

Inclusion-body myositis, a multifactorial muscle disease associated with aging: current concepts of pathogenesis

Valerie Askanas and W. King Engel

Purpose of review

Sporadic inclusion-body myositis, the most common muscle disease of older persons, has no known cause or persistently beneficial treatment. The unfolding pathogenesis could lead to new treatment strategies and it is now of growing interest among clinicians and basic scientists. About 100 papers related to the subject were published in 2006 and the first part of 2007 (we cite only articles most relevant to this review).

Recent findings

This review focuses on the current concepts of the pathogenesis of sporadic inclusion-body myositis. Both degeneration and mononuclear-cell inflammation are components of the pathology, but how each relates to the pathogenesis remains unclear. We suggest that an intramuscle fiber degenerative component is primary, leading to muscle-fiber destruction, while the lymphocytic inflammatory component may only slightly contribute to sporadic inclusion-body myositis muscle-fiber damage. Intracellular accumulation of amyloid- β precursor protein, amyloid- β , and amyloid- β oligomers in an aging muscle-fiber cellular milieu, and other abnormalities, appear to be key pathogenic factors. We summarize intracellular molecular events and their consequences, and correlate findings in sporadic inclusion-body myositis muscle biopsies with inclusion-body myositis experimental models in tissue culture and in transgenic mice.

Summary

Treatment of sporadic inclusion-body myositis remains a challenge. Antiinflammatory approaches used so far are without major or enduring benefit. Possible new treatment avenues are suggested.

Keywords

amyloid- β , amyloid- β intracellular toxicity, amyloid- β precursor protein, degeneration, misfolded proteins, muscle fiber aging, sporadic inclusion-body myositis

Abbreviations

α-syn	α -synuclein
Aβ	amyloid- β
AβPP	amyloid- β precursor protein
αBC	α B-crystallin
ERS	endoplasmic reticulum stress
h-IBM	hereditary inclusion-body myopathy
MHC	major histocompatibility complex
MstnPP	myostatin precursor protein
NFκB	nuclear factor κ B
s-IBM	sporadic inclusion-body myositis
p-tau	phosphorylated tau
UPR	unfolded protein response

© 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins
1040-8711

Introduction

Sporadic inclusion-body myositis (s-IBM) is the most common, progressive muscle disease of persons age 50 years and older, and it leads to severe disability. There is no persistently successful treatment. Clinical features of s-IBM, pathologic diagnostic criteria, and various treatment approaches were recently reviewed in detail [1^{••},2[•],3,4]. Figure 1 illustrates currently used pathologic diagnostic criteria, based on the following staining techniques: Engel trichrome (Fig. 1a); immunoperoxidase reaction with SMI-31 antibody recognizing phosphorylated tau (p-tau) (Fig. 1b); either congo-red visualized through Texas-red filters and fluorescence microscopy (Fig. 1d) or crystal violet (Fig. 1d) for β -pleated sheet amyloid [1^{••},3]. While crystal violet can visualize larger clumps of amyloid (Fig. 1d), the more sensitive congo-red fluorescence allows visualization of even very small amyloid foci (Fig. 1c).

This review emphasizes the most recent research advances leading to our newest considerations of the s-IBM pathogenic cascade.

Pathogenetic considerations

Two aspects will be discussed.

The inflammatory component

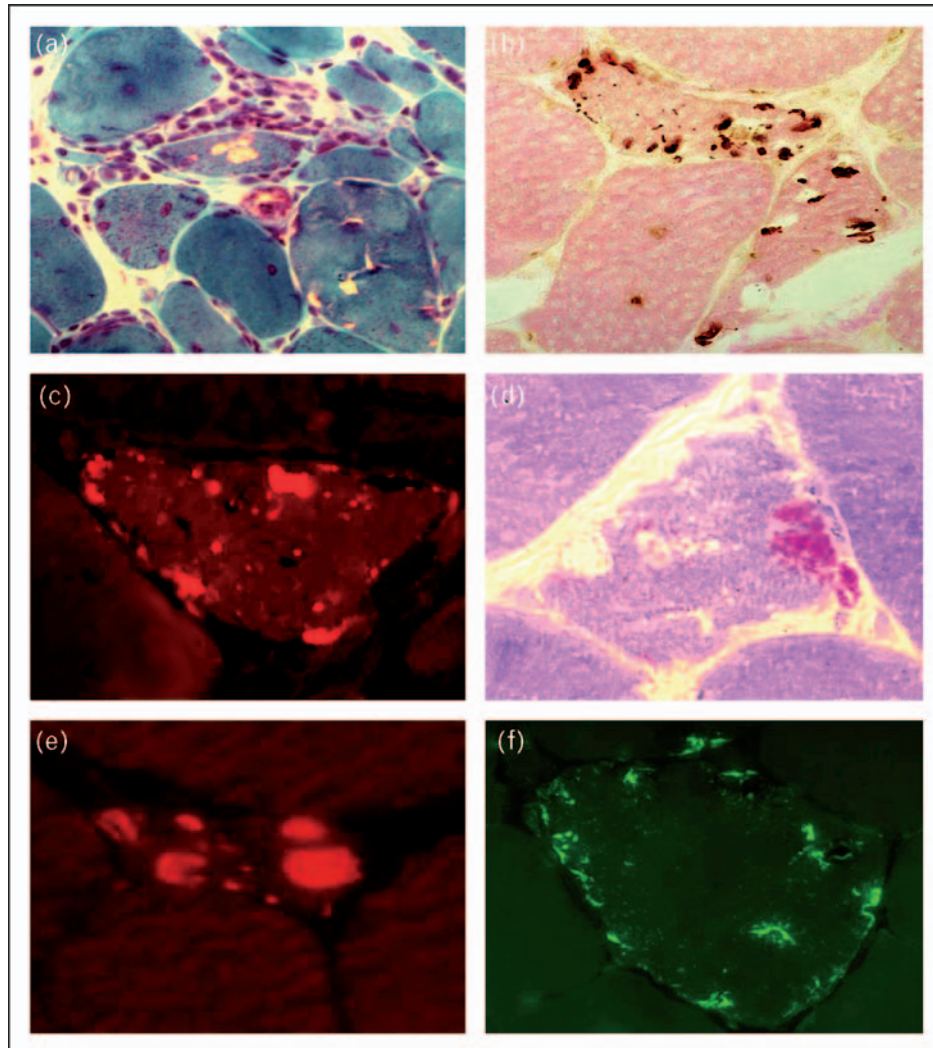
As the name indicates, s-IBM has a definite mononuclear-cell inflammation and thereby is usually grouped, descriptively but probably incorrectly pathogenetically, with polymyositis and dermatomyositis. Recent reviews address the inflammatory aspect and the possibility that it may have a primary role in pathogenesis [2[•],5]. One argument is that s-IBM muscle fibers abundantly express major histocompatibility complex type 1 (MHC)-1, and

Curr Opin Rheumatol 19:550–559.
© 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.

USC Neuromuscular Center, Department of Neurology, University of Southern California Keck School of Medicine, Good Samaritan Hospital, Los Angeles, California, USA

Correspondence to Dr Valerie Askanas, USC Neuromuscular Center, Good Samaritan Hospital, 637 S. Lucas Ave., Los Angeles, CA 90017-1912, USA
Tel: +1 213 975 9950; fax: +1 213 975 9955; e-mail: askanas@usc.edu

Current Opinion in Rheumatology 2007, 19:550–559

Figure 1 Light-microscopic features of sporadic inclusion-body myositis muscle biopsy

(a) Engel Trichrome staining demonstrating vacuolated and atrophic muscle fibers, and mononuclear-cell inflammation. (b) Characteristic diagnostic inclusions stained with SMI-31 antibody, identifying phosphorylated tau, in two abnormal muscle fibers. (c) Congo-red staining visualized through Texas-red filters and epifluorescence illumination shows various sized amyloid deposits within abnormal muscle fibers. (d) Crystal-violet staining showing a large clump of metachromatically red amyloid inclusion in a muscle fiber. (e) Large, plaque-like intramuscle fiber amyloid- β immunoreactivity is in multiple foci, visualized through a Texas-red filter and epifluorescence illumination. (f) Characteristic, delicate squiggly inclusions identifying phosphorylated tau on paired helical filaments in an abnormal muscle fiber. (a-c, e) original magnification, $\times 1250$; (d, f) original magnification, $\times 2100$.

one expressing MHC-1 are invaded by clonally expanded cytotoxic CD8⁺ lymphocytes [2^{*},5], as also occurs in polymyositis. Despite that T-cell activation, s-IBM patients as a group respond poorly to antidyimmune treatment, in contrast to polymyositis and dermatomyositis patients [1^{**},2^{*},4], suggesting that other pathogenic factors in s-IBM may be more important and more likely primary.

MHC-1 expression is also present on regenerating muscle fibers in various myopathies [2^{*}] (O. Paciello and V. Askanas, unpublished observations). Furthermore, MHC-1 is strongly expressed on muscle fibers in genetic

dysferlin deficiency, where it elicits a CD4 rather than a CD8 T-lymphocyte response [6], and in limb-girdle muscular dystrophy 2-I, when it is accompanied by inflammation [7]. While MHC-1 is critical for a muscle fiber to become antigen presenting, the mechanisms causing MHC-1 expression in s-IBM fibers are not known. Possibly, MHC-1 expression in s-IBM results from the demonstrated endoplasmic reticulum stress [8] (see below), and additionally may be induced by amyloid- β precursor protein (A β PP) that associates with endoplasmic reticulum chaperones GRP78 and GRP94 [8]. Our suggestion is supported by studies of others

showing that binding of endoplasmic reticulum chaperones to some peptides leads to expression of MHC-1, cellular antigen display, and activation of MHC-restricted T cells [9–11]. MHC-1 was also shown to associate with GRP78 on the cell surface, and immunization of mice with GRP94 elicited cytotoxic T cells [12]. Also, in accord with our proposal that the lymphocytic inflammation in s-IBM is secondary, is the report that transgenic mice overexpressing A β PP plus a mutated presenilin1 have CD8 T-cell inflammation [13*].

We propose, therefore, that the prominent degenerative component in s-IBM muscle fibers (see below) may be eliciting the T-cell inflammatory reaction. We further postulate that the aging milieu of the s-IBM muscle fiber and of the total patient may be facilitating the lymphocytic inflammation. Interestingly, some of the older patients with hereditary IBM (h-IBM), caused by the missense mutations in the UDP-N-acetylglucosamine-2 epimerase/N-acetylmannosamine-kinase (GNE) gene, have various degrees of lymphocytic inflammation [14–16], even though that form of h-IBM is not considered immune mediated. The reason is not understood, but we postulate that the aging cellular environment, and perhaps other individual intrinsic abnormalities may, in older h-IBM patients, make some of the accumulated proteins appear ‘foreign’ to the immune system and produce the lymphocytic inflammation.

The degenerative component

Degeneration of s-IBM muscle fibers is characterized by a progressive fiber vacuolization and atrophy, accompanied by accumulation of intramuscle-fiber multiprotein aggregates (‘inclusion bodies’). In a given section of an s-IBM muscle biopsy, the aggregates are present mainly in vacuole-free regions of vacuolated muscle-fiber cytoplasm and in cytoplasm of ‘nonvacuolated’ fibers. The vacuoles themselves usually do not contain the IBM-characteristic inclusions [3,17**]. The IBM autophagic vacuoles, usually containing membranous debris, are lysosomal and an end result of muscle-fiber destruction. Recently it was reported that s-IBM vacuolated muscle fibers, and those in some other vacuolar myopathies, contain a marker of autophagosomes, but only in s-IBM is it colocalized with A β PP [18], suggesting that s-IBM muscle fibers may be attempting through the autophagosome to degrade A β PP, perhaps bound to other simple or complex proteins.

Two major types of aggregates/inclusions in s-IBM muscle fibers are the rounded, plaque-like aggregates prominently containing amyloid- β (A β) immunoreactivity; and various-sized delicate, squiggly, linear aggregates containing p-tau (Fig. 1e, f). Both aggregates contain proteins in the β -pleated-sheet configuration indicating amyloid. In addition, both also contain other proteins

with a propensity to misfold, including α -synuclein and presenilin1; markers of oxidative stress; endoplasmic reticulum chaperones indicative of the unfolded protein response (UPR); 26S proteasome components; mutated ubiquitin (UBB⁺); and heat-shock proteins (reviewed in [17**]; see below). Accordingly, we consider that unfolding and misfolding of proteins plays a major role in formation of multiprotein aggregates in s-IBM fibers. Various demonstrated proteins and mechanisms putatively participating in the s-IBM pathogenesis are illustrated in Fig. 2. Concurrently, in s-IBM fibers there is expression of some putatively protective proteins and mechanisms (Fig. 2, details below). Those defensive mechanisms, however, appear insufficient because the muscle fiber continues to deteriorate and eventually dies. Overall, in s-IBM compared with other myopathies, there is a definite paucity of regenerating muscle fibers (V. Askanas, personal observation). One mechanism possibly related to that defective regeneration may involve defective mesoangioblasts from s-IBM muscle biopsies, which were reported in culture to not fuse into muscle fibers unless they are transfected with MyoD [19].

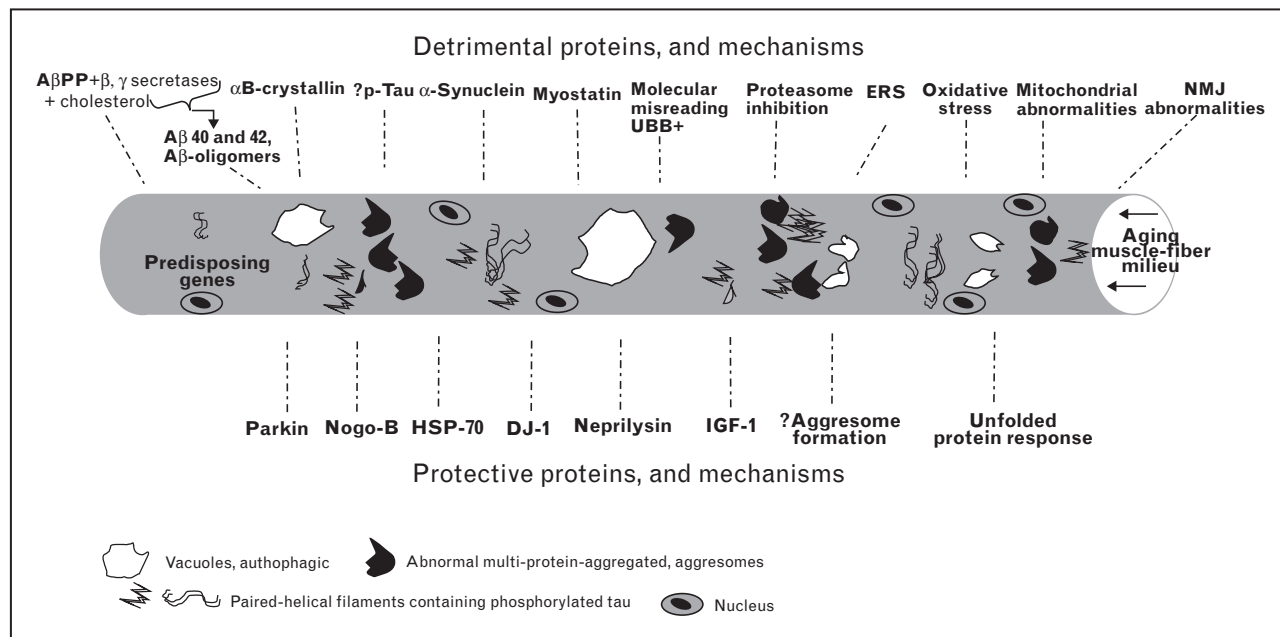
Importance of A β PP/A β in sporadic inclusion-body myositis pathogenesis

Key aspects will be discussed.

Intracellular toxicity of A β PP/A β

We have proposed for several years that increased intracellular expression of A β PP and of its proteolytic fragment A β plays a key upstream, toxic, role in s-IBM pathogenesis [17**,20,21]. Several studies provide strong evidence for intracellular toxicity of A β PP/A β in s-IBM, including the following. Experimental overexpression of A β PP through direct A β PP gene transfer into human normal, cultured muscle fibers induced most of the basic aspects of the IBM phenotype (reviewed in [17**]). In cultured muscle fibers of h-IBM due to GNE mutation, the ‘genetically determined’ A β PP overexpression preceded other IBM-type abnormalities [22]. Both experimentally and ‘genetically’ A β PP-overexpressing cultured muscle fibers cocultured with fetal-rat spinal-cord neurons had morphologically abnormal neuromuscular junctions (NMJs) and could not become innervated [22] – we therefore postulated that spontaneous A β PP overexpression in s-IBM patient muscle may be responsible for a ‘myogenous dysinnervation’ [22] and for the observed NMJ structural abnormalities [23]. A β PP overexpression in normal cultured human muscle fibers leads to proteasome inhibition [24]. A β PP overexpression in normal cultured human muscle fibers leads to increased expression of myostatin [25*], which is also increased in s-IBM muscle fibers [26] (see below). In a GNE-knockout mouse overexpressing the GNE V572L mutation, A β accumulations preceded by several weeks of other identified abnormalities within the muscle fibers, such as

Figure 2 Intracellular molecular aspects of sporadic inclusion-body myositis muscle-fiber degeneration



Various detrimental and protective proteins and mechanisms operating within the s-IBM muscle fiber are diagrammatically illustrated. Aβ, amyloid-β; AβPP, amyloid-β precursor protein; ERS, endoplasmic reticulum stress; p-tau, phosphorylated tau; HSP, heat shock protein; IGF, insulin-like growth factor; NMJ, neuromuscular junction.

weakness, increased serum creatine kinase activity, vacuolization and general muscle atrophy [27^{••}]. This model suggests that accumulation of Aβ, and perhaps its decreased sialylation, is an important upstream or mid-stream component of the h-IBM pathogenesis. Importantly, aging appears to be a significant component contributing to both the weakness and abnormal pathological phenotype in those GNE mutant transgenic mice, as it is in patients. Additional support of our toxic intracellular AβPP/Aβ hypothesis of IBM pathogenesis is provided by transgenic mouse models overexpressing AβPP in muscle fibers, which exhibit some aspects of the IBM phenotype [28–30]. A new mouse model – in which overexpression of AβPP in muscle fibers, combined with elimination of native presenilin 1 and its replacement with a mutated presenilin 1, leading to the increased generation of Aβ42 – had, in addition to some other aspects of IBM, CD8 T-cell inflammation, increased CD8 T-cell mRNA, increased tau phosphorylation, and increased expression of two kinases, GSK-3β and CDK5 [13[•]], both of which participate in tau phosphorylation. Those studies accorded with previous ones of s-IBM muscle biopsies, in which there was an increased preferential deposition of Aβ42 [31], and increased expression of GSK-3β and CDK5, which were immuno-co-localized with p-tau on IBM-paired helical filaments [32,33]. Another aspect of those AβPP transgenic mouse models was dependence on aging in the development of IBM-like abnormalities [13[•],28–30].

In the Alzheimer disease brain, it was traditionally considered that extraneuronally accumulated Aβ exerts a cytotoxic influence on neurons and other cells [34,35]. In 1998 [36] we proposed that in Alzheimer's disease, similarly to our databased concept of intracellular s-IBM pathogenesis, intracellular, viz. intraneuronal, toxicity of Aβ may be playing an important pathogenic role. This is now becoming more generally accepted in Alzheimer's disease, as evidenced by a recent review [37[•]].

Mechanisms of AβPP overexpression and its abnormal processing

In s-IBM muscle fibers there is increased transcription of AβPP-751 [38]. The mechanism of that AβPP overproduction is not established, but our studies suggest that they may include the activator protein-1 transcription complex, Redox-factor-1 (Ref-1), and transcription factor nuclear factor κB (NFκB) (reviewed in [3,17^{••}]). Our recent studies have shown that NFκB binding to DNA is increased in s-IBM muscle fibers [39^{••}]. The following components and mechanisms for abnormal processing AβPP found in s-IBM muscle fibers include β-secretase (BACE1), which cleaves AβPP at the N-terminal of Aβ [40–42]; nicastrin and presenilins, which are components of the γ-secretase system that cleaves AβPP at the C-terminal of Aβ, generating either Aβ40 or Aβ42 [43,44]; accumulated free cholesterol, which colocalizes with Aβ [45] – similarly to nonmuscle cells, in AβPP-overexpressing cultured

human muscle fibers, exposure to cholesterol induces A β deposition [46,47]; accumulation of cystatin C, an endogenous cysteine protease inhibitor, which was previously proposed to participate in A β deposition within the amyloid plaques Alzheimer disease brain [48,49]; transglutaminases 1a and 2, which contribute to A β aggregation and insolubility by cross-linking A β molecules [50].

Accordingly, it appears that mechanisms participating in both increased transcription of A β PP and its abnormal processing are overly active within s-IBM muscle fibers. In them, putatively protective mechanisms are concurrently expressed, including neprilysin, which participates in A β degradation [51,52]; insulin-like-growth factor1 (IGF1), which protects against A β toxicity [53]; and Nogo-B, which prevents binding of BACE1 to A β PP, thereby inhibiting A β production [54]. These data together further support our hypothesis that both cytotoxic and protective mechanisms are concurrently operating in s-IBM muscle fibers (Fig. 2).

Putative role of A β oligomers

Several studies in nonmuscle cells have demonstrated that A β and other amyloidogenic proteins exert cytotoxicity when in the form of oligomeric intermediates or 'preamyloid' protofibrils, and not while in the form of insoluble amyloid fibrils or aggregates [54,55,56,57,58,59]. We have postulated that in s-IBM muscle fibers, A β toxicity may not be related to A β in the insoluble aggregates, but rather to an intracellular toxicity of its soluble oligomers and protofibrils [17**]. A similar cytotoxicity of 'electronmicroscopically invisible amyloid-precursor molecules' having a 'selective affinity' for certain important cellular molecules was hypothesized in systemic amyloidosis peripheral neuropathy many years ago (Engel, 1979, discussed in [1**]).

α B-crystallin (α BC) specifically recognizes and stabilizes proteins that have a propensity to aggregate and precipitate [60,61]. In cell-free systems, α BC prevents A β fibril growth and spontaneous fibril formation, binds A β , and prevents its aggregation [62]. When applied extracellularly, however, concomitantly with A β to cultured rat neurons, α BC increases A β cytotoxicity [63], possibly due to the influence of α BC on maintaining A β in its soluble oligomeric, highly cytotoxic form [62,63]. Our recent study [64*] provided a novel demonstration that in human muscle fibers α BC was experimentally increased by A β PP overexpression; proteasome inhibition; and a combination of both, which showed an additive effect. α BC physically associated with A β PP and A β oligomers in human muscle fibers, both in A β PP-overexpressing cultures and in s-IBM biopsied muscle fibers. We suggested [64*] that the binding of α BC to A β oligomers may retard and diminish their fibrillization and aggregation into

visible nontoxic aggregates, thereby prolonging their existence as toxic oligomers.

Other putatively toxic proteins and mechanisms

Several of these are discussed.

Phosphorylated tau

As in the Alzheimer's disease brain, p-tau is accumulated intracellularly in s-IBM muscle fibers in the form of aggregates of congophilic paired helical filaments [65]. Most of the therapeutic studies related to Alzheimer's disease have been focusing on decreasing A β , since the tau pathology has been regarded as downstream from A β . This approach was recently challenged by studies in transgenic mice expressing human A β PP, in which reducing endogenous tau levels prevented behavioral deficits and reduced mortality without reducing A β plaque deposition [66**]. In contrast to A β exerting an intramuscle fiber cytotoxicity, there is no direct evidence that p-tau may be toxic; however, this possibility should be explored.

α -Synuclein

α -Synuclein is being implicated in the pathogenesis of several neurodegenerative diseases [67,68]. Its overexpression has been associated with, and possibly causative of, oxidative stress, impaired proteasome function, and mitochondrial abnormalities [67,69,70].

We have shown that α -synuclein is accumulated in s-IBM muscle fibers [71], and that its 22 kDa O-glycosylated form is more frequently expressed than its native 16 kDa form [72**]. The 22 kDa form, but not the native 16 kDa form, was shown by others to be a target of ubiquitination by parkin [73]. The preferential increase of the 22 kDa O-glycosylated form of α -syn in s-IBM muscle fibers may be due to the proteasome inhibition previously demonstrated in s-IBM fibers [24].

Oxidative and nitric oxide-induced stress, and mitochondrial abnormalities, are also aspects of the s-IBM muscle fiber pathology [3,17**,74]. Accordingly, a putative toxicity of α -syn, in addition to the demonstrated cytotoxicity of A β , may contribute to muscle-fiber degeneration in s-IBM. Such toxicities seem more likely related to an intracellular accumulation of their soluble oligomers and protofibrils [17**].

Parkin, an E3-ubiquitin ligase, is greatly increased in s-IBM muscle fibers, where it accumulates in the form of aggregates or aggresomes [72**]. In brains of sporadic Parkinson disease patients, parkin and α -syn accumulate in Lewy bodies, which are considered aggresomes [68,75]. Parkin, in addition to enhancing proteasome function through ubiquitination of proteins, also protects cells against toxicity induced by α -syn, endoplasmic reticulum stress, and other stresses, perhaps by helping

to aggregate toxic oligomers and promote their degradation [76,77]. Accordingly, we propose that in s-IBM muscle fibers parkin plays a protective role [72**] (Fig. 2).

Myostatin

Myostatin, a secreted protein, is considered a negative regulator of muscle growth during development and of muscle mass during adulthood [78]. In biopsied s-IBM muscle fibers, myostatin precursor protein (MstnPP) and myostatin dimer were significantly increased; and MstnPP was physically associated with A β PP, and colocalized by light and electron-microscopic immunocytochemistry with A β /A β PP [26].

Recently, A β PP overexpression into cultured normal human muscle fibers increased MstnPP expression, and subsequent experimental inhibition of proteasome caused accumulation of both MstnPP/myostatin and A β PP/A β colocalized within aggresomes, and their physical association [25*]. The mechanism by which A β PP/A β increases MstnPP is not known. Possibly, A β PP binding to MstnPP causes its posttranslational modification that lessens its degradation and traffic, resulting in accumulation.

Oxidative stress

There is increasing evidence that free-radical toxicity may participate in the IBM pathogenesis. Indicators of oxidative stress, as well as enzymes participating in the cellular defense against oxidative stress, are accumulated in IBM muscle fibers [3,74]. IBM muscle fibers contain increased NF κ B [39**]. Recently, we showed that DJ-1 is increased and oxidized in s-IBM fibers [79]. Although its precise functions are not yet known, DJ-1 was reported to have antioxidative and neuroprotective properties [80,81]. During oxidative stress, DJ-1 becomes oxidized [80]. Its experimental downregulation sensitizes cells to oxidative stress. DJ-1 was also reported to be an important mitochondrial protective agent [82,83]. Our studies indicated for the first time that DJ-1 may play a role in human muscle disease.

Proteasome inhibition

The 26S proteasome is a multisubunit protease complex of around 700 kDa present in the cytoplasm and nucleus of eukaryotic cells having a major role in degrading normal and abnormal proteins through a ubiquitin-mediated ATP-independent process [84]. Our study found significant proteasomal inhibition in s-IBM muscle fibers [24]. A β and proteasome subunits colocalized at the light and electron-microscopy levels and there was a physical association of A β /A β PP and proteasome protein, suggesting that the A β PP/A β proteasome interrelationship may be important in inducing proteasome abnormalities in s-IBM fibers [24]. Moreover, proteasome activity was inhibited in cultured human muscle fibers

overexpressing A β /A β PP [24]. Other factors in s-IBM muscle fibers that may contribute to proteasome inhibition are illustrated in Fig. 3 and include an aging muscle-fiber environment, protein overcrowding, oxidative stress, accumulated p-tau, α -synuclein, and UBB⁺¹. UBB⁺¹, a product of 'molecular misreading' [85,86] is accumulated in s-IBM muscle fibers [87], and can inhibit proteasome [86]. 'Molecular misreading' designates acquired, non-DNA-encoded dinucleotide deletions occurring within mRNAs, resulting in production of potentially toxic mutant proteins [85]. Speculatively, some such uncatabolized unfolded/misfolded proteins may, in susceptible s-IBM patients, elicit MHC-1, be presented by the muscle fiber, and induce a secondary CD8 T-cell response.

Aggresomes form when proteasome is inhibited [88]. Whether aggresomes, in general, contribute to cellular death or protect cells from toxic effects of misfolded proteins remains uncertain. We have recently demonstrated that most of the s-IBM multiprotein aggregates contain γ -tubulin and have other features of aggresomes, and aggresomes were also induced in cultured human muscle fibers by overexpressing A β PP with or without proteasome inhibition [24].

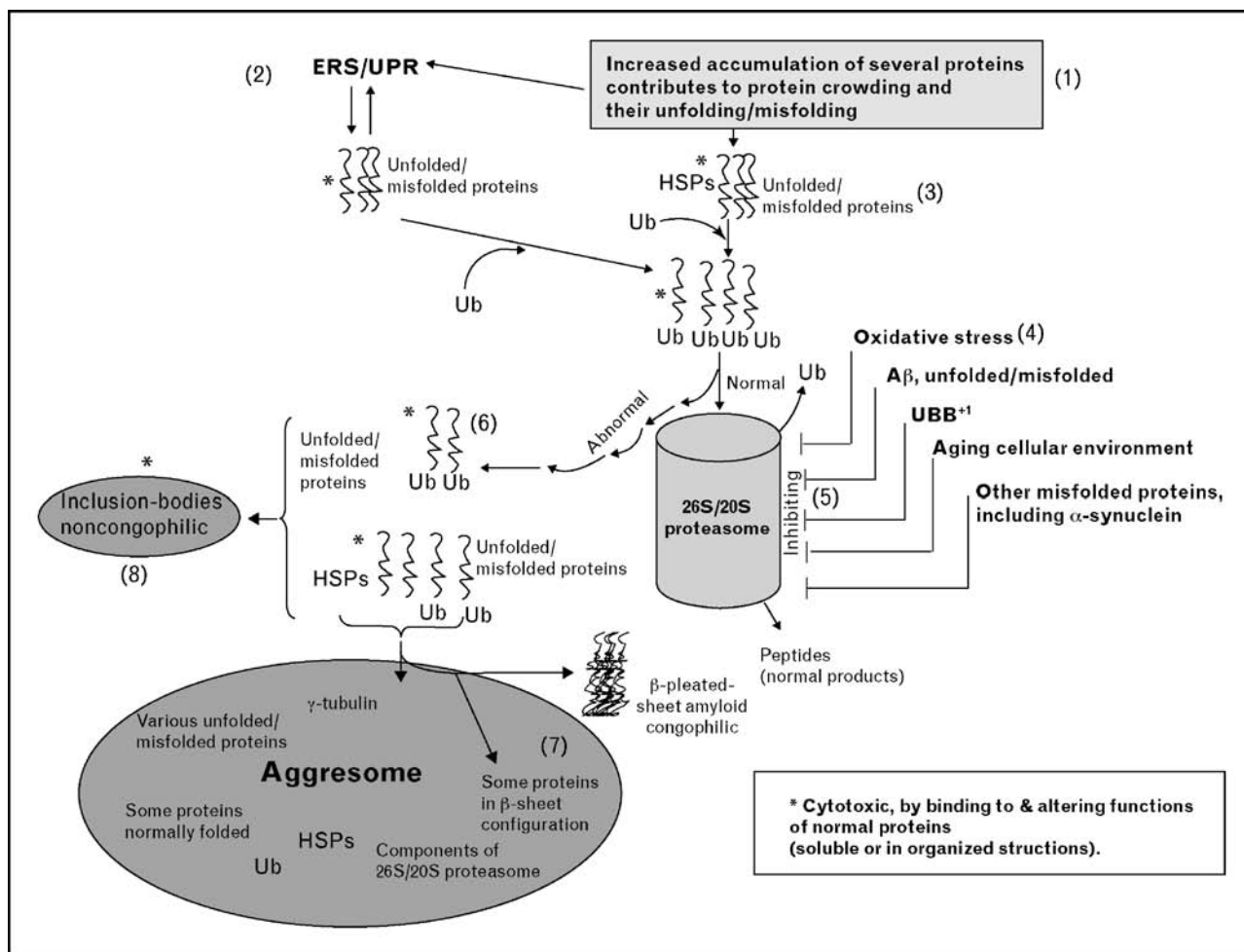
Endoplasmic reticulum stress and unfolded protein response

The endoplasmic reticulum is an intracellular compartment having a critical role in the processing, folding and exporting of newly synthesized proteins into the secretory pathway [89*]. In the endoplasmic reticulum, molecular chaperones are required to assure proper folding of unfolded or misfolded proteins [89*]. Unfolded proteins accumulating in the endoplasmic reticulum cause endoplasmic reticulum stress (ERS) [89*]. This elicits the UPR, a functional mechanism by which a cell attempts to protect itself against ERS [89*]. We reported in s-IBM muscle fibers evidence of the UPR [8]. We also demonstrated for the first time that the endoplasmic reticulum chaperones calnexin, calreticulin, GRP94, BiP/GRP78, and ERp72 physically associate with A β PP in s-IBM muscle fibers, suggesting they play a role in A β PP folding and processing [8]. Recently, we showed that HERP, another endoplasmic reticulum chaperone, is upregulated in both the s-IBM biopsied muscle fibers and in the ERS-induced cultured human muscle fibers [90*]. Our newest studies demonstrated that in cultured normal human muscle fibers, ERS induces myostatin through an NF κ B related mechanism [39**].

Mitochondrial abnormalities

Mitochondrial abnormalities include ragged-red fibers [91], cytochrome-c-oxidase (Cox) negative muscle fibers, and multiple mitochondrial DNA deletions (reviewed in [92]). They are more common in s-IBM muscle than

Figure 3 Proposed mechanisms (simplified) of unfolded/misfolded protein accumulation, proteasome inhibition, and aggresome formation within sporadic inclusion-body myositis (s-IBM) muscle fibers



(1) Abnormal accumulation of several proteins leads to molecular crowding, resulting in accumulation of normal and unfolded/misfolded proteins (2), which evoke endoplasmic reticulum stress (ERS) and unfolded protein response (UPR) (3). Heat shock proteins (HSPs) (3, 6) can aid proper protein folding. (4) Oxidative stress contributes to protein misfolding. The 26S/20S proteasome is inhibited (5) by several factors operating in the aging environment of the s-IBM muscle, including: oxidative stress, accumulated oligomerized (unfolded/misfolded) amyloid-β (Aβ), other unfolded/misfolded proteins, and UBB⁺¹. An inhibited proteasome (5) is unable to degrade ubiquitinated (Ub) proteins, which subsequently (6) are retained within the muscle fiber. This leads to their accumulation, further unfolding/misfolding, and binding to important normal proteins, and also forming β-pleated-sheet amyloid and aggregation into aggresomes (7) and other inclusions (8). Unfolded/misfolded proteins gradually accumulating within the muscle fibers are putatively cytotoxic (indicated by *).

expected for the patient’s age [92]. In s-IBM, there can be a moderate number to many Cox-negative fibers. With their electron-transport generation of ATP blocked, those fibers must be surviving on anaerobic glycolysis, at least in their Cox-negative regions. We have shown that excessive APP and Aβ contributes to the mitochondrial abnormalities [93]. This concept of AβPP/Aβ mitochondrial toxicity is now supported by studies in other systems, especially as putatively related to Alzheimer’s disease and Parkinson’s brain [70,82,94]. Alpha-synuclein accumulated in s-IBM muscle fibers may, in misfolded and oligomeric forms, also contribute to mitochondrial toxicity [70]. The mitochondrial abnormalities pre-

sumably contribute to muscle-fiber malfunction and degeneration.

Conclusion

There is convincing evidence that s-IBM is a multifactorial myopathy associated with an aging muscle-fiber environment. The igniting factor leading to the s-IBM pathogenic cascade remains unknown. Our proposed pathogenic cascade was recently illustrated [17**]. Evidence against the lymphocytic inflammatory component playing a primary pathogenic role is suggested by unsatisfactory responses to various antidysimmune treatments.

Based on our studies, we propose that the following approaches may be of therapeutic value in s-IBM: stimulate A β degradation; diminish oligomerization and fibrilization of A β , and other proteins; diminish the intracellular increase in various unfolded/misfolded proteins by reducing their formation or increasing their disposal; upregulate heat shock proteins; diminish adverse effects of intramuscle fiber cholesterol (use of statins is of uncertain benefit and potentially myotoxic); diminish oxidative stress and protect mitochondria, especially those not yet affected, perhaps with a very high dose of coenzyme Q10 and L-carnitine (neither of proven efficacy), until better molecules are developed. Greater understanding of molecular mechanisms associated with human muscle-fiber aging may also provide new avenues toward s-IBM therapy.

Acknowledgements

Our studies described in this review were supported by the National Institutes of Health (NINDS grant NS34103 and NIA Merit Award AG16768), the Muscular Dystrophy Association, the Myositis Association, and the Helen Lewis Research Funds. We thank our many research team colleagues who participated over the years in the studies described herein. The most recent collaborators include A. Nogalska, S. Wojcik, G. Vattermi, P. Fratta, J. McFerrin and C. Terracciano.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 651–652).

- 1 Engel WK, Askanas V. Inclusion-body myositis: clinical, diagnostic, and pathologic aspects. *Neurology* 2006; 66:S20–S29. Detailed review of clinical and pathological considerations, including novel pathogenetic ideas.
- 2 Dalakas MC. Sporadic inclusion body myositis: diagnosis, pathogenesis and therapeutic strategies. *Nat Clin Pract* 2006; 2:437–447. Interesting review discussing inflammatory aspects of s-IBM.
- 3 Askanas V, Engel WK. Molecular pathology and pathogenesis of inclusion-body myositis. *Microsc Res Tech* 2005; 67:114–120.
- 4 Needham M, Mastaglia FL. Inclusion body myositis: current pathogenetic concepts and diagnostic and therapeutic approaches. *Lancet Neurol* 2007; 6:620–631.
- 5 Dalakas MC. Inflammatory, immune, and viral aspects of inclusion-body myositis. *Neurology* 2006; 66:S33–S38.
- 6 Brunn A, Schröder, Deckert M. The inflammatory reaction pattern distinguishes primary dysferlinopathies from idiopathic inflammatory myopathies: an important role for the membrane attack complex. *Acta Neuropathol* 2006; 112:325–332.
- 7 Darin N, Krokmark AK, Ahlander AC, *et al.* Inflammation and response to steroid treatment in limb-girdle muscular dystrophy 21. *Eur J Paediatr Neurol* 2007; 17 April [Epub ahead of print].
- 8 Vattermi G, Engel WK, McFerrin J, Askanas V. Endoplasmic reticulum stress and unfolded protein response in inclusion body-myositis muscle. *Am J Pathol* 2004; 164:1–7.
- 9 Suto R, Srivastava PK. A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. *Science* 1995; 269:1585–1588.
- 10 Blachere NE, Li Z, Chandawarkar RY, *et al.* Heat shock protein-peptide complexes, reconstituted in vitro, elicit peptide-specific cytotoxic T lymphocyte response and tumor immunity. *J Exp Med* 1997; 186:1315–1322.
- 11 Triantafilou M, Fradelizi D, Triantafilou K. Major histocompatibility class one molecule associates with glucose regulated protein (GRP) 78 on the cell surface. *Hum Immunol* 2001; 62:764–770.
- 12 Arnold D, Faath S, Rammensee H, Schild H. Cross-priming of minor histocompatibility antigen-specific cytotoxic T cells upon immunization with the heat shock protein gp96. *J Exp Med* 1995; 182:885–889.
- 13 Kitazawa M, Green KN, Caccamo A, LaFerla FM. Genetically augmenting A β 42 levels in skeletal muscle exacerbates inclusion body myositis-like pathology and motor deficits in transgenic mice. *Am J Pathol* 2006; 168:1986–1997. A novel transgenic mouse model having some aspects of IBM.
- 14 Krause S, Schlotter-Weigel B, Walter MC, *et al.* A novel homozygous missense mutation in the GNE gene of a patient with quadriceps-sparing hereditary inclusion body myopathy associated with muscle inflammation. *Neuromuscul Disord* 2003; 13:830–834.
- 15 Amouri R, Driss A, Murayama K, *et al.* Allelic heterogeneity of GNE gene mutation in two Tunisian families with autosomal recessive inclusion body myopathy. *Neuromuscul Disord* 2005; 15:361–363.
- 16 Askanas V, Engel WK. Hereditary inclusion-body myositis. In: Rosenberg RN, DiMauro S, Paulson H, *et al.*, editors. The molecular and genetic basis of neurologic and psychiatric disease. Lippincott, Williams and Wilkins (in press).
- 17 Askanas V, Engel WK. Inclusion-body myositis: a myodegenerative conformational disorder associated with A β , protein misfolding, and proteasome inhibition. *Neurol* 2006; S39–S48. Detailed analysis of s-IBM being a protein-conformational muscle disorder.
- 18 Lünemann JD, Schmidt J, Schmid D, *et al.* β -Amyloid is a substrate of autophagy in sporadic inclusion body myositis. *Ann Neurol* 2007; 61:476–483.
- 19 Morosetti R, Mirabella M, Gliubuzzi C, *et al.* MyoD expression restores defective myogenic differentiation of human mesoangioblasts from inclusion-body myositis muscle. *Proc Natl Acad Sci U S A* 2007; 103:16995–17000.
- 20 Askanas V, Engel WK. Inclusion-body myositis: newest concepts of pathogenesis and relation to aging and Alzheimer disease. *J Neuropathol Exp Neurol* 2001; 60:1–14.
- 21 Askanas V, Engel WK. Proposed pathogenetic cascade of inclusion-body myositis: importance of amyloid- β , misfolded proteins, predisposing genes, and aging. *Curr Opin Rheumatol* 2003; 15:737–744.
- 22 McFerrin J, Engel WK, Askanas V. Impaired innervation of cultured human muscle overexpressing β APP experimentally and genetically: relevance to inclusion-body myopathies. *Neuroreport* 1998; 9:201–205.
- 23 Alvarez RB, Engel WK, Askanas V. Ultrastructural abnormalities of neuromuscular junctions in sporadic inclusion-body myositis. *Neurol* 2000; 54:240–241.
- 24 Fratta P, Engel WK, McFerrin J, *et al.* Proteasome inhibition and aggresome formation in sporadic inclusion-body myositis and in amyloid-beta precursor protein-overexpressing cultured human muscle fibers. *Am J Pathol* 2005; 167:517–526.
- 25 Wojcik S, Nogalska A, McFerrin J, *et al.* Myostatin precursor protein is increased and associates with amyloid-beta precursor protein in inclusion-body myositis culture model. *Neuropathol Appl Neurobiol* 2007; 33:238–242. Novel demonstration that A β PP overexpression induces MstnPP in human muscle.
- 26 Wojcik S, Engel WK, McFerrin J, Askanas V. Myostatin is increased and complexes with amyloid-beta within sporadic inclusion-body myositis muscle fibers. *Acta Neuropathol* 2005; 110:173–177.
- 27 Malicdan MC, Noguchi S, Nonaka I, *et al.* A GNE knockout mouse expressing human V572L mutation develops features similar to distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy. *Hum Mol Genet* 2006; 16:115–128. A novel model of the h-IBM transgenic mouse.
- 28 Jin LW, Hearn MG, Ogburn CE, *et al.* Transgenic mice over-expressing the C-99 fragment of betaPP with an alpha-secretase site mutation develop a myopathy similar to human inclusion body myositis. *Am J Pathol* 1998; 153:1679–1686.
- 29 Fukuchi K, Pham D, Hart M, *et al.* Amyloid-beta deposition in skeletal muscle of transgenic mice: possible model of inclusion body myopathy. *Am J Pathol* 1998; 153:1687–1693.
- 30 Sugarman MC, Yamasaki TR, Oddo S, *et al.* Inclusion body myositis-like phenotype induced by transgenic overexpression of β APP in skeletal muscle. *Proc Natl Acad Sci U S A* 2002; 99:6334–6339.
- 31 Vattermi G, Checler F, Engel WK, Askanas V. Amyloid- β 42 is preferentially deposited in muscle biopsies of patients with sporadic inclusion-body myositis (s-IBM). *Neurology* 2003; 60:333–334.
- 32 Wilczynski GM, Broccolini A, Engel WK, Askanas V. Novel proposed role of glycogen synthase kinase-3 β (GSK-3 β) in the pathogenesis of inclusion-body myositis (IBM). *Neurol* 2001; 56:A464.

- 33 Wilczynski GM, Engel WK, Askanas V. Cyclin-dependent kinase 5 colocalizes with phosphorylated tau in human inclusion-body myositis paired-helical filaments and may play a role in tau phosphorylation. *Neurosci Lett* 2000; 293:33–36.
- 34 Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001; 81:741–766.
- 35 Selkoe DJ. Aging, amyloid, and Alzheimer's disease: a perspective in honor of Carl Cotman. *Neurochem Res* 2003; 28:1705–1713.
- 36 Askanas V, Engel WK. Does overexpression of BetaAPP in aging muscle have a pathogenic role and a relevance to Alzheimer's disease. *Am J Pathol* 1998; 153:1673–1677.
- 37 LaFerla FM, Green KN, Oddo S. Intracellular amyloid- β in Alzheimer's disease. • *Nat Rev Neurosci* 2007; 8:499–509.
A detailed review of the proposed pathogenic role of intracellular A β in Alzheimer's disease.
- 38 Sarkozi E, Askanas V, Johnson SA, *et al.* β -amyloid precursor protein mRNA is increased in inclusion-body myositis muscle. *Neuroreport* 1993; 4:815–818.
- 39 Nogalska A, Wojcik S, Engel WK, *et al.* Endoplasmic reticulum stress induces myostatin precursor protein and NF- κ B in cultured human muscle fibers: relevance to inclusion-body myositis. *Exp Neurol* 2007; 204:610–618.
- A novel demonstration that ERS-induced NF κ B upregulates MstnPP in human muscle.
- 40 Sinha S, Anderson JP, Barbour R, *et al.* Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 1999; 402:537–540.
- 41 Vattemi G, Engel WK, McFerrin J, *et al.* Presence of BACE1 and BACE 2 in muscle fibres of patients with sporadic inclusion-body myositis. *Lancet* 2001; 358:1962–1964.
- 42 Vattemi G, Engel WK, McFerrin J, *et al.* BACE1 and BACE2 in pathologic and normal human muscle. *Exp Neurol* 2003; 179:150–158.
- 43 Sisodia S, St. George-Hyslop PH. Gamma-secretase, Notch, Abeta, and Alzheimer's disease: where do the presenilins fit in? *Nat Rev Neurosci* 2002; 3:281–290.
- 44 Vattemi G, Kefi M, Engel WK, Askanas V. Nicastrin, a novel protein participating in amyloid- β production, is overexpressed in sporadic inclusion-body myositis muscle. *Neurology* 2003; 60:315.
- 45 Jaworska-Wilczynska M, Wilczynski GM, Engel WK, *et al.* Three lipoprotein receptors and cholesterol in inclusion-body myositis muscle. *Neurology* 2002; 58:438–445.
- 46 Fielding CJ, Fielding PE. Cholesterol and caveolae: structural and functional relationships. *Biochem Biophys Acta* 2000; 1529:210–222.
- 47 McFerrin J, Engel WK, Leclerc N, Askanas V. Combined influence of amyloid- β precursor protein (A β PP) gene transfer and cholesterol excess on cultured normal human muscle fibers. *Neurology* 2002; 58:489.
- 48 Levy E, Sastre M, Kumar A, *et al.* Co-deposition of cystatin C with amyloid-beta protein in the brain of Alzheimer-disease patients. *J Neuropathol Exp Neurol* 2001; 60:94–104.
- 49 Vattemi G, Engel WK, McFerrin J, Askanas V. Cystatin C colocalizes with amyloid- β and co-immunoprecipitates with amyloid- β precursor protein in sporadic inclusion-body myositis muscle. *J Neurochem* 2003; 85:1539–1546.
- 50 Choi Y-C, Park GT, Kim T-S, *et al.* Sporadic inclusion body myositis correlates with increased expression and cross-linking by transglutaminases 1 and 2. *J Biol Chem* 2000; 275:8703–8710.
- 51 Farris W, Schütz SG, Cirrito JR, *et al.* Loss of neprilysin function promotes amyloid plaque formation and causes cerebral amyloid angiopathy. *Am J Pathol* 2007; 171:1–11.
- 52 Broccolini A, Gidaro T, Morosetti R, *et al.* Neprilysin participates in skeletal muscle regeneration and is accumulated in abnormal muscle fibres of inclusion body myositis. *J Neurochem* 2006; 96:777–789.
- 53 Broccolini A, Ricci E, Pescatori M, *et al.* Insulin-like growth factor 1 in inclusion-body myositis and human muscle cultures. *J Neuropathol Exp Neurol* 2004; 63:650–659.
- 54 Wojcik S, Engel WK, Yan R, Askanas V. In sporadic inclusion-body myositis (s-IBM) muscle fibers, NOGO-B might play a novel protective role in inhibiting the abnormal processing of amyloid- β precursor protein (A β PP). *Neurol* 2006; 66:A150–A151.
- 55 Klein WL. ADDLs and protofibrils: the missing links? *Neurobiol Aging* 2002; 23:231–235.
- 56 Ferreira ST, Vieira MNN, De Felice FG. Soluble protein oligomers as emerging toxins in Alzheimer's and other amyloid diseases. *IUBMB Life* 2007; 59:332–345.
Review emphasizing the importance of soluble protein oligomers.
- 57 Glabe CG, Kaye R. Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. *Neurology* 2006; 66:S74–S78.
- 58 Walsh DM, Selkoe DJ. A β oligomers: a decade of discovery. *J Neurochem* • 2007; 10:1–13.
Review emphasizing the importance of soluble protein oligomers.
- 59 Watson D, Castano E, Kokjohn TA, *et al.* Physicochemical characteristics of soluble oligomeric Abeta and their pathologic role in Alzheimer's disease. *Neurol Res* 2005; 27:869–881.
- 60 Derham BK, Harding JJ. Alpha-crystallin as a molecular chaperone. *Prog Retin Eye Res* 1999; 18:463–509.
- 61 Yu S, MacRae TH. The small heat shock proteins and their role in human disease. *FEBS J* 2005; 272:2613–2627.
- 62 Raman B, Ban T, Sakai M, *et al.* α B-crystallin, a small heat-shock protein, prevents the amyloid fibril growth of an amyloid- β peptide and beta2 microglobulin. *Biochem J* 2005; 392:573–581.
- 63 Stege GJ, Renkawek K, Overkamp PS, *et al.* The molecular chaperone alphaB-crystallin enhances amyloid beta neurotoxicity. *Biochem Biophys Res Commun* 1999; 262:152–156.
- 64 Wojcik S, Engel WK, McFerrin J, *et al.* AbetaPP-overexpression and proteasome inhibition increase α B-crystallin in cultured human muscle: relevance to inclusion-body myositis. *Neuromuscul Disord* 2006; 16:839–844.
Putatively novel role of α BC in s-IBM muscle fibers.
- 65 Mirabella M, Alvarez RB, Bilak M, *et al.* Difference in expression of phosphorylated tau epitopes between sporadic inclusion-body myositis and hereditary inclusion-body myopathies. *J Neuropathol Exp Neurol* 1996; 55:774–786.
- 66 Roberson ED, Searce-Levie K, Palop JJ, *et al.* Reducing endogenous tau •• ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* 2007; 316:750–754.
Novel demonstration of the role of tau in neurodegeneration.
- 67 Cookson MR. The biochemistry of Parkinson's disease. *Annu Rev Biochem* 2005; 74:29–52.
- 68 Bossy-Wetzel E, Schwarzenbacher R, Lipton SA. Molecular pathways to neurodegeneration. *Nat Med* 2004; 10:2–9.
- 69 Lindersson E, Beedholm R, Hojrup P, *et al.* Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J Biol Chem* 2004; 279:12924–12934.
- 70 Hashimoto M, Rockenstein E, Crews L, Masliah E. Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. *Neuromol Med* 2003; 4:21–36.
- 71 Askanas V, Engel WK, Alvarez RB, *et al.* Novel immunolocalization of α -synuclein in human muscle of inclusion-body myositis, regenerating and necrotic muscle fibers, and at neuromuscular junctions. *J Neuropathol Exp Neurol* 2000; 59:592–598.
- 72 Paciello O, Wojcik S, Engel WK, *et al.* Parkin and its association with •• α -synuclein and A β PP in inclusion-body myositis and A β PP over-expressing cultured human muscle fibers. *Acta Myologica* 2006; 25:13–22.
Novel demonstration that parkin and α -syn may play an important role in s-IBM pathogenesis.
- 73 Shimura H, Schlossmacher MG, Hattori N, *et al.* Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* 2001; 293:263–269.
- 74 Yang Ch-C, Alvarez RB, Engel WK, Askanas V. Increase of nitric oxide synthases and nitrotyrosine in inclusion-body myositis. *Neuroreport* 1996; 8:153–158.
- 75 Schlossmacher MG, Frosch MP, Gai WP, *et al.* Parkin localizes to the Lewy bodies of Parkinson disease and dementia with Lewy bodies. *Am J Pathol* 2002; 160:1655–1667.
- 76 Tsai YC, Fishman PS, Thakor NV, Oyler GA. Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. *J Biol Chem* 2003; 278:22044–22055.
- 77 Imai Y, Soda M, Takahashi R. Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. *J Biol Chem* 2000; 275:35661–35664.
- 78 Gonzalez-Cadavid NF, Bhasin S. Role of myostatin in metabolism. *Curr Opin Clin Nutr Metab Care* 2004; 7:451–457.
- 79 Terracciano C, Nogalska A, Engel WK, *et al.* Parkinson-disease-associated DJ-1 is oxidized and might play a novel pathogenic role in sporadic inclusion-body myositis (s-IBM) muscle fibers. *Neurology* 2007; 69:121.
- 80 Choi J, Sullards MC, Olzmann JA, *et al.* Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. *J Biol Chem* 2006; 281:10816–10824.

- 81** Xu J, Zhong N, Wang H, *et al.* The Parkinson's disease-associated DJ-1 protein is a transcriptional co-activator that protects against neuronal apoptosis. *Hum Mol Genet* 2005; 14:1231–1241.
- 82** Abou-Sleiman PM, Muqit MMK, Wood NW. Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Nat Rev Neurosci* 2006; 7:207–219.
- 83** Zhang Li, Shimoji M, Thomas B, *et al.* Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. *Hum Mol Genet* 2005; 14:2063–2073.
- 84** Voges D, Zwickl P, Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem* 1999; 68:1015–1068.
- 85** van Leeuwen FW, de Kleijn DP, van den Hurk HH, *et al.* Frameshift mutants of β -amyloid precursor protein and ubiquitin in Alzheimer's and Down patients. *Science* 1998; 279:242–247.
- 86** Fischer DF, De Vos RA, Van Dijk R, *et al.* Disease-specific accumulation of mutant ubiquitin as a marker for proteasomal dysfunction in the brain. *FASEB J* 2003; 17:2014–2024.
- 87** Fratta P, Engel WK, van Leeuwen FW, *et al.* Mutant ubiquitin UBB+1 is accumulated in sporadic inclusion-body myositis muscle fibers. *Neurology* 2004; 63:1114–1117.
- 88** Kopito RR. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol* 2000; 10:524–530.
- 89** Zhang K, Kaufman RJ. The unfolded protein response: a stress signaling pathway critical for health and disease. *Neurology* 2006; 66:S102–S109. Detailed review of UPR.
- 90** Nogalska A, Engel WK, Kokame K, *et al.* Homocysteine-induced endoplasmic reticulum protein (Herp) is up-regulated in sporadic inclusion-body myositis and in endoplasmic reticulum stress-induced cultured human muscle fibers. *J Neurochem* 2006; 96:1491–1499.
- Experimental demonstration of a novel endoplasmic reticulum protein in s-IBM and in cultured human muscle fibers.
- 91** Engel WK. Dagen Des Oordeels: pathokinetic mechanisms and molecular messages (a dramatic view). *Arch Neurol* 1979; 36:329–339.
- 92** Oldfors A, Moslemi AR, Jonasson L, *et al.* Mitochondrial abnormalities in inclusion-body myositis. *Neurol* 2006; 66:S49–S55.
- 93** Askanas V, McFerrin J, Baque S, *et al.* Transfer of beta-amyloid precursor protein gene using adenovirus vector causes mitochondrial abnormalities in cultured normal human muscle. *Proc Natl Acad Sci U S A* 1996; 93:1314–1319.
- 94** Hong WK, Han EH, Kim DG, *et al.* Amyloid- β -peptide reduces the expression level of mitochondrial cytochrome oxidase subunits. *Neurochem Res* 2007; 32:1483–1488.