer p62-immunoreactivity because it is much stronger and more specific since it does not stain muscle fiber nuclei.

**Ubiquitin immunoreactivity**

Ubiquitin immunoreactivity is localized in both Aβ- and p-tau containing inclusions within s-IBM muscle fibers, and it can be used to differentiate s-IBM from polymyositis [42]. Importantly, ubiquitin-positive inclusions were also identifiable in formalin-fixed paraffin-embedded muscle biopsies of s-IBM patients, but not in any other inflammatory myopathy [43]. This could be important in some laboratories where freshly-frozen muscle biopsy specimens are not available for studies.

**Alkaline phosphatase staining**

In s-IBM muscle biopsies, perimysial connective tissue, even in regions of active disease, lacks the typically-strong alkaline phosphatase (AP)-positivity seen in similarly-active regions of polymyositis (PM) and dermatomyositis biopsies—that activity is attributable to active fibroblasts [44,45]. It is a convenient and inexpensive stain to differentiate between s-IBM and PM.
Electronmicroscopy

The characteristic aggregates containing p-tau are, at the electronmicroscopy (EM) level, clusters of PHFs. Commonly they are located within non-vacuolated areas of vacuolated muscle fibers (figure 4 A). These 15–21 nm diameter twisted filaments (figure 4 B), which are essentially identical to PHFs of Alzheimer disease brain, and are immunopositive with several antibodies reacting with various epitopes of p-tau (figure 5 A–C) [40].

Figure 3

Accumulation of p62 within s-IBM muscle fibers

A–C. By light microscopy, p62-immunoreactivity appears as squiggly and skein-like–and large inclusions, or small dots. No other structures, including muscle fiber nuclei, are immunoreactive. A–C: ×3100.

D. By gold-immunoelectronmicroscopy, p62 associates with the periphery of a cluster of paired helical filaments (PHFs). ×48,000.
Figure 4

Transmission electronmicroscopy of s-IBM muscle fibers

A. A large cluster of PHFs located within a normally-appearing myofiber.
B. PHFs have their characteristic twisted-pair appearance. A: ×28,000; B: ×62,000.
C. A cluster of 6–10 nm amyloid-like fibrils, ×42,000.
D. A vacuole containing inclusions consisting of numerous, various-sized membranous whorls of autophagosomal/lysosomal debris, ×25,000.
The s-IBM muscle fiber cytoplasm also contains collections of 6–10 nm amyloid-like fibrils (figure 4 C), which contain Aβ42-immunoreactivity [46] (figure 5 D). The combination of tau-positive PHFs plus Aβ42-positive collections of 6–10 nm amyloid-like fibrils is essentially diagnostic of s-IBM. Numerous, various-sized membranous structures, such as myelin-like whorls and multilaminar bodies, as well as osmophically-dark amorphous material, and other types of lysosomal debris, are virtually always present (figure 4 D). They are characteristic, but not diagnostic. Also typical, but not diagnostic, are various mitochondrial abnormalities including paracrystalline inclusions.

TDP-43 immunoreactivity
Immunoreactivity of the transactive response DNA-binding protein 43 (TDP-43) in the form of cytoplasmic inclusions within s-IBM muscle fibers was reported by several investigators [47–50], and was confirmed by us [51]. However, quantitative comparison of TDP-43-immunoreactive inclusions to p62-immunoreactive inclusion, revealed that fibers positive for p62-inclusions were three times more frequent than ones containing TDP-43-inclusions [51]. Moreover, p62-inclusions were more sharply defined, and two biopsies displaying several p62 inclusions did not contain any TDP43-inclusions. Therefore, we do not recommend TDP-43 for diagnostic evaluation of s-IBM biopsies.

Abnormal protein aggregation and misfolding, and abnormalities of protein disposal
In normal cells quality-control mechanisms assure that any malfunctioning or damaged intracellular structures, including proteins and organelles, are identified and repaired, or removed (reviewed in [29,52–55]). The cellular mechanisms for ensuring proper protein quality also influence protein transcription, thereby preventing proteins from being over- or under-produced. To eliminate misfolded proteins, a cell utilizes several mechanisms: protein refolding through the endoplasmic reticulum (ER)-chaperones; protein refolding through heat shock proteins; protein degradation through the 26S ubiquitin-proteasome system (UPS); and protein degradation through autophagy. Under various pathological conditions, and in aging, protein quality-control is disturbed (reviewed in [52,56]). This results in accumulation and aggregation of pathologically modified proteins and damaged organelles.
Within s-IBM muscle fibers accumulation of misfolded proteins, free or in multiprotein aggregates can result from their increased production and/or inadequate clearance, or both. Aggregation of abnormal proteins is considered to be caused by binding of partly unfolded or misfolded polypeptides induced by interaction between their inappropriately exposed hydrophobic surfaces [26]. Fully-formed, insoluble amyloid fibrils may not be cytotoxic, but their simpler pre-amyloid oligomers occurring either diffusely or in the protofibril stage, are considered very cytotoxic [57].

Diseases characterized by protein misfolding and aggregation are termed “conformational diseases” (reviewed in [58]). Unfolding or misfolding of proteins can occur under several circumstances, including oxidative stress, macromolecular crowding, defective protein disposal, and “ageing” [55]. Abnormal glycosylation and other deleterious chemical reactions can lead to protein unfolding and misfolding, with resultant protein and cellular malfunction. In s-IBM muscle fibers very large, abnormal protein aggregates occur mainly in the form of plaque-like or dotty inclusions, or

**Figure 6**

**Light-microscopic immunohistochemistry of s-IBM muscle fibers**
A. Amyloid-β42 is present in several plaque-like and some dotty inclusions.
B, C. p-tau immunolocalized with Alz-50 and AT8 antibodies is present in the form of large, squiggly and skein-like inclusions.
D–F. Show close immunocolocalization of p-tau identified by monoclonal AT100 antibody, which identifies p-tau of Alzheimer brain paired helical filaments (D, red), with p62 identified by green fluorescence (E). There is a very close colocalization between p62 and p-tau as seen by a merged picture (C). All: ×2600.
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Squiggly-linear inclusions, all of which contain several accumulated proteins (detailed in [2,59]). Plaque-like and dotty inclusions contain: amyloid-β precursor protein (AβPP); Aβ40 and Aβ42 ([figure 6 A]), and Aβ42 oligomers, as well as proteins participating in AβPP proteolysis, such as β-secretase (BACE1) and the γ-secretase complex (containing presenilin1 and nicastrin) ([2,59]). p-tau is accumulated mainly in the form of squiggly inclusions ([figure 6 B–D]), where it closely co-localizes with p62 ([figure 6 D–F]). Also accumulated within some of those aggregates are α-synuclein and cellular prion protein, as well as a number of other proteins having various functions and significance including: markers of oxidative stress; ER-chaperones indicative of the unfolded-protein-response (UPR); 26S proteasome components, and the proteasome shuttle protein p62; autophagosome-related protein LC3; mutated ubiquitin (UBB+1); various transduction and transcription factors; and mutated ubiquitin (UBB+)3; various transduction and transcription factors; and several other proteins (reviewed in [1–6]).

In an aggregate, an abnormal protein could accumulate due to its impaired catabolism (related to lysosome or proteasome inhibition), overproduction, or being captured by or “glued” to another accumulated protein. While it is not clear whether protein-aggregates impair normal cellular functions, it is very likely that they are not welcome by the muscle fiber, because masses of aggregates—which are visible in many muscle fibers on a given transverse section and known to be present in various places along the muscle fibers—could severely impair muscle fiber integrity and mechanical function. Large aggregates can physically displace and disturb the function of normal cytoplasmic proteins and organelles, such as mitochondria and endoplasmic reticulum. Proteins that accumulate within the aggregates often show oxidative damage [60], which may have been caused them to be incorporated there. Oxidative and nitrotyrosine stress occurs in s-IBM muscle fibers ([27,61] and below)—and nitration and oxidative stress affect assembly and phosphorylation of tau [62,63], and probably damage various other proteins and muscle fiber function.

In this review we describe a few of the major proteins that accumulate, emphasizing their possible pathogenic roles (details of other accumulated proteins are available in [59]).

Putative detrimental role of some of the proteins abnormally accumulated within s-IBM muscle fibers

Intra-cellular accumulation of Aβ42 and its putative toxicity

Our s-IBM Studies from nearly two decades ago were the first to identify an intracellular accumulation of Aβ in any disease [46,64,65]. They were the basis for our proposal of a crucial cytotoxic role of intra-cellular Aβ, not only for s-IBM muscle fibers but also, by analogy, for AD neurons [66].

Several experimental studies, including our cultured human muscle IBM model, and transgenic mouse models of others, including a GNE transgenic mouse model, provide strong evidence for an intracellular toxicity of amyloid-β precursor protein (AβPP) and Aβ in s-IBM (reviewed in detail in [6,59]). Increased AβPP mRNA and abnormal accumulation of both AβPP and Aβ are identified early within s-IBM abnormal muscle fibers [4,5]. Normally, Aβ is released from AβPP as a 40 or 42 amino acid peptide. In s-IBM muscle fibers there are abnormalities of the AβPP-processing machinery that lead to increased generation of Aβ1-42. BACE1, which cleaves AβPP at the N-terminal of Aβ, and components of the γ-secretase systems are increased [67–70], and associate with AβPP, which could suggest that they participate in its processing to generate the Aβ. Our most recent studies showed that in s-IBM muscle fibers AβPP is phosphorylated [71] and according to others, phosphorylation of AβPP increases its toxicity and assembly into Aβ toxic oligomers [72,73].

In s-IBM muscle fibers, there is preferential accumulation of the Aβ42 fragment [33], which is known to be more hydrophobic and more prone to self-association and oligomerization than Aβ40, and as such is much more cytotoxic [74–78]. In s-IBM muscle fibers, all congophilic (i.e., fibrillar, amyloidic) Aβ inclusions contain Aβ42 [33] ([figure 6 A]). Recently, the putative importance of Aβ42 cytotoxicity in s-IBM muscle fibers was strengthened by our novel demonstration of Aβ42 oligomers, in the form of Aβ42-dimers, -trimers and -tetramers [79]. None of the control muscle biopsies had Aβ oligomers [79]. Nonfibrillar cytotoxic “Aβ-derived diffusible ligands” (ADDLs), originally derived from Aβ42 [80], are increased in in s-IBM muscle fibers [79]–ADDLs are also increased in AD brain, where they were proposed to play an important pathogenic role [81].

Our novel demonstration of Aβ42 oligomers in s-IBM muscle biopsies, provides additional evidence that their intramuscle fiber accumulation may contribute importantly to the s-IBM pathogenic cascade. There are several other factors acting in s-IBM muscle fibers that might contribute to Aβ production, oligomerization and deposition. These include increased expression and accumulation of: cystatin C, an endogenous cysteine protease inhibitor, which was previously proposed to participate in Aβ deposition within the amyloid plaques AD brain [82]; transglutaminases 1 and 2, which contribute to Aβ aggregation and insolubility by cross-linking Aβ molecules [83], and free cholesterol [84], which increases Aβ production and amyloidogenesis (referenced in [84]), and αβ-crystallin (αBC) [85], which specifically recognizes and stabilizes proteins that have a propensity to aggregate and precipitate [86,87]. Our studies have demonstrated that in s-IBM biopsied muscle (αBC) physically associates with AβPP and Aβ oligomers, and AβPP-overexpression in cultured human muscle fibers significantly increases (αBC) [85]. Therefore, binding of αBC to Aβ oligomers conceivably might retard and diminish their fibrillation and aggregation into visible presumably non-toxic aggregates—if so, that could adversely
Accumulation of phosphorylated tau

In s-IBM muscle fibers, as in AD brain [88–90], p-tau is accumulated intracellularly in the form of congophilic, linear, squiggly inclusions [40,91] and (figure 6 B,C), which by EM appear as PHFs (figure 5 A–C). Various antibodies recognizing several epitopes of the p-tau present in AD brain, including AT-100, AT-8, PHF-1, and ones selectively recognizing only AD-specific conformational tau, such as Alz-50 [92], exclusively associate with s-IBM PHFs by both the light- and electron-microscopic immunohistochemistry (figure 6 B,C and figure 5 A–C) [40,91]. Several kinases known to phosphorylate-tau [89,93,94] are also accumulated within s-IBM muscle fibers, where they colocalize with p-tau-positive inclusions. These include extracellular signal-regulated kinase (ERK) [95], CDK5 [96], glycogen synthase kinase 3β (GSK-3β) [97] and casein kinase 1 [98]. Additionally, GSK-3β is hyperphosphorylated and activated in s-IBM muscle fibers [71].

s-IBM-PHFs also contain RNA and the RNA-binding-protein survival motor neuron (SMN), both of which were proposed to contribute to PHF formation [99]. New studies related to neurodegeneration strongly suggest that increase of p-tau is cytotoxic to neurons (reviewed in [89,100]). The mechanisms leading to the abnormal phosphorylation and accumulation of tau, and its consequences in s-IBM, are not yet clarified. In addition to overexpression of various kinases that participate in tau phosphorylation, it has been recently reported that Aβ42 oligomers induce tau phosphorylation [78,101]. Tau is known to be ubiquitinated, and its accumulation has been at least partially attributed to inhibition of the 26S proteasome (reviewed in [102]). Recently, it has been reported that in AD brain p-tau is hyperacetylated due to deficiency thereof of SIRT1 [103]. That modification was reported to impair p-tau degradation by the 26S proteasome [103]. Impaired autophagy has also been implicated as a factor contributing to tau oligomerization and accumulation [104]. Whether Aβ2-oligomers, and impaired functions of both the 26S proteasome and autophagy, both identified in s-IBM muscle fibers [20,24,33,79], contribute to tau phosphorylation remains to be investigated. It is of interest that the deacetylation activity of SIRT1 is decreased in s-IBM muscle fibers ([105] and below).

α-Synuclein and parkin

Abnormal expression of α-Synuclein (α-syn) occurring spontaneously in brains of various neurodegenerative disorders has been associated with, and possibly causative of, oxidative stress, impaired proteosome function, and mitochondrial abnormalities [106,107]. We have shown that α-syn is accumulated in s-IBM muscle fibers [108], and that its 22 kDa O-glycosylated form is more expressed than its native 16 kDa form [109]. The 22 kDa form, but not the native 16 kDa form, was shown by others to be a target of ubiquitination by parkin [110]. The preferential increase of the 22 kDa O-glycosylated form of α-syn in s-IBM muscle fibers might be due to the proteasome inhibition we previously demonstrated in them ([20], and below).

Because oxidative- and nitric-oxide-induced stress, and mitochondrial abnormalities, are also aspects of the s-IBM muscle fiber pathology (reviewed in [3,111]), a putative toxicity of α-syn, may contribute to the muscle fiber degeneration. Parkin, an E3-ubiquitin ligase that ubiquitinates α-syn [112], is increased in s-IBM muscle fibers, where it accumulates in the form of aggregates [109]. In brains of sporadic Parkinson disease patients, parkin and α-syn accumulate in Lewy bodies, which are considered aggresomes [112]. Parkin, in addition to ubiquitinating several proteins, also protects cells against toxicity induced by α-syn, ER and other stresses, perhaps by helping to aggregate toxic α-syn oligomers and promote their degradation [113]. Accordingly, we propose that increase of parkin in s-IBM muscle fibers is their attempt to protect themselves against various toxicities.

Interestingly, in diseased human muscle, α-syn and parkin also accumulate in ragged-red fibers in various muscle diseases [114]. This accumulation is not related to their accumulation in s-IBM muscle fibers [114]. Ragged-red fibers, originally described in 1970 [115] using the Engel-modified trichrome staining [30], represent muscle fibers containing enlarged and otherwise abnormal mitochondria that are often accumulated at the periphery of the fibers [30]. Ragged-red fibers are abundantly present in muscle biopsies of patients with various mitochondriopathies, including ones with genetically-determined mitochondrial DNA mutations. They are also present in muscle biopsies of some ageing patients, and their number is significantly increased in s-IBM muscle biopsies [116]. We propose that abnormal mitochondria within the ragged-red fibers are destined for autophagic degradation, and parkin is recruited to facilitate their clearance, as has been reported in other systems [117,118].

Myostatin

Myostatin (MSTN), a protein secreted from skeletal muscle, is considered a negative regulator of muscle growth during development and of muscle mass during adulthood [119]. In biopsied s-IBM muscle fibers, MSTN precursor protein (MSTNPP) and MSTN dimer are significantly increased on immunoblots, and MSTNPP immuno-co-localizes with AβPP/Aβ [120]. Interestingly, AβPP-overexpression into cultured normal human muscle fibers increased MSTNPP expression, and subsequent experimental inhibition of proteasome caused accumulation and colocalization of both MSTNPP/myostatin and AβPP/Aβ, and their physical association [121]. The mechanism(s) by which overexpressed AβPP/Aβ increases MSTNPP is not known. Possibly, AβPP binding to MSTNPP causes its posttranslational modification that lessens its traffic and degradation, resulting in accumulation.
Recently, the importance of MSTNPP accumulation in s-IBM was emphasized by the studies of others [122] demonstrating that MSTNPP is capable of forming intracellular β-pleated sheet amyloid. Since MSTN physically associates with AβPP/Aβ [120,121], it is possible, as we have previously proposed [120], that these two proteins might enhance each other's aggregation, oligomerization and β-pleated sheet formation.

**Accumulation of other proteins**

The scope of this article does not permit a detailed description of several other proteins aggregated in s-IBM muscle fibers, such as cellular prion protein, mutated ubiquitin (UBB+1), α-1 antichymotrypsin, and several others. They are illustrated and referenced in [2,59].

**Abnormalities of protein degradation pathways in s-IBM muscle fibers**

In eukaryotic cells, the 26S proteasome and the autophagic/lysosomal systems are two major protein degradation pathways [123]. The 26S proteasome, also called the ubiquitin-proteasome system (UPS), is a major degradation mechanism for normal regulatory and other short-lived proteins, and misfolded proteins exported from the endoplasmic reticulum (ER) through a ubiquitin-mediated ATP-independent process [22,123]. However, long-lived, structural proteins and/or variously damaged or misfolded proteins, and obsolescent cellular organelles are degraded through "autophagy" [29,54,124].

**UPS and its malfunction in s-IBM muscle fibers**

The 26S proteasomes are composed of a catalytic 20S core and a 19S regulatory complex. The 20S core contains the protease-activity sites having trypsin-like (TI), chymotrypsin-like (CTI) and peptidyl glutamyl-peptide hydrolytic (PGPH) activities [125,126], while the 19S, is thought to mediate the recognition of both polyubiquitinated moieties and unfolded proteins, thereby permitting their access into the interior of the 20S component to be catabolized [52,125,127]. Decreased proteasome function has been recently reported in several neurodegenerative diseases characterized by accumulation of multiprotein aggregates in the brain [22,128,129]. AβPP/Aβ have been reported to inhibit proteasomal activity in our culture IBM model and in other cells [129–131].

In s-IBM muscle fibers, we have demonstrated reduced activities of the three major proteasomal proteolytic enzymes [20]. Several factors, in addition to increased AβPP and Aβ, present in s-IBM muscle fibers might contribute to inhibiting proteasome function, including an ageing muscle fiber environment; protein over-crowding; oxidative stress [5,6,11], and accumulated p-tau [5,6,11], α-synuclein [108], and UBB+1 [132] – all of these are capable of inhibiting proteasome activity in other systems [130,131,133–135].

We propose that the ageing cellular environment of s-IBM muscle fibers, combined with factors such as oxidative stress and perhaps other detrimental molecular events, leads to abnormal production and accumulation of UBB+1 [132]. A failure to degrade/remove surplus proteins, including abnormal damaged proteins, is presumably detrimental to muscle fibers as it is to other cells. Furthermore, accumulated ubiquitinated, misfolded, and oxidized protein aggregates by themselves can cause proteasome inhibition. Moreover, the still-soluble, early intermediates of protein aggregates, in the form of dimers and trimers can also induce proteasome inhibition [134], and they are highly toxic to cells [26,78].

**Autophagosomal-lysosomal pathway**

The autophagosomal-lysosomal pathway (ALP) is the major degrading mechanism for long-lived, structural proteins and/or damaged or misfolded proteins, and obsolescent cellular organelles [29,54,124]. ALP is composed of three main pathways: macroautophagy, chaperone-mediated-autophagy (CMA), and microautophagy, all of which lead the material to be degraded to lysosomes (reviewed in [29,54,56,123]). Lysosomes are the main compartments in which degradation of a various proteins and molecules actually occurs, through the activity of various lysosomal proteolytic enzymes. The other components of the ALP serve mainly as crucial delivery pathways to the lysosomes of the molecules to be degraded. The term "autophagy" in reference to the lysosomal degradation, has been used for decades, but the molecular aspects of delivering the cargo destined for lysosomal degradation have been delineated only recently [29,54,56,136]. We recommend that the term “autophagy” be reserved for lysosomal degradation, and not to “macroautophagy” as it often occurs in the literature.

"Macroautophagy" designates formation and maturation of “autophagosomes”, which are structures carrying degradation-destined proteins and organelles to the lysosomes for their lysosomal degradation. After an autophagosome fuses with the lysosomal membrane, it disposes its cargo into the lysosome, where it is then degraded by the lysosomal enzymes [29,54,56,137]. Autophagosomes proliferate and mature when the lysosomal function is inhibited, because the cargo that they are carrying cannot be received and cleared by the lysosomes. That situation is detrimental to the cell and can result in formation of autophagosomal vacuoles [137]. This occurs in s-IBM muscle fibers and in neurons of some of the neurodegenerative disorders. In some situations, proliferation of autophagosomes in neurons has been associated with abnormal Aβ over-production and vacuolization [124,138]. Accordingly, before suggesting to s-IBM patients or to those with various neurodegenerative disorders the use of drugs to enhance macroautophagy, the function of the entire ALP must be evaluated.

For example, inhibition, rather than stimulation of macroautophagy increased neuronal survival under some pathologic conditions (reviewed in [55]).
Existence of autophagic vacuoles (AV) in s-IBM muscle biopsies (figure 1 A–C) has been known for many years (recently reviewed in [2,7,139]), but the mechanism of their formation was not well understood. Our newest studies have demonstrated for the first time [24] that in s-IBM muscle fibers there is increased formation and maturation of vacuolar autophagosomes, as indicated by: the autophagosomal marker LC3-II [140], and mTOR-mediated phosphorylation of p70S6K [137,140]. These observations suggest that activated macroautophagy is an important factor leading to formation of the vacuoles. Most importantly, our studies provided important evidence of impaired autophagy in s-IBM muscle fibers as related to the inhibition of lysosomal enzymes Cathepsin D and B [24]. The decrease of lysosomal cathepsin D and B enzymatic activities was specific to s-IBM, because in polymyositis muscle fibers their activities were actually increased in our study [24] and in studies by others (referenced in [24]). In contrast to several neurodegenerative diseases in which ALP functions have been extensively studied [29,54,55,124], ALP functions in s-IBM muscle fibers have been virtually unexplored.

Impaired autophagy in s-IBM muscle fibers might be, at least partially, responsible for the abnormal accumulation of various proteins, including AβPP, Aβ, α-syn, BACE1 and tau, all reported to be partially degraded through autophagy [29,129,141,142]. Moreover, Aβ has been shown to be produced, at least partially, within the autophagosomes [124,138,143].

Our newest studies demonstrated that inhibition of lysosomal activity in cultured human muscle fibers induces Aβ oligomerization in them [79].

Other important intracellular abnormalities in s-IBM muscle fibers

Endoplasmic reticulum stress and the unfolded protein response

The ER is an intracellular compartment having a critical role in the processing, folding and exporting of newly-synthesized proteins into the secretory pathway (reviewed in [144,145]). In the ER, molecular chaperones are required to assure proper folding of unfolded or misfolded proteins [144,145]. Unfolded proteins accumulating in the ER cause ER stress [144,145]. This elicits the UPR, a mechanism by which a cell attempts to protect itself against ERS [144,145]. In s-IBM muscle fibers we have previously reported evidence of ER stress and the UPR, and the activation of all three branches of the UPR [25,28,146]. Since in s-IBM muscle fibers misfolded proteins continue to accumulate and aggregate, we propose that in them the UPR is not adequate, probably because it is overwhelmed and/or impaired by the misfolded proteins. Our most recent studies have suggested that ERS is detrimental to the muscle fiber because experimentally-produced ER stress in cultured normal human muscle fibers induced myostatin, a negative regulator of muscle mass, through an NF-κB related mechanism [147], and decreased SIRT1 deacetylase activity [105], reviewed in details in [1], and decreased activities of Cathepsin D and B [24]. Accordingly, ER stress may importantly contribute to the s-IBM pathogenesis.

Decreased deacetylase activity of SIRT1

SIRT1 belongs to the mammalian sirtuin family of NAD+-dependent histone deacetylases (HDACs) [147,148]. Targets known to be deacetylated by SIRT1 include histone 4 (H4), NF-κB, and p53 [147,148]. SIRT1 activation has been proposed to play a role in neuroprotection, including AD [147]. In various cell lines, increase of SIRT1 or its activation was reported to protect against Aβ toxicity, attributed to either decreasing the amount of Aβ by activating α-secretase [149], or by inhibiting NF-κB activation and its subsequent disturbance of signaling [150]. In AD brain a deficiency of SIRT1 [151] is postulated, among other factors to detrimentally influence tau acetylation ([103] and above).

In s-IBM muscle fibers SIRT1 activity and deacetylation of SIRT1 targets, including NF-κB, are significantly decreased despite increased SIRT1 protein expression [105]. Because increased acetylation (or decreased deacetylation) of NF-κB leads to its increased activity [152], decreased SIRT1 deacetylase activity might be directly responsible for the presumably-detrimental NF-κB activation in s-IBM muscle fibers, thereby contributing to the abnormal accumulation of Aβ and increased MSTN.

To our knowledge, our study was the first demonstration of decreased SIRT1 deacetylase activity in any human muscle disorder, namely s-IBM, which is a disease associated with ageing.

Oxidative stress

There is increasing evidence that free-radical toxicity may participate in the s-IBM pathogenesis. Indicators of oxidative stress, as well as enzymes participating in the cellular defense against oxidative stress, are accumulated in s-IBM muscle fibers [61,153], for example increased NF-B [154]. Recently, we showed in s-IBM fibers that Dj-1 is increased and is oxidized [27]. Although its precise functions are not yet known, Dj-1 was reported to have anti-oxidative and neuroprotective properties [155]. During oxidative stress, Dj-1 becomes oxidized [155]. Its experimental downregulation sensitizes cells to oxidative stress [155]. Dj-1 was also reported to be an important mitochondrial protective agent [156]. Our studies indicated for the first time that Dj-1 might play a role in human muscle disease.

Heat shock proteins (HSPs)

Chaperones of the HSP70 family play a very important role in protein folding and refolding, and in disaggregation of partially unfolded proteins (reviewed in [157,158]).
biopsies, HSP70 is prominently increased and it associates with Aβ by immunoprecipitation. Vacuolated muscle fibers and some non-vacuolated fibers contain strongly-immunoreactive HSP70 inclusions, in which HSP70 colocalizes with Aβ [159] and reviewed and illustrated in [2]). Accordingly, in s-IBM muscle fibers HSP70 may participate in Aβ refolding, and may facilitate the refolding of other proteins.

**Mitochondrial abnormalities**

These include: ragged-red fibers [115], cytochrome-c-oxidase (Cox) negative muscle fibers, and multiple mitochondrial DNA deletions (reviewed in [116]). They are more common in s-IBM muscle than expected for the patient’s age [116]. Cox-negativity can be intermittently segmental along the muscle fiber [117]. With their electron-transport generation of ATP blocked, Cox-negative fibers may be surviving by anaerobic glycolysis in their Cox-negative regions, or possibly by ATP diffusing from an adjacent region. We have shown that excessive APP and Aβ contributes to the mitochondrial abnormalities [160]. This concept of AβPP/Aβ mitochondrial-toxicity is now supported by studies in other systems, especially as putatively related to AD and Parkinson brain [156,161,162]. α-synuclein accumulated in s-IBM muscle fibers may, in oligomeric and misfolded forms, also contribute to mitochondrial toxicity [161]. The mitochondrial abnormalities presumably contribute to the overall muscle fiber malfunction and degeneration.

**Conclusion**

In conclusion, s-IBM is a degenerative, conformational muscle disease of multifactorial pathogenesis. We reviewed molecular mechanisms responsible for multiprotein aggregation and accumulation within s-IBM muscle fibers. We also propose that the accumulation of post-translationally-modified proteins in the degenerated muscle fibers, can be perceived as “foreign” (not self) and be responsible for inducing T-cell inflammation in the s-IBM muscle. Accordingly, we propose that s-IBM is not a primary inflammatory disease—that would explain lack of benefit with anti-dysimmune treatment.

Although we do not yet know a unique-primary abnormality or mechanism, the multiplicity of abnormalities in s-IBM muscle fibers present various potential targets for therapeutic drugs.

**Conflicts of interests:** none

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Sporadic inclusion-body myositis


