Pathogenic Considerations in Sporadic Inclusion-Body Myositis, a Degenerative Muscle Disease Associated With Aging and Abnormalities of Myoproteostasis

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Abstract

The pathogenesis of sporadic inclusion-body myositis (s-IBM) is complex; it involves multidimensional pathways and the most critical issues are still unresolved. The onset of muscle fiber damage is age related and the disease is slowly, but inexorably, progressive. Muscle fiber degeneration and mononuclear cell inflammation are major components of s-IBM pathology, but which is precedent and how they interrelate is not known. There is growing evidence that aging of the muscle fiber associated with intramyofiber accumulation of conformationally modified proteins plays a primary pathogenic role leading to muscle fiber destruction. Here, we review the presumably most important known molecular abnormalities that occur in s-IBM myofibers and that likely contribute to s-IBM pathogenesis. Abnormal accumulation within the fibers of multiprotein aggregates (several of which are congophilic and, therefore, generically called “amyloid”) may result from increased transcription of several proteins, their abnormal posttranslational modifications and misfolding, and inadequate protein disposal, that is, abnormal “myoproteostasis,” which is combined with and may be provoked or abetted by an aging intracellular milieu. The potential cytotoxicity of accumulated amyloid β protein (Aβ42) and its oligomers, phosphorylated tau in the form of paired helical filaments and α-synuclein, and the putative pathogenic role and cause of the mitochondrial abnormalities and oxidative stress are reviewed. On the basis of our experimental evidence, potential interventions in the complex, interwoven pathogenic cascade of s-IBM are suggested.

Key Words: Aging, Autophagy, Inclusion-body myositis, Misfolded proteins, Multiprotein aggregates, Proteasome, Proteostasis.

INTRODUCTION

Sporadic inclusion-body myositis (s-IBM) is the most common muscle disease of older persons. Although the course of the disease varies from patient to patient, s-IBM typically progresses rather slowly. Nevertheless, its relentless course eventually leads to severe disability and wheelchair dependency (1–5). Effective long-term treatment is not currently available (1–5). Sporadic inclusion-body myositis was initially considered to be a rare muscle disease. However, during the last 2 decades, due both to greater physicians awareness of this disease and the existence of reliable pathologic markers of s-IBM muscle biopsies, the diagnosis of s-IBM has become more prevalent.

Pathologically, the s-IBM muscle biopsy exhibits an unusual and specific pathologic phenotype, which combines multifaceted muscle fiber degeneration with extracellular T-cell inflammatory infiltrates. How each relates to s-IBM pathogenesis remains unknown (2, 5, 6); however, it is becoming more likely that s-IBM-characteristic muscle fiber degeneration leads to the muscle fiber weakness and atrophy.

The muscle fiber degeneration is characterized by vacuolization and intramuscle fiber accumulations of ubiquitinated, congophilic, postranslationally modified proteins (2, 6). We propose that those misfolded, conformationally modified proteins provoke an inflammatory response. In support of this hypothesis is the well-established observation that patients with s-IBM typically do not satisfactorily respond to various antisyndrome/anti-inflammatory treatments that have been extensively tested (1, 4, 5, 7). Moreover, some older patients with hereditary IBM (h-IBM) caused by missense mutations in the UDP-N-acetylgalactosamine-2 epimerase/N-acetylmannosaminylkinase gene have varying degrees of lymphocytic inflammation, although that form of h-IBM is not considered to be immune mediated (8, 9). Muscle biopsies of patients with h-IBM are similar to those of s-IBM about having a similar spectrum of accumulated abnormal proteins (10, 11). It is possible that, in older patients with h-IBM, their “aging” muscle fiber milieu (and perhaps other individual intrinsic muscle fiber abnormalities) make some of the accumulated proteins interpreted as “foreign” by the immune system, thereby inducing the T-cell lymphocyte inflammatory response.

The possibility that the inflammation in s-IBM might be secondary to the ongoing degeneration and production of abnormal proteins within the muscle fibers (assuming they are either leaked or expressed on the myofiber surface) would, if confirmed, be supported by an aged transgenic mouse overexpressing mutated gelsolin (12). In this reported “model of s-IBM,” there is intramyofiber accumulation of misfolded and congophilic proteins, including amyloid β (Aβ) and gelsolin, but interestingly there is also perivascular and endomysial
lymphocytic infiltration, strongly suggesting that inflammation is consequent to the overexpressed abnormal mutant protein or other secondarily accumulated proteins within the muscle fibers.

There are also several phenomena in the degeneration of muscle fibers in s-IBM that are similar to the complex neuronal degenerative processes that occur both in Alzheimer (AD) and Parkinson (PD) diseases. These include (a) abnormal accumulations of many of the same putatively pathogenic proteins, (b) similar posttranslational modifications of the accumulated proteins, (c) similarly defective mechanisms of protein disposal, including inhibition of both the 26S proteasome and autophagy, and (d) mitochondrial abnormalities (2, 6, 13). Thus, s-IBM, similarly to AD and PD, is considered a "conformational disorder," caused by protein unfolding/misfolding and associated with the formation of ubiquitinated multiprotein inclusion bodies (aggregates) (2, 13, 14). Another important aspect of the pathogenesis of s-IBM is an impaired regeneration ability of muscle fibers (15).

Here, after a brief summary of muscle biopsy diagnostic criteria, we describe the most recent research findings, unveiling some of the mechanisms underlying impaired protein degradation and its consequences. We will focus on what we consider to be the most critical proteins and events participating in the s-IBM pathogenic cascade (Fig. 1). On the basis of our experimental evidence (Fig. 2), we will discuss the likely relationships between various IBM-characteristic pathologic pathways, which may help enlighten the complex, interwoven pathogenic cascade of s-IBM.

**SUMMARY OF DIAGNOSTIC CRITERIA FOR s-IBM IN A MUSCLE BIOPSY**

Proper evaluation of the muscle biopsy is the most important diagnostic aspect of s-IBM, and it is important to apply reliable diagnostic criteria. Because various degrees of lymphocytic inflammation and expression on muscle fibers of the major histocompatibility complex I (MHC-I) can occur in muscle biopsies of both s-IBM and polymyositis (as well as in other muscle diseases such as necrotizing myopathy), biopsies of patients with s-IBM are often misdiagnosed as polymyositis, particularly in the earlier stages of s-IBM (16). Therefore, we, contrary to some other investigators, consider that the expression of MHC-I on muscle fibers is not a diagnostic criterion of s-IBM. Sporadic inclusion-body myositis patients misdiagnosed as polymyositis sometimes undergo long-term treatment with various ineffective immunosuppressant drugs that can engender unpleasant side effects (4).

Below is a brief summary of s-IBM muscle biopsy pathologic diagnostic criteria. Details can be found elsewhere (2, 6):

On Engel-trichrome staining (17), the standard general stain used for fresh-frozen sections of muscle biopsies, there are some muscle fibers that contain one or a few vacuoles in a given transverse-section. Most of these vacuoles appear to be autophagic because they contain poorly differentiated bluish-reddish material interiorly or at their periphery, indicating lipoprotein membranous material or proteinaceous material (Figs. 3A–D). This distinguishes the pathogenic vacuoles from empty freeze- Artifacts holes. Vacuoles that are definitely "rimmed" are rare; most vacuoles in s-IBM do not have a sharply defined peripheral red rim. The number of vacuolated muscle fibers varies not only between patients with s-IBM but also on different transverse sections of the same muscle biopsy and even more in 2 different muscle biopsies obtained at the same time from the same patient. Accordingly, we consider that evaluating potential treatments of patients with s-IBM by examining muscle biopsies taken before and after treatment, as often done in some therapeutic trials, to be unreliable and potentially misleading.

Multiple foci of intracellular amyloid deposits identified by Congo red fluorescence visualized through Texas Red filters (18) are evident within approximately 40% to 70% of the s-IBM vacuolated muscle fibers in a given transverse section; they are located mostly in their nonvacuolated regions or in a number of "nonvacuolated," normal-appearing muscle fibers (Figs. 3F, G). This technique is the best, most sensitive method for highlighting β-pleated-sheets amyloid inclusions, which sometimes are very small or few. Congo Red staining visualized in polarized light is widely used to seek amyloid, but it is much less precise, more difficult to interpret, and can miss identifying amyloid deposits. Thus, it should not be used routinely for s-IBM diagnosis. We have shown that, similarly to the brain with AD, in s-IBM muscle. both amyloid-β42 (Aβ42) and phosphorylated tau (p-tau), the latter in the form of twisted paired helical filaments (PHFs), are congophilic (19, 20). A number of other proteins accumulated in s-IBM muscle fibers, including normal cellular prion and α-synuclein (α-syn) (2, 6, 21), also have the propensity to self-aggregate into β-pleated-sheet amyloid. Crystal violet metachromatically reddish pink staining can also show the intramyofiber amyloid deposits in s-IBM muscle fibers. This method is more convenient because it does not require fluorescence microscopy, but it is less precise because small amyloid deposits can be difficult to identify (2).

The intramuscle fiber clusters of p-tau-containing PHFs by SQTSM1/p62 staining are diagnostically very important (22). p62 is a shuttle protein transporting polyubiquitinated proteins for both proteasomal and lysosomal degradation (23, 24). In s-IBM muscle fibers, p62 is increased both at the mRNA and protein levels and is an integral component of p-tau-containing PHFs (22). Staining of p62 appears in the form of strongly immunoreactive, various-sized, mainly squiggly, linear, or small rounded aggregates (Fig. 3E). These are in the nonvacuolated cytoplasm of approximately 80% of the s-IBM vacuolated muscle fibers and in approximately 20% to 25% of the muscle fibers that appear “nonvacuolated” in a given 10-μm-thick transverse section.

Previously, electron microscopic (EM) identification of PHFs was considered important for s-IBM diagnosis; however, now, because of the availability of new histochemical and immunohistochemical markers, that importance has diminished. For example, typical clusters of PHFs are easily visualized by light microscopy after staining with the antibodies against p62 or p-tau (Figs. 3E and 4D–H). We have demonstrated by EM immunocytochemistry that p62 and p-tau are strongly associated with PHFs (Fig. 5).

We do not find a transactive response DNA-binding protein 43 (TDP-43) immunoreactivity, reported by others
(25–28) and confirmed by us (29), to be diagnostically useful in s-IBM muscle biopsies because it is much less abundant and inferior to p62 immunoreactivity. We do not recommend TDP-43 for evaluating s-IBM biopsies.

**FIGURE 1.** Intracellular abnormalities present in sporadic inclusion-body myositis (s-IBM) muscle fibers. We propose that some aspects of the environment and predisposing genes in an aging muscle cell milieu lead to several abnormal mechanisms and accumulations of several proteins. These constitute the typical profile of s-IBM muscle fiber abnormalities. AβPP, amyloid-β precursor protein (AβPP); Aβ42, amyloid β protein; NF-κB = nuclear factor κB; p-Tau, phosphorylated tau; SIRT1, sirtuin 1; UBB⁺¹, mutant ubiquitin.

**EVIDENCE OF ABNORMAL MYOPROTEOSTASIS**

The term proteostasis describes the integrated cellular network that controls the life of proteins from their initiation to termination (30, 31). Proteostasis is considered a broader
Induction of:

**AβPP/Aβ Overexpression**

*Leads to:*
1) Vacuolation, 2) Impaired 26S proteasome,
3) Congophilic inclusions,
4) Mitochondrial abnormalities, COX - negativity.

*Increases:*
1) Cholesterol, 2) Myostatin,
3) αB-crystallin; 4) Parkin.

**Endoplasmic Reticulum Stress**

*Leads to:*
1) Ubiquitinated aggregates, 2) Formation of aggresomes, 3) GSK-3β activation,
4) Phosphorylation of AβPP.

*Increases:*
1) Myostatin, 2) p62 & NBR1, 3) Parkin.

**26S Proteasome Inhibition**

*Decreases:*
Lysosomal enzyme activities, leading to vacuolation.

*Increases:*
1) AβPP, 2) Aβ1-42, 3) Aβ42 oligomers,
4) γ-Secrete Components and Activity, 5) p62 & NBR1.

**Autophagy Impairment**

FIGURE 2. Experimental induction of sporadic inclusion-body myositis (s-IBM)-related mechanisms in cultured human muscle fibers produces s-IBM-characteristic abnormalities. AβPP, amyloid-β precursor protein (AβPP); Aβ42, amyloid β protein; BACE1, β-site amyloid-β precursor protein cleaving enzyme; COX, cytochrome C oxidase; GSK-3β, glycogen synthase kinase 3β; NBR1, neighbor of BRCA1 gene 1; NF-κB = nuclear factor κB; SIRT1, sirtuin 1; UPR, unfolded protein response.

Among several proteins (2), we highlight here the ones that are likely to be pathogenetically most important. We discuss that proteins identified as increased or present in immunocytochemistry aggregates could be the result of (i) impaired catabolism (related to the lysosome or proteasome inadequacy), (ii) overproduction, (iii) posttranslational modifications, or (iv) being "attached" to other accumulated proteins. Accumulated proteins might or might not have their normal cellular function and/or structure and collectively justify our term of abnormal myoproteostasis.

Increased Transcription of Amyloid-β Precursor Protein and Abnormalities of AβPP Processing

Sporadic IBM muscle fibers have increased mRNA of amyloid-β precursor protein (AβPP) 751 (32), the most abundant form in the peripheral tissues (33). The underlying mechanism of AβPP overproduction in s-IBM is not yet clarified. In addition, AβPP in s-IBM myofibers is posttranslationally modified, as indicated by its increased phosphorylation (34). There are also distinct abnormalities in AβPP processing, including (a) increased β-site amyloid-β precursor protein cleaving enzyme (BACE1) (35, 36), which cleaves AβPP at the N-terminal of Aβ (37), and (b) increased components of the γ-secretase system (Nogalska et al, unpublished data), which cleaves AβPP at the C-terminal of Aβ to generate either Aβ-40 or Aβ-42 (38). Both BACE1 and γ-secretase components are increased both on the protein and mRNA levels in s-IBM (35, 36, 39). In addition, Nicastrin, a component of γ-secretase, is strongly hyperglycosylated, indicating its posttranslational modification (Nogalska et al, unpublished data). Recently, in s-IBM muscle fibers, we demonstrated increased levels of BACE1-antisense transcript, which has been shown to regulate BACE1 mRNA and protein expression in vivo and in vitro (39), and was reportedly increased in brains with AD (40).

Other factors likely contributing to Aβ production, deposition and oligomerization, such as cystatin C, transglutaminase 1 and 2, and cholesterol are also increased in s-IBM muscle fibers (2, 21). Accordingly, the milieu within the s-IBM muscle fiber combined with an increased transcription of AβPP is a facilitating environment for Aβ production and accumulation.
Accumulation of Aβ42 and Evidence of Putatively Toxic Aβ42 Oligomers

In our s-IBM studies from 2 decades ago, we were the first to identify an intracellular accumulation of Aβ in any disease (41, 42). They were the basis for our proposal of an important cytotoxic role of intracellular Aβ, not only for s-IBM muscle fibers but, by analogy, also for neurons in AD (43). For years, it was considered that only extracellular Aβ...
is detrimental in the brain with AD (44). Now, however, the presence of intraneuronal Aβ is well established, and its possible toxicity and importance in the pathogenesis of AD is being considered (31, 45, 46).

Compared with Aβ40, Aβ42 is more cytotoxic, more prone to self-associate and oligomerize (45–47), and much more increased in s-IBM muscle fibers (20) (Figs. 4A, B); and Aβ42 is the form associated with the Congo Red–positive amyloid inclusions (20). The putative importance of Aβ42 cytotoxicity in s-IBM muscle fibers is strengthened by our recent novel demonstration of actual Aβ42 oligomers in them (48). Increased plasma Aβ42 was also demonstrated in patients with s-IBM (49). In general, cytotoxicity of Aβ is considered to depend on its initial assembly into detrimental oligomers. In contrast to Aβ monomers, which, in other systems, are considered not cytotoxic (45, 46), small oligomers and protofibrils are thought to be the cytotoxic forms of Aβ42 (31, 45, 46, 50). In s-IBM muscle biopsies, Aβ42 dimers, trimers, and tetramers were evident by immunoblots (48), thereby providing additional evidence that intramuscle fiber accumulation of Aβ42 oligomers may contribute importantly to the s-IBM pathogenic cascade. Nonfibrillar, cytotoxic “Aβ-derived diffusible ligands,” originally derived from Aβ42 (50), are increased in the brain with AD and were proposed to play an important pathogenic role (50). Importantly, Aβ-derived diffusible ligands are also increased in s-IBM muscle (48), thereby further strengthening the role of Aβ in s-IBM pathogenesis. Indeed, cytotoxicity of soluble, ultrastructurally invisible prefibrillar abnormal amyloidogenic proteins was postulated 3 decades ago for extracellular amyloidoses (51).

**p-tau**

In s-IBM muscle fibers, as in the brain with AD (52, 53), p-tau is accumulated intracellularly in the form of congophilic, delicate squiggly or linear inclusions (54). These can be identified using various antibodies that recognize several epitopes of p-tau present in the brain with AD, including ones that identify AD-specific conformational tau (22, 54, 55) (Figs. 4D, F–H). By immuno-EM, p-tau appears as PHFs (Figs. 5C, D). Several kinases that phosphorylate tau (52, 53, 56) are also accumulated within s-IBM muscle fibers, where they colocalize with p-tau–positive inclusions. These include extracellular...
signal-regulated kinase (57), CDK5 (58), glycogen synthase kinase 3β (GSK-3β), and casein kinase 1 (59). Also, GSK-3β is hyperphosphorylated and activated in s-IBM muscle fibers (34), but whether there is also an increase in soluble preaggregated form of p-tau is not yet known.

Several other proteins are also accumulated within the bundles of p-tau–containing PHFs (2). Hypothetically, those proteins might be either passively captured within the p-tau–containing PHFs and thereby be removed from their normal locale, which could impair their normal physiological

FIGURE 5. Electron microscopy (EM) and immuno-EM of sporadic inclusion-body myositis (s-IBM) paired helical filaments (PHF). (A) Transmission EM of a typical cluster (bundle) of PHFs. (B) Gold immuno-EM labeling with an anti-p62 antibody of a bundle of PHF. (C) Gold immuno-EM labeling with an anti–phosphorylated tau (p-tau, antibody Alz50) of a bundle of PHFs. (D) Horseradish peroxidase immuno-EM illustrates a bundle of PHFs labeled with anti-p-tau antibody PHF1; the rest of the myofiber (upper portion) is unstained. Note: Both Alz50 and PHF1 typically label PHFs in Alzheimer disease brain within neurofibrillary tangles. Magnifications: (A, C) 53,000×; (B, D) 25,000×.
functions, or they might be “actively” and disruptively bound to tau or other proteins within PHFs by an interaction with exposed hydrophobic surfaces of misfolded proteins associated with the PHFs. Multiple clusters of PHFs might displace and impair the function of other cytoplasmic proteins and organelles, such as mitochondria and endoplasmic reticulum (ER). Proteins that accumulate within the p-tau aggregates are susceptible to oxidative damage (60). Oxidative and nitrotyrosine stresses have been identified in s-IBM muscle fibers (61, 62), and those stresses can affect tau assembly and phosphorylation (63, 64).

**α-Syn**

α-Syn, a protein with poorly understood normal cellular functions, is a major component of Lewy bodies in the brain with PD (65, 66). Although abnormalities of α-syn were usually considered central to the pathogenesis of PD and abnormalities of Aβ were considered specific to the pathogenesis of AD, it has been recently proposed that α-syn and its oligomers might also play a role in AD (65, 67). Abnormal expression of α-syn occurring spontaneously in the brains of patients with various neurodegenerative disorders has been associated with, and possibly causative of, oxidative stress, impaired proteasome function, and mitochondrial abnormalities (65, 67). Oxidative stress can induce aggregation of α-syn into amyloid-like fibrils (65, 67).

In 2000, we demonstrated by immunocytochemistry that abnormal accumulation of α-syn occurs in diseased human muscle, namely s-IBM, and thus is not unique to brain disorders (68) (Fig. 4C). In s-IBM muscle, α-syn increases are also found by immunoblots (69), and its mRNA is increased by real-time polymerase chain reaction, suggesting increased production (Nogalska et al, 2011, unpublished data). In s-IBM muscle, increased α-syn might also involve its decreased degradation. As discussed below, both the 26S proteasomal and the lysosomal degradations are impaired in s-IBM muscle fibers, and α-syn has been shown to be degraded by both of these pathways (70). Moreover, impaired autophagy was reported to contribute to the formation of α-syn aggregates of Lewy bodies in PD (71). Recently, α-syn has been shown to be associated with mitochondria, and its overexpression induced mitochondria abnormalities (72). Because oxidative- and nitric oxide–induced stresses and mitochondrial abnormalities are also aspects of s-IBM pathology (2, 13, 61, 62), putative toxicity of α-syn may contribute to myofiber degeneration. Recently, α-syn was reported to stimulate tau phosphorylation directly through activation of GSK-3β (73).

**Mitochondrial Abnormalities**

Mitochondrial abnormalities, including ragged-red fibers (74), cytochrome C oxidase (COX)–negative muscle fibers, and multiple mitochondrial DNA deletions, are more common in the muscle of patients with s-IBM than are expected for the patient’s age (75). Although the mechanisms causing ragged-red fibers and prominent COX negativity of muscle fibers in s-IBM are not clarified, possibilities include toxic soluble oligomers of Aβ42, α-syn, or other proteins; factors resulting from oxidative and ER stress; and impaired autophagy. We have recently demonstrated that in diseased human muscle, α-syn and parkin accumulate in ragged-red fibers (2). We propose that abnormal mitochondria within ragged-red fibers, in various diseases including s-IBM, are destined for autophagic degradation and that parkin is recruited to facilitate their clearance as has been reported in other systems (76, 77). However, decreased autophagy in s-IBM muscle fibers impairs mitochondria degeneration. This process, referred to as abnormal mitophagy, has also been linked to aging and various neurodegenerative disorders (77). Thus, mitochondrial abnormalities in s-IBM muscle likely contribute to the muscle fiber malfunction, degeneration, and weakness.

Abnormalities of DJ-1 provide another evidence of mitochondrial perturbation (61). DJ-1 is a ubiquitously expressed protein of the Thiip/Pfpl/DJ1 superfamily. Mutations in the DJ-1 gene that prevent its expression are considered a cause of early-onset autosomal-recessive PD (78–80). In brains with sporadic AD and PD, DJ-1 was reported to be increased and highly oxidized (79). Although its precise functions are not yet known, DJ-1 has been proposed to be an antioxidant (80), and an important mitochondrial protective agent (80). Increased oxidation of DJ-1 itself was proposed to decrease its antioxidant activity (80).

DJ-1 is quantitatively increased in s-IBM muscle fibers where it is highly oxidized and abnormally accumulated in the mitochondria (61), suggesting that the increased DJ-1 may be attempting to mitigate mitochondrial oxidative damage but may be ineffective because it is itself excessively oxidized. Several other proteins, including mutated ubiquitin and myostatin, and some of the possibly detrimental mechanisms in s-IBM, including ER stress and decreased deacetylation, have recently been reviewed in detail (2).

**Abnormal Protein Disposal**

Unfolding or misfolding of proteins can occur in vivo and in vitro under several circumstances, including macromolecular crowding, defective protein disposal, oxidative stress, and “aging.” To eliminate abnormal, unfolded or misfolded proteins, and/or aggregated proteins, cells use several mechanisms, including protein refolding through the ER chaperones, protein refolding through heat shock proteins, protein degradation through the 26S ubiquitin-proteasome system (UPS), and protein degradation through autophagy. Intracellular mechanisms to maintain a proper quality and balance of proteins and organelles ensure that any malfunctioning or damaged intracellular structures, including proteins and organelles, are identified and repaired or cleared (2, 70, 81). This control or surveillance machinery is particularly important for nondividing postmitotic cells because their accumulated abnormal proteins cannot be diluted during cell division (70).

Protein quality control is disturbed under various pathologic conditions and in aging (31, 70, 82, 83). In eukaryotic cells, 2 major pathways of cellular protein degradation relate to the 26S proteasome and the autophagic/lysosomal systems (70, 81–83). The 26S proteasome (also called the UPS) is a major degradation mechanism for normal regulatory and other short-lived proteins and misfolded proteins exported from the ER through a ubiquitin-mediated ATP-independent process.
Impairment of the 26S Proteasome Function in s-IBM Muscle Fibers

Decreased proteasome function has been recently reported in several neurodegenerative diseases characterized by accumulation of multiprotein aggregates in the brain, and Aβ has been reported to inhibit proteasomal activity in cultured cells (84). In s-IBM muscle fibers, we demonstrated proteasomal abnormalities, as evidenced by 1) abnormal accumulation of 26S proteasome subunits by immunocytochemistry and immuno-EM, 2) increased expression of 26S proteasome subunits by immunoblots, but, contrastingly, 3) reduced activities of the 3 major proteasomal proteolytic enzymes (85). This indicates accumulation of hypoactive or inactive proteasomal subunits. Alterations of the proteasomal system in s-IBM muscle have also been described by others (86). Interestingly, our experimental studies suggested that AβPP/β may inhibit proteasome function (85). Other factors present in the s-IBM muscle fibers that might contribute to inhibiting proteasome function include an aging myofiber environment, protein overcrowding, oxidative stress, and accumulated p-tau, α-syn, and mutant ubiquitin (UBB⁺) (87)—all of these are capable of inhibiting proteasome activity in other systems (84, 88–90).

A failure to degrade/remove unnecessary proteins, including abnormal damaged proteins, is presumably detrimental to muscle fibers as it is to other cells. For example, accumulated ubiquitinated, misfolded, and oxidized proteins aggregate by themselves and can cause proteasome inhibition. However, it is more likely that the still-soluble, early intermediates of protein aggregates, in the form of dimers and trimers, can also induce proteasome inhibition (89), and some are known to be highly toxic to cells (45). There are other diverse functions controlled by the UPS, including regulation of gene transcription through monoubiquitination and deubiquitination of histones and presentation of MHC I molecules (91). Whether proteasomal abnormalities participate in antigen presentation and T-cell inflammation in s-IBM muscle fibers is not yet known.

Impaired Autophagy in s-IBM Muscle Fibers

Autophagic pathways are composed of 3 main components: macroautophagy, chaperone-mediated autophagy, and microautophagy, all of which lead to cargo degradation within lysosomes (70, 83). Lysosomes comprise the main compartment in which degradation of various proteins and molecules occurs through the activity of various lysosomal proteolytic enzymes. The term autophagy referring to lysosomal degradation has been used for decades, but the molecular aspects of delivering the cargo destined for lysosomal degradation have only recently been delineated (70, 83, 92). When lysosomal function is inhibited, there is proliferation and enlargement of autophagosomes, which constitute the route by which the cargo is transferred to the lysosomes, 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 the formation of autophagosomal vacuoles (83). This occurs in s-IBM muscle fibers, as well as in neurons of some neurodegenerative disorders (2, 6, 21, 70, 83). In certain situations, proliferation of autophagosomes in neurons has been associated with experimental Aβ overproduction and vacuolization (83).

Although autophagic vacuoles associated with accumulated lysosomal-membranous structures within s-IBM muscle fibers have been associated with s-IBM pathology for many years (93), the mechanism of their formation was not well understood. Our recent studies have demonstrated that, in s-IBM muscle fibers, there is increased formation and ‘‘maturation’’ of pathologic vacuolar autophagosomes (94). These observations suggested that excessive activated macroautophagy is an important factor leading to the formation of the vacuoles.

In contrast to several neurodegenerative diseases in which the autophagic pathways have been extensively studied, autophagic pathways in s-IBM muscle fibers have been virtually unexplored.

In addition to activated macroautophagy in s-IBM muscle fibers, our recent studies provided important evidence that autophagy related to lysosomal function is impaired (94). Those changes seemed to be specific to s-IBM because, in polymyositis muscle fibers, lysosomal activities were actually increased in our study and in studies by others (94). This suggests that the lymphocytic inflammation present in both s-IBM and polymyositis does not contribute to impairment of the autophagic/lysosomal degradation in s-IBM.

Impaired autophagy in s-IBM muscle fibers might be, at least partially, responsible for the abnormal accumulation of various proteins, including Aβ, α-syn, BACE1, and tau, all of which are reported to be degraded through autophagy (37, 70, 95, 96); Aβ has been shown to be produced within the autophagosomes (83, 97).

Two partner proteins, p62 and neighbor of BRCA1 gene 1 (NBR1), which are both shuttle proteins that transport polyubiquitinated proteins to either proteasomal or lysosomal degradation (23, 24, 98), are greatly increased in s-IBM muscle fibers, wherein they accumulate especially in the p-tau-containing PHFs structures (22, 99). p62 has been reported to be accumulated in ubiquitinated inclusions in AD neurofibrillary tangles and in Lewy bodies in PD (100, 101). In the former, p62 localized in neurofibrillary tangles was associated with p-tau (101).

Our recent studies demonstrated that, in s-IBM muscle, both p62 and NBR1 are increased at both the protein and mRNA levels and are strongly accumulated in aggregates within muscle fibers, where they closely colocalize with p-tau by both light and EM immunocytochemistry (22, 99). This is another example of impaired protein degradation in s-IBM muscle fibers.

Experimental Evidence for the Interrelationship Between Various Components of the Pathology of s-IBM

Studies of the s-IBM muscle biopsies do not permit precise evaluation of the causes and effects of various pathologic findings. Therefore, one of our approaches is to explore molecular mechanisms participating in the s-IBM
pathogenesis by using well-differentiated cultured normal human muscle fibers, the cellular microenvironment of which we experimentally modify to mimic various pathologic aspects of the s-IBM myofiber milieu. This provides our “IBM-Human Muscle Culture Models (IBM-HM-TC-Models)” studies of which demonstrate relationships and suggest crosstalk among various molecular events occurring in s-IBM muscle (Fig. 2), and provide possibly relevant models to seek the efficacy of potential therapies for s-IBM. Others have used experimental transgenic mouse models, which have also helped elucidate some aspects of s-IBM pathogenesis (102–107); however, because of space limitations, they are not discussed here.

Overexpression of AβPP/Aβ into Mature Cultured Normal Human Muscle Fibers (AβPP(+)-IBM-HM-TC-Model)

In this model, there are several aspects of the IBM pathologic phenotype, including (a) pronounced vacuolization of most of the muscle fibers, (b) crenophagic inclusions in some myofibers, (c) proteasome inhibition and aggresome formation, (d) nuclear PHFs, (e) mitochondrial abnormalities including COX deficiency, (f) cholesterol accumulation, (g) increased αB-crystallin, (h) increased parkin, (i) increased myostatin, and (j) the inability to become innervated (69, 85, 108–113). This cultured human muscle model clearly demonstrates that AβPP/Aβ overexpression can be central to the induction of an IBM-characteristic phenotype.

In this model, AβPP/Aβ overexpression precedes other IBM-type abnormalities (108, 109), including an inability to become properly innervated by normal fetal spinal cord neurons, having morphologically abnormal neuromuscular junctions (110). We therefore postulated that spontaneous AβPP/Aβ overexpression in the patient with s-IBM muscle may be responsible for a “myogenous dysinnervation” (110) and for the observed neuromuscular junction structural abnormalities (114).

Consequences of 26S Proteasome Inhibition

Experimental inhibition of proteasome by epoxomicin (with or without concomitant AβPP overexpression) created our “Prof(-)-IBM-HM-TC-Model,” which exhibited the following abnormalities: accumulation of ubiquitinated aggregates, development of aggresomes, αB-crystallin increase and accumulation in aggregates, parkin increase and aggregation, myostatin aggregation, GS-3β activation, induction of AβPP phosphorylation, and increase in p62/SQSTM1 and NBR1 (22, 34, 69, 85, 112, 113).

Consequences of Autophagy Inhibition

Experimental inhibition of autophagy using chloroquine or bafilomycin creates an “Autoph(-)-IBM-HM-TC-Model” in which, thus far, there are several abnormalities similar to those present in s-IBM muscle biopsies. They include the following: increased production of Aβ42 (115), increased production of the putatively cytotoxic Aβ oligomers (48, 115), posttranslational modification of AβPP (Nogalska et al, 2012, unpublished data), increased transcription and accumulation of the γ-secretase components (115), and increased p62/SQSTM1 and NBR1 (22, 99).

Evidence Suggesting the Importance of ER Stress in the s-IBM Pathogenic Cascade

Experimental pharmacological induction of ER stress in well-differentiated cultured human muscle fibers created our “ER(+)-IBM-HM-TC-Model,” which exhibits several abnormalities that are present in s-IBM muscle biopsies as follows: a strong induction of the unfolded protein response; increased activation of nuclear factor κB (NF-κB); increased myostatin mRNA and protein as a response to abnormal NF-κB activation; decreased sirtuin 1 activity resulting in hyperacetylation and activation of NF-κB; increased BACE1 protein, its mRNA, and its noncoding regulatory transcript; and impaired autophagy (39, 94, 116–119).

EXPERIMENTALLY BASED TREATMENT SUGGESTIONS

On the basis of our IBM culture models and various experimental AD models, the following compounds might be suggested for treating patients with s-IBM. For most of them, however, their possible toxicity and potential benefits in patients are not known.

Lithium

Lithium was reported to diminish tau and Aβ pathologies in various experimental models of AD (120). In a transgenic mouse model whose skeletal muscle bears some aspects of IBM muscle fibers, lithium was reported to decrease tau phosphorylation through decreasing activity of GSK-3B (121). In cultured human muscle fibers in our AβPP(+) IBM-HM-TC-Model, lithium significantly decreased total AβPP, phosphorylated AβPP, and Aβ oligomers (34). In addition, it significantly increased the inactive form of GSK-3B (34). Accordingly, treating patients with s-IBM with lithium, which is widely used in treating bipolar disorders in humans, might be beneficial if used in “adequate” safe dosage.

Polyphenols

Polyphenols have recently been reported to benefit an AD experimental mouse model and an IBM culture model. For example, treatment of our ER stress–induced cultured human muscle fibers (ERS(+) -IBM-HM-TC-Model) with resveratrol (trans-3,4’,5-trihydroxystilbene), an antioxidant polyphenol and potent activator of sirtuin 1 (122), significantly decreased myostatin mRNA and protein, which was associated with NF-κB deacetylation (deactivation) and increased muscle fiber size (123), suggesting that resveratrol might be beneficial in treating patients with s-IBM, if one can use a stable nontoxic compound in adequate dosage. Other phenolic compounds, including curcumin and grape seed–derived polyphenols, have been reported to decrease the amyloid burden and Aβ fibrillation in AD transgenic mice and in vitro (124).

Sodium Phenylbutyrate

Sodium phenylbutyrate PBA, an orally active chemical chaperone approved by the US Food and Drug Administration, allegedly mimics the function of intracellular molecular chaperones in preventing protein aggregation and oligomerization.
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In our experimental Autoph(−/−)-IBM-HM-TC-Model based on chloroquine-inhibited autophagy, treatment with PBA virtually eliminated vacuolization, increased activities of both cathepsin D and B, decreased NBR1, and substantially decreased Aβ42 and Aβ42 oligomerization (Nogalska et al., 2012, unpublished data). Those data provide a rationale for considering therapeutic trials of patients with s-IBM with PBA. In patients with amyotrophic lateral sclerosis, PBA was tried with dosages up to 21 g/d and was reportedly well tolerated (126); however, no beneficial effect has yet been reported. In some patients with Huntington disease, dosages greater than 15 g/d were not well tolerated (127).

CONCLUSIONS

In conclusion, s-IBM is a degenerative muscle disease associated with abnormal myoproteostasis, in which aging seems to be a key risk factor. Several molecular mechanisms responsible for multiprotein aggregation and accumulation within s-IBM muscle fibers were reviewed. We again propose that s-IBM is not a primary disimmune/inflammatory disease but that the accumulation of postranslationally modified, misfolded proteins in aging milieu of s-IBM muscle fibers, can be perceived as “foreign” (not self) and may be responsible for inducing a T-cell immune response in the s-IBM muscle. Because antidisimmune treatments are essentially not effective in s-IBM and have even reported to be detrimental (4), other therapeutic measures are needed. Some are suggested herein.

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