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Autophagy Defects in Skeletal Myopathies

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Abstract

Autophagy is an evolutionarily conserved catabolic process that targets different types of cytoplasmic cargo (such as bulk cytoplasm, damaged cellular organelles, and misfolded protein aggregates) for lysosomal degradation. Autophagy is activated in response to biological stress and also plays a critical role in the maintenance of normal cellular homeostasis; the latter function is particularly important for the integrity of postmitotic, metabolically active tissues, such as skeletal muscle. Through impairment of muscle homeostasis, autophagy dysfunction contributes to the pathogenesis of many different skeletal myopathies; the observed autophagy defects differ from disease to disease but have been shown to involve all steps of the autophagic cascade (from induction to lysosomal cargo degradation) and to impair both bulk and selective autophagy. To highlight the molecular and cellular mechanisms that are shared among different myopathies with deficient autophagy, these disorders are discussed based on the nature of the underlying autophagic defect rather than etiology or clinical presentation.

INTRODUCTION

Autophagy is a catabolic pathway by which various types of cytoplasmic cargo (whether endogenous or exogenous in origin) are targeted to the lysosome, where they are degraded and ultimately recycled back into the cytoplasm. Autophagic degradation has two major roles in maintaining cellular homeostasis: (*a*) to eliminate damaged cellular organelles and misfolded protein aggregates generated by baseline cellular processes and (*b*) to respond to various external stressors, such as starvation, hypoxia, and oxidative stress, among others. Three types of autophagy have been described to date: chaperone-mediated autophagy [in which a cytoplasmic protein destined for degradation is directly targeted to the lysosome via its interaction with the lysosomal proteins HSPA8 (Hsp70) and LAMP2A (lysosome-associated membrane protein 2A)], microautophagy (in which cytoplasmic cargo is taken up directly by the lysosome through a membrane invagination), and macroautophagy (in which cytoplasmic cargo is sequestered into a special organelle, the autophagosome, prior to its delivery to the lysosome); of the three, only macroautophagy (hereafter referred to as autophagy) plays a role in the pathogenesis of skeletal myopathies and, therefore, is the focus of this review. Importantly, autophagy can be either selective (if a specific type of substrate is preferentially targeted for degradation, usually based on its interaction with one or more autophagy receptors) or nonselective, or bulk (if an entire region of cytoplasm is engulfed and then degraded in the lysosome). In some muscle diseases, there is a block in the overall autophagic flux that impairs both bulk and selective autophagy, while in others, the main deficit is specific for one or more subtypes of selective autophagy.

Detailed reviews of autophagic machinery and the mechanisms of autophagy regulation are available elsewhere (1–5), so only a few relevant aspects of mammalian autophagy are highlighted here (**Figure 1**). Autophagy induction involves nucleation of the phagophore, a de novo–generated double-layered membrane cisterna, or isolation membrane; this step is followed by phagophore expansion, cargo sequestration, and phagophore closure, all of which require one of eight mammalian orthologs of the yeast protein ATG8 [most commonly microtubule-associated protein 1 light chain 3 (LC3)] and ultimately result in formation of the autophagosome. Upon autophagy induction, LC3-I (a soluble form of LC3 that is present in the cytosol) is conjugated to phosphatidylethanolamine; the conjugated form of LC3 (LC3-II) is bound to both inner and outer autophagosome membranes and is often used as a proxy measure for the size of the overall autophagosomal compartment. In addition to its role in the elongation and closure of the phagophore, LC3 plays a key role in selective cargo sequestration by directly interacting either with the cargo itself or, more often, with various autophagy receptors, such as p62 (also called sequestosome 1 or SQSTM1; gene name *SQSTM1*). p62 is required for selective autophagy of many different types of cargo, including mitochondria and stress granules; however, its main function is to phase separate ubiquitinated proteins into larger protein aggregates or condensates that are ultimately targeted for autophagic degradation in a process termed aggrephagy (6). After an autophagosome is formed, it undergoes maturation that involves changes in the associated protein machinery, binding to the dynein–dynactin molecular motor, and long-range transport along the microtubule network to the perinuclear region, where it fuses with a lysosome to form an autolysosome; after the autolysosome is formed, the inner membrane of the original autophagosome and all the cargo within it are degraded by lysosomal acid hydrolases. Importantly, a static measurement that shows an increase in the size of the LC3-II-positive autophagosomal compartment can reflect either autophagy activation (increased on-rate) or autophagy block (decreased off-rate); to appropriately interpret the nature and direction of changes in the autophagic flux, it is necessary to assess not only the level of LC3-II but also the level of one or more autophagic receptors, which are normally degraded together with their cargo. The autophagic receptor most commonly used

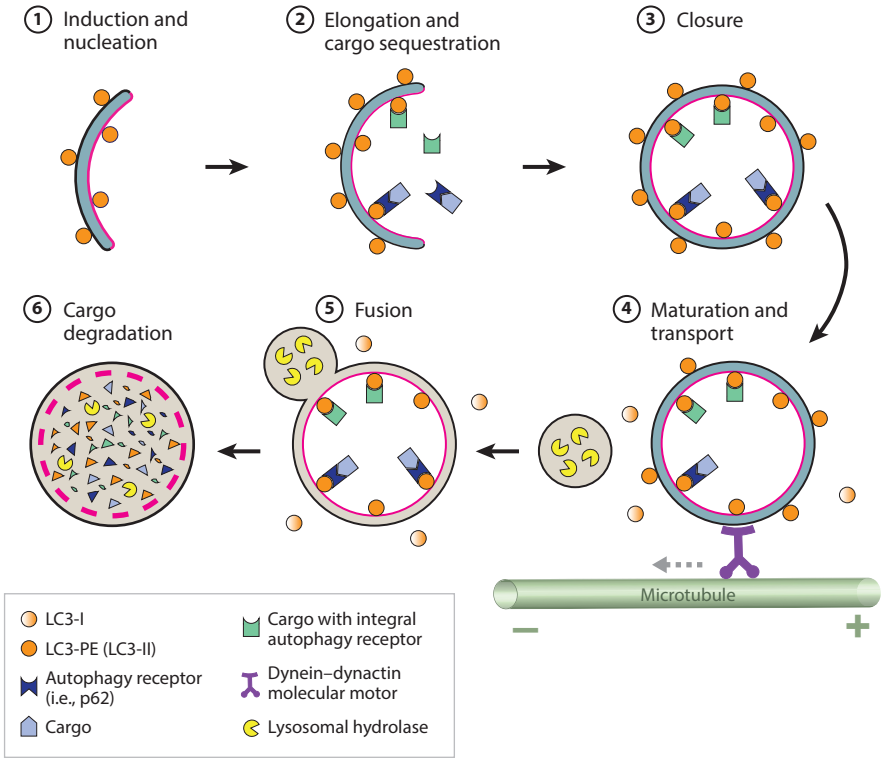

Figure 1

Key steps in mammalian macroautophagy. Macroautophagy is a complex process that starts with (①) nucleation of the phagophore (also known as the isolation membrane), a two-layered membrane cisterna that contains phosphatidylethanolamine (PE)-conjugated LC3-II (or another mammalian ATG8 ortholog) bound to its inner and outer membranes. (②) The phagophore then expands, surrounding the cargo that is being sequestered through its interaction with LC3-II; although LC3-II–cargo binding can be direct, more often it is mediated via an autophagic receptor such as p62. (③) Eventually, the expanding membrane closes, resulting in the formation of a double-walled vesicle called the autophagosome. (④) The autophagosome then matures; this involves (*a*) changes in the protein machinery bound to its external surface (including the release of LC3, which can be reused, and binding of various proteins that are required for autophagosome–lysosome fusion) and (*b*) binding to the dynein–dynactin molecular motor, which then transports the maturing autophagosome centripetally toward the lysosomes located at the minus end of the microtubules (i.e., in the center of the cell). (⑤) The lysosome and autophagosome then fuse, which enables the lysosomal hydrolases to be released into the resulting vesicle (the autolysosome). (⑥) Through the action of these lysosomal enzymes (which requires an acidic intravesicular milieu), the autolysosomal contents are degraded; this degradation includes not only the sequestered cargo but also the inner membrane, LC3-II bound to the inner membrane, and autophagic receptors. Not pictured are two additional steps: (*a*) kinesin- and FYCO1-mediated centrifugal transport of the phagophore from the perinuclear area (where nucleation occurs) to the periphery of the cell (where cargo is sequestered and the autophagosomes are formed) and (*b*) fusion of the autophagosome with the late endosome, which sometimes (but not always) occurs prior to the fusion of this combined vesicle (the amphisome) with the lysosome.

for this purpose is p62; if the p62 level increases along with the LC3-II level, the findings suggest a block in the late stages of autophagy and an overall decrease in the rate of autophagic flux (3).

Skeletal muscle diseases with impaired autophagy (i.e., autophagic myopathies in a broad sense of the term) are usually classified based on their etiology into genetic, toxic, and sporadic

or idiopathic forms. However, many of these entities show morphologic similarities that reflect their shared pathogenesis, as established through investigation of cellular and animal models of disease; to emphasize these mechanistic connections, in this review myopathies with impaired autophagy are grouped based on the nature of the underlying autophagic defect.

MYOPATHIES WITH DEFECTS OF AUTOPHAGIC FLUX

In spite of large differences in their etiology and clinical presentation, autophagic vacuolar myopathies (AVMs) are grouped together based on their unique histopathology that includes (*a*) sarcoplasmic vacuolation, (*b*) a significant increase in the number of autophagic vacuoles as seen by electron microscopy, and/or (*c*) marked accumulation of LC3-II-positive autophagosomes and p62-positive undegraded cargo as seen by light microscopy. The extensive morphologic overlap among different AVMs (which was recognized well before the autophagic pathway was fully elucidated) raised the possibility that all of these diseases share a common molecular mechanism; indeed, subsequent mechanistic studies have established that AVMs are caused by a block in the autophagic flux that occurs after autophagy induction (i.e., at steps ④–⑥ in **Figure 1**). Because the underlying autophagic defect plays a critical role in the pathogenesis of AVMs, they are generally considered to be true autophagic myopathies (i.e., autophagic myopathies in a narrow sense of the term).

Defects of Cargo Degradation

Although AVMs can be caused by a block of the autophagic flux at any of the postinduction autophagy steps, a defect of cargo degradation (**Figure 1**, step ⑥) is encountered most often and can be either genetic or drug-induced (toxic) in etiology.

X-linked myopathy with excessive autophagy. X-linked myopathy with excessive autophagy (XMEA), first described in 1988 (7), exclusively affects males and usually presents with a slowly progressive limb girdle weakness that becomes apparent in the first or second decade of life; however, late-onset, infantile, and congenital cases have also been reported (reviewed in Reference 8). In contrast to many other AVMs, XMEA is restricted to skeletal muscle and spares the heart (9); however, cardiac involvement was recently documented in a severe case with a congenital onset (10). Muscle biopsies from patients with XMEA show stereotypical AVM features, such as sarcoplasmic vacuolation, basophilic stippling on hematoxylin and eosin (H&E) staining, and increased enzyme histochemistry for a lysosomal enzyme acid phosphatase; less typically for a nonimmune myopathy, muscle fibers in XMEA also show upregulation of the MHC-1 protein and sarcolemmal deposition of the complement membrane attack complex C5b-9 (but no fiber necrosis or inflammation) (8). In addition, XMEA muscle fibers contain unusual vacuoles (so-called autophagic vacuoles with sarcolemmal features, or AVSFs) that are lined by sarcolemmal and basal lamina proteins synthesized by the muscle fiber (such as dystrophin, dysferlin, merosin, and perlecan) but that lack the basal lamina proteins produced by fibroblasts (such as collagen IV) (8). AVSFs were originally thought to be specific to XMEA and Danon disease, the two AVMs in which they represent a prominent finding; however, closer examination has shown that they are likely present in all AVMs to a certain degree (8, 11–13). Although the origin of AVSFs in not entirely clear, they are thought to be derived from the late endosomes (which occasionally fuse with autophagosomes before fusing with lysosomes). Ultrastructurally, classic autophagic vacuoles filled with electron-dense cellular debris are seen between sarcomeres and beneath the sarcolemma (8); essentially identical autophagic vacuoles are seen in other AVMs and are illustrated in later sections.

XMEA is caused by mutations in VMA21, a chaperone that is critical for the proper assembly of the vacuolar ATPase; multiple *VMA21* mutations have been reported, all of which result in a decreased expression (but not complete absence) of VMA21 mRNA and protein. Vacuolar ATPase is a widely expressed proton pump that has many cellular roles; in the lysosome, its main function is to maintain the low pH that is necessary for the normal function of lysosomal acid hydrolases (14). Thus, it is currently thought that mutations in *VMA21* ultimately lead to a failure of lysosomal hydrolysis, the final step in autophagic cargo degradation (**Figure 1**, step ⑥).

Chloroquine and hydroxychloroquine myopathy. Chloroquine (CQ) and its analog hydroxychloroquine (HCQ) were developed in the mid-1950s to treat malaria, but currently they are most frequently used to treat autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis (15). While generally considered safe and well tolerated, both drugs can have serious side effects, including retinopathy (16), skeletal myopathy (17), and potentially life-threatening cardiomyopathy (18). CQ/HCQ skeletal myopathy, which presents with proximal muscle weakness and elevation of creatine kinase levels, was originally thought to be rare, with an incidence of 1 case per 100 patient-years of therapy (19); however, a subsequent prospective study showed that this complication is more frequent than originally thought, with a cumulative prevalence of 12.6% over 3 years of treatment (20). Clinically evident CQ- or HCQ-induced heart disease (which usually manifests as restrictive cardiomyopathy, conduction abnormalities, or both) is less frequent, although subclinical heart involvement is probably present in most patients with skeletal myopathy. On light microscopy, skeletal muscle in CQ/HCQ myopathy shows classic AVM features: fiber vacuolation, basophilic stippling, and an increase in the number of LC3- and p62-positive puncta, which in CQ/HCQ myopathy typically accumulate in the core of the fiber (17) (**Figure 2*a–d***); AVSFs are sometimes but not always present (11). In addition,

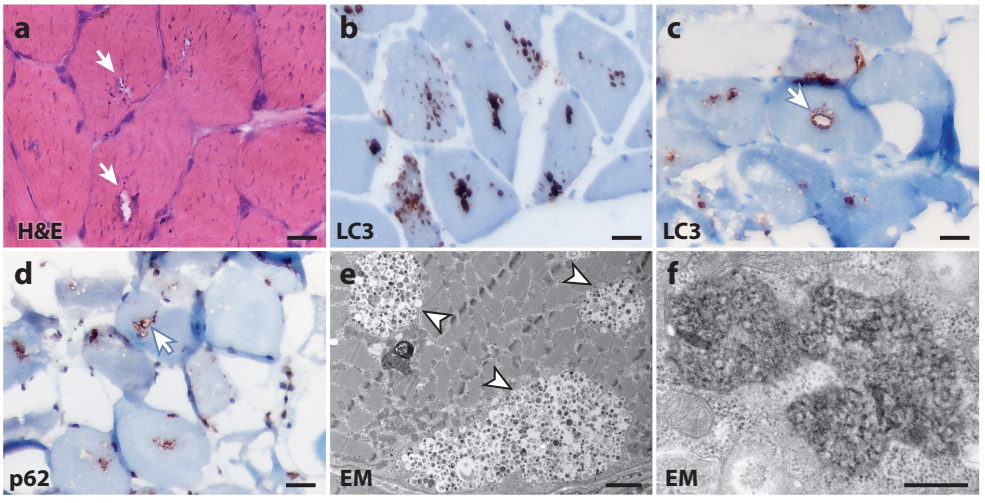

Figure 2

Hydroxychloroquine myopathy. A representative hematoxylin and eosin (H&E)–stained cryosection shows (*a*) prominent basophilic stippling and rimmed vacuoles (*arrows*). LC3 immunohistochemistry highlights (*b*) the accumulation of coarse sarcoplasmic puncta and (*c*) the rim of rimmed vacuoles (*arrow*). (*d*) The same structures are also labeled by p62 immunohistochemistry (*arrow* highlights a cluster of rimmed vacuoles). Electron microscopy (EM) shows (*e*) autophagic vacuoles (*arrowheads*) and (*f*) curvilinear bodies. Scale bars *a–d*: 20 μm; scale bar *e*: 2 μm; scale bar *f*: 0.5 μm.

some fibers contain so-called rimmed vacuoles, empty-appearing spaces in the fiber sarcoplasm surrounded by a rim of basophilic granular material (**Figure 2*a***) that can be highlighted by either LC3 or p62 immunohistochemistry (**Figure 2*c*,*d***); essentially identical rimmed vacuoles can be induced in rat muscle by CQ treatment in vivo (21, 22) and are a common feature of many myopathies in which autophagic impairment plays a key role in pathogenesis. Of note, rimmed vacuoles are not actually empty; rather, their content is lost during routine specimen processing but can be visualized in electron microscopy preparations (see the section titled Sporadic Inclusion Body Myositis). Ultrastructurally, CQ/HCQ myopathy is characterized by the accumulation of autophagic vacuoles (**Figure 2*e***) and curvilinear bodies (**Figure 2*f***) (further discussed in the section titled CLN3 Disease). Morphologic features of CQ and HCQ cardiomyopathy are similar, with marked cardiomyocyte vacuolation accompanied by the accumulation of LC3- and p62-positive puncta, autophagic vacuoles, and curvilinear bodies (18).

CQ and HCQ are 4-aminoquinoline cationic compounds that accumulate in lysosomes and raise lysosomal pH (23–25), thereby attenuating the function of lysosomal hydrolases (26); as in XMEA, this ultimately leads to a block in the autophagic flux and the accumulation of undigested autophagosomes in the affected tissues. (This effect of CQ has been extensively employed to experimentally measure autophagic flux in vitro as well as in vivo; see Reference 27.) The pathogenesis of CQ/HCQ retinopathy is less well understood, but it appears to involve the same underlying mechanism (16, 28). Given that the molecular mechanism underlying autophagic failure is similar in XMEA and CQ/HCQ-induced toxicity, it is not clear why XMEA primarily affects skeletal muscle while CQ and HCQ are also toxic to the retina and heart (the two tissues that are often involved in other AVMs, such as CLN3 disease and Danon disease).

CLN3 disease. Neuronal ceroid lipofuscinoses (NCLs) are degenerative diseases of childhood characterized by excessive accumulation of lipopigments (lysosomal residual bodies) throughout the body; they encompass a spectrum of diverse clinical phenotypes and are caused by mutations in a number of different proteins (termed CLN1–14) (29). While NCLs primarily lead to neuronal degeneration in the retina and brain (29), skeletal muscle involvement with AVM features has recently been reported in patients with CLN3 disease, the most frequent type of NCL (30–32). Muscle specimens from patients with CLN3 disease show pathologic features somewhat similar to XMEA, with increased acid phosphatase activity, MHC-1 upregulation, and C5b-9 deposition in muscle fiber membranes; AVSFs are present but are much less frequent than in XMEA or Danon disease (30–32). In addition, CLN3 muscle fibers often demonstrate central core-like regions that are filled with granular content but do not show basophilic rimming; prominent accumulation of LC3- and p62-positive puncta or granules is seen in those central areas (32) and is reminiscent of the central autophagosome buildup seen in CQ/HCQ myopathy (17) (**Figure 2**), Pompe disease (33–35), and colchicine myopathy (17). Ultrastructurally, muscle in CLN3 disease demonstrates the expected accumulation of autophagic vacuoles, but it also shows curvilinear bodies (the intralysosomal accumulations of subunit C of mitochondrial ATP synthase) (30, 32); while these curvilinear bodies are often considered to be fairly specific for NCLs (29), they are also a key diagnostic feature of CQ/HCQ myopathy (**Figure 2*f***).

CLN3 is a lysosomal membrane protein with six transmembrane domains. The most common genetic alteration (seen in ~80% of patients with CLN3 disease) is a 1-kb deletion that creates a premature stop codon and likely results in a complete loss of CLN3 protein expression (31, 36); however, there are also many disease-causing point mutations, all of which are located in the second lumen-facing loop, which therefore seems to be critical for the function of this protein (30). Based on the current evidence, CLN3 has multiple cellular roles that include regulating cell migration, the cell cycle, and apoptosis (36). However, and likely of the greatest significance from

the standpoint of AVM pathogenesis, CLN3 also plays a key role in the maintenance of lysosomal acidity: Fibroblasts derived from patients with CLN3 disease show a 0.6 increase in lysosomal pH (37), a small but significant effect that is similar in magnitude to the increase in lysosomal pH measured in cells derived from XMEA patients (14) or in peritoneal macrophages treated with CQ (23).

Pompe disease. Pompe disease, first described in the 1930s and also known as glycogen storage disease type II or acid maltase deficiency, is caused by loss-of-function mutations in the gene coding for the lysosomal hydrolase acid α-glucosidase (GAA), which is responsible for glycogen catabolism in the lysosome (recently reviewed in Reference 38). Depending on the severity of the mutation and the resulting level of residual GAA activity, the clinical presentation of Pompe disease varies from very severe (infantile-onset disease, or IOPD; with <1% of residual GAA activity) to relatively mild (late-onset disease; with up to 30% of residual GAA activity). GAA is a ubiquitously expressed enzyme, but cardiac and skeletal muscle symptoms (hypertrophic cardiomyopathy and severe muscle weakness) usually dominate the clinical picture of patients with untreated IOPD; after the successful introduction of enzyme replacement therapy in 2006 (which significantly improves cardiac function and has led to a significant reduction in mortality in IOPD), it became clear that the spectrum of tissues affected by Pompe disease is actually quite broad and includes the central and peripheral nervous systems, gastrointestinal tract, urinary tract, and bones (39, 40).

In Pompe skeletal myopathy, the pathologic findings (**Figure 3**) include vacuolation of muscle fibers, accumulation of glycogen (which is largely localized within the membrane-bound lysosomal compartment) (**Figure 3*c*,*f***), and accumulation of autophagic vacuoles that can be visualized either by electron microscopy, which was first noted in 1970 (33) (**Figure 3*f***), or by LC3

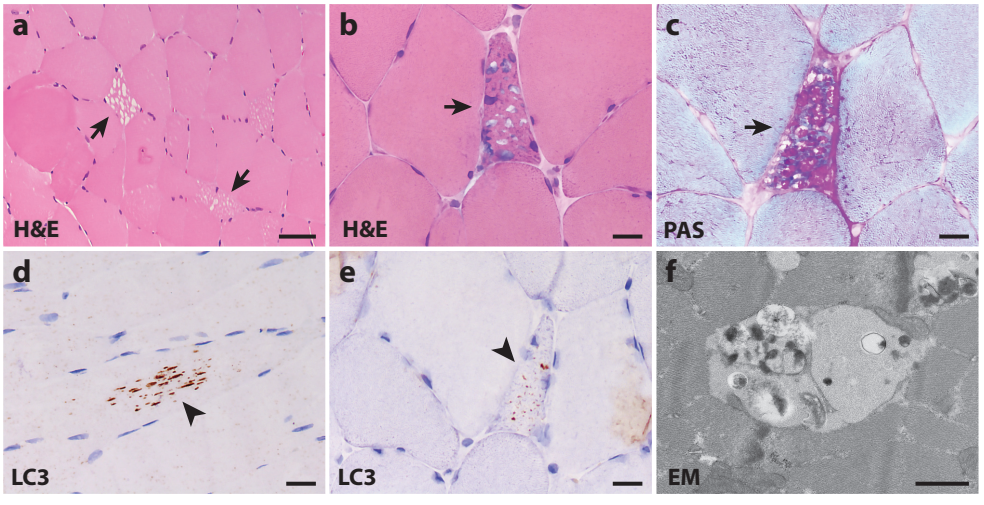

Figure 3

Late-onset Pompe disease. (*a*,*b*) Scattered vacuolated fibers (*arrows*) are seen on (*a*) hematoxylin and eosin (H&E)–stained paraffin sections and (*b*) H&E–stained cryosections. (*c*) Periodic acid–Schiff (PAS) stain highlights glycogen accumulation in the vacuolated fiber (*arrow*). (*d*,*e*) Fibers with an accumulation of LC3-positive sarcoplasmic puncta (*arrowheads*) are seen in both (*d*) paraffin sections and (*e*) cryosections. (*f*) Electron microscopy (EM) shows glycogen within membrane-bound spaces that are associated with the accumulation of autophagic material. Scale bar *a*: 50 μm; scale bars *b*–*e*: 20 μm; scale bar *f*: 0.8 μm.

immunohistochemistry (**Figure 3*d*,*e***); the findings in cardiac muscle are similar (41). As already mentioned, these undigested autophagosomes or autophagic vacuoles accumulate largely in the core of muscle fibers in a pattern that is reminiscent of drug-induced AVMs and CLN3 disease; an essentially identical pattern is seen in *GAA* knockout mice, which successfully model Pompe disease (34, 35). Initially, it was thought that the massive accumulation of glycogen in Pompe skeletal muscles leads to mechanical disruption of the myofibrillar apparatus and a consequent loss of muscle contractility; however, the emerging consensus is that autophagic impairment, which progressively worsens long after glycogen accumulation has peaked (42, 43), plays a significant role in the pathogenesis of Pompe myopathy (44) and is responsible for the relatively poor response of skeletal muscle to enzyme replacement therapy (38). It is not entirely clear how the dysfunction of glycogen catabolism leads to a more general defect in autophagic cargo degradation; however, a parallel with CLN3 disease—another lysosomal storage disorder—suggests that a defect of lysosomal acidification may play a role in the pathogenesis of Pompe disease. Alternatively (or perhaps additionally), there could be a defect in autophagosome–lysosome fusion (**Figure 1**, step ⑤), the phase of autophagy that seems to be impaired in several lysosomal storage disorders (45), as well as in Danon disease.

DEFECT OF AUTOPHAGOSOME–LYSOSOME FUSION: DANON DISEASE

Like XMEA, Danon disease, first described in 1981 (46), is an X-linked disorder; unlike XMEA, which primarily targets skeletal muscle, Danon disease affects multiple tissues and is characterized by a triad of clinical features: life-threatening cardiomyopathy (usually hypertrophic but sometimes dilated), proximal muscle weakness, and mild-to-moderate cognitive impairment (recently reviewed in References 47 and 48). The disease is more severe and has an earlier onset in males, but heterozygous females are also affected. Skeletal muscle pathology overlaps with the other AVMs discussed thus far: muscle biopsies from patients with Danon disease show fiber size variation without overt degeneration, regeneration, or inflammation; basophilic stippling or the accumulation of small sarcoplasmic basophilic granules akin to those frequently seen in CQ/HCQ myopathy (**Figure 2*a***); AVSFs; and prominent LC3- and p62-positive sarcoplasmic puncta (47, 49). Ultrastructurally, there is the accumulation of autophagic vacuoles and also glycogen, which is why Danon disease was initially classified as "lysosomal glycogen storage disease with normal acid maltase" (46). Cardiomyocytes show marked vacuolation accompanied by the accumulation of autophagic vacuoles (41) and LC3-positive puncta (47, 48); again, these findings are reminiscent of those seen in CQ/HCQ cardiomyopathy (18). Brain changes in Danon disease are less well understood; however, an autopsy of a 31-year-old patient who died from heart failure showed pale granular neurons with abundant lipofuscin accumulation but without typical autophagic vacuoles or LC3-positive inclusions (50).

Danon disease is caused by a number of different loss-of-function mutations in *LAMP2* (47, 48). Alternative splicing of the *LAMP2* gene results in three different splice isoforms (LAMP2A, -2B, and -2C), all of which are primarily localized to the lysosomes and late endosomes, but have different functional roles: LAMP2A is involved in chaperone-mediated autophagy, LAMP2B in autophagosome–lysosome fusion (**Figure 1**, step ⑤), and LAMP2C in RNA and DNA autophagy (47). Interestingly, both clinical and experimental data suggest that the loss of LAMP2B and consequent disruption of macroautophagy are necessary and sufficient to cause Danon disease, although the cardiac and brain phenotypes (but not the skeletal muscle phenotype) are more severe when all three isoforms are affected (47, 48, 51). A recent study has shown that LAMP2B is required for autophagosome–lysosome fusion in human cardiomyocytes, where it functions independently of

syntaxin 17 (which is essential for autophagosome–lysosome fusion in many other cell types) (51); it is not known whether LAMP2B plays an equally important role in autophagosome–lysosome fusion in skeletal muscle fibers.

LAMP2-deficient mice show an accumulation of autophagic vacuoles in both cardiac and skeletal muscles, but their cardiac phenotype is less severe than the one observed in patients with Danon disease. In addition, these mice show autophagy impairment in organs that are not thought to be affected in Danon disease (such as the liver, pancreas, and kidneys), prompting the hypothesis that the severe cardiac impairment in Danon disease overshadows subclinical defects in other human tissues (47). It is not clear why cardiac disease is more severe in LAMP2-deficient humans compared with LAMP2-deficient mice, nor is it clear why the heart is severely involved in Danon disease, Pompe disease, and CQ or HCQ intoxication, but is only rarely and mildly affected in XMEA.

DEFECT OF AUTOPHAGOSOME MATURATION: COLCHICINE MYOPATHY

Colchicine is a plant-derived alkaloid that is approved by the US Food and Drug Administration to treat and prevent gout and to treat familial Mediterranean fever; however, its therapeutic index is quite narrow, with a significant overlap between therapeutic and toxic doses (52). Acute colchicine intoxication leads to multisystem organ failure and death, usually due to hemodynamic collapse most likely occurring secondary to myocardial toxicity (52). In contrast, chronic colchicine toxicity primarily manifests as a neuromuscular injury that affects skeletal muscle more prominently than peripheral nerves and is reversible upon discontinuation of the drug (53). Colchicine myopathy usually presents with proximal muscle weakness and creatine kinase elevation; its epidemiology has not been well studied, but a recent study reported a relatively high incidence of 1.4%, with a higher risk associated with concurrent chronic kidney or liver disease, a higher dose of colchicine, and concomitant use of a drug that inhibits the CYP3A4 isoform of cytochrome p450 (54). Histologically, muscle fibers in colchicine myopathy typically show a central, apparently disorganized zone that has a basophilic appearance on H&E staining (the basophilic core) (17, 53, 55) (**Figure 4*a***) and stains with a higher intensity than the rest of the fiber on NADH-TR (nicotinamide adenine dinucleotide tetrazolium reductase) histochemistry (**Figure 4*b***); a prominent accumulation of LC3- and p62-positive puncta is seen in the disorganized area of the fiber (17) (**Figure 4*c*,*d***). Rimmed vacuoles and vacuoles reminiscent of AVSFs are occasionally seen (55). Muscle fiber necrosis is usually absent, but can develop in the most severe cases (52, 56). Electron microscopy shows the accumulation of autophagic vacuoles (**Figure 4*e***) as well as disorganization of the normal myofibrillar apparatus (**Figure 4*f***). Thus, morphologic findings in colchicine myopathy show an overlap between a classic AVM and a myofibrillar myopathy (MFM).

Colchicine binds to tubulin, preventing microtubule polymerization; this cytoskeletal disruption is thought to block the autophagic flux by interfering with autophagosomal transport during autophagosome maturation (53) (**Figure 1**, step ④). In further support of this mechanism, deposits of the molecular motor dynein were demonstrated within the disorganized fiber cores (57). In addition, a similar myopathy can develop following treatment with vincristine, another microtubule-disrupting agent (53). Indeed, colchicine-induced blocking of autophagosome maturation can be experimentally employed to measure the autophagic flux in the mature skeletal muscle of living experimental animals (58). In a mouse model, colchicine myotoxicity is augmented by co-administration of an inducer of autophagic flux, such as rapamycin or a statin (59); based on a number of case reports, it was thought that a similar drug interaction might occur in humans, but a recent retrospective cohort study showed that the concomitant use of colchicine and a statin was not associated with a higher risk of myopathy (54).

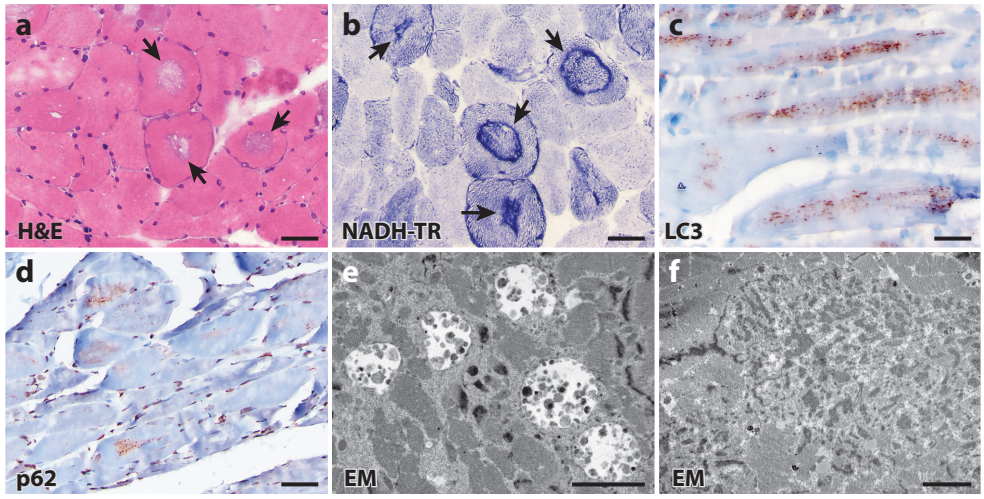

Figure 4

Colchicine myopathy. (*a*,*b*) Centrally disorganized areas that accumulate pale basophilic material (known as basophilic cores; *arrows*) can be seen in many muscle fibers on (*a*) hematoxylin and eosin (H&E)–stained cryosections, but are better appreciated with (*b*) NADH-TR (nicotinamide adenine dinucleotide tetrazolium reductase) histochemistry. (*c*) LC3- and (*d*) p62-positive puncta accumulate within the disorganized basophilic cores. (*e*,*f*) Electron microscopy (EM) highlights (*e*) the accumulation of autophagic vacuoles and (*f*) disorganization of the myofibrillar apparatus with a resulting accumulation of filaments and filament fragments that mimic the granulofilamentous material seen in myofibrillar myopathies. Scale bars *a*–*d*: 50 μm; scale bars *e*–*f*: 2 μm.

MYOPATHIES WITH DEFECTS OF CARGO RECOGNITION

While AVMs are caused by a defect in general autophagic flux, a more selective autophagy dysfunction has been associated with the pathogenesis of other skeletal myopathies that show the accumulation of rimmed vacuoles, protein aggregates, or both. For some of these disorders, the nature of the underlying autophagy defect has been identified and involves impairment of selective cargo sequestration (**Figure 1**, step ②); for others, which are described in later sections, the exact nature and mechanisms of the underlying autophagy defects are not yet understood but are being actively investigated.

Granulophagy Defect: Multiple System Proteinopathies

Multiple system proteinopathy 1 (MSP1) is a pleiotropic degenerative disease that was originally abbreviated as IBMPFD based on its three most common clinical presentations (inclusion body myopathy, Paget's disease of the bone, and frontotemporal dementia) (60). It is caused by autosomal dominant mutations in the gene encoding valosin-containing protein (VCP; also known as p97 or Cdc48). Subsequent to the original disease description, it was discovered that *VCP* mutations can also cause amyotrophic lateral sclerosis, Parkinson disease, Charcot–Marie–Tooth disease type 2, and other conditions (reviewed in Reference 61). Simultaneously, it was recognized that a similar constellation of diverse but overlapping clinical syndromes can be caused by mutations in four other genes (*HNRNPA1*, *HNRNPA2B1*, *SQSTM1*, and *MATR3*); consequently, these disorders are now grouped together and classified as multiple system proteinopathies (MSP1–MSP5) (62). Of the five currently identified MSPs, *VCP*-associated MSP1 is the most common and affects ~50% of all MSP patients (62).

The skeletal muscle phenotype is seen in ~90% of patients with *VCP* mutations and is usually the presenting feature of MSP1; the bone and brain phenotypes are less frequent (~40% and 30% of patients, respectively), while the other reported phenotypes are comparatively rare (<10%) (63). Interestingly, the presentation varies even among individuals who carry the same mutation, suggesting a considerable role for environmental and genetic disease modifiers (63). The weakness in MSP1 patients is most commonly proximal, with involvement of the shoulder and hip girdle muscles, but it can also be distal or axial (61). Muscle biopsy findings are variable, but typically include chronic myopathic features reminiscent of a muscular dystrophy, including fiber size variation, endomysial fibrosis, and central nucleation; rimmed vacuoles; and LC3-, p62-, and TDP43-positive protein aggregates. If the patient also has motor neuron disease or peripheral neuropathy, there can be concurrent neurogenic changes (61). Inflammation and MHC-1 upregulation can sometimes be present, but they are generally less prominent than in sporadic inclusion body myositis (sIBM; further discussed below). Finally, myofibrillar disorganization accompanied by the accumulation of Z-disc and intermediate filament proteins can also be seen; interestingly, an analysis of laser-dissected protein aggregates obtained from MSP1 muscle showed a considerable overlap with MFMs but not with the protein aggregates seen in GNE [UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase] myopathy (61), both of which are discussed below.

VCP belongs to a large family of AAA+ ATPases that have a number of diverse cellular functions, many of which depend on their interactions with different protein cofactors. VCP itself plays a key role in several types of cellular damage responses by serving as a protein extractor or segregase; through the generation of ATP hydrolysis–dependent mechanical force, it leads to partial unfolding of its protein substrates and disruption of their interactions with other proteins, cellular membranes, or nuclear chromatin (recently reviewed in Reference 64). The segregase function of VCP is critical for the role it plays in endoplasmic reticulum–associated protein degradation, degradation of damaged mitochondria, and degradation of various chromatin-associated proteins in the context of DNA damage (64); in addition, it was recently hypothesized that VCP-mediated extraction of ubiquitinated proteins from the lysosomal membrane represents a critical step in lysophagy (the removal of damaged lysosomes via autophagy), which is critical for lysosomal homeostasis (65). Furthermore, a recent study used live imaging to show that VCP plays a key role in normal lysosomal dynamics, the impairment of which leads to muscle degeneration (66). In an elegant series of experiments, the authors of that study demonstrated that in *Drosophila* skeletal muscle, lysosomes do not exist as individual vesicular structures but as a dynamic tubular network that undergoes continuous fusion and fission; maintenance of this lysosomal network requires VCP and is impaired by MSP1-causing VCP mutations, which lead to network disintegration, the accumulation of ubiquitinated protein aggregates, and muscle weakness (66). (Interestingly, maintenance of this lysosomal tubular network also requires intact microtubules; thus, it is possible that disruption of normal lysosomal dynamics also plays a role in the autophagosome maturation defect caused by cytoskeletal dysfunction, which was discussed above.)

What is the mechanistic relationship between the five different subtypes of MSP? MSP4 is caused by mutations in *SQSTM1*, further substantiating the causal link between autophagic dysfunction and MSP pathogenesis. The other three MSP genes—*HNRNPA1*, *HNRNPA2B1*, and *MATR3*—all encode RNA-binding proteins found in stress granules, which are aggregates of nontranslating messenger ribonucleoprotein complexes that form under cellular stress conditions to arrest further mRNA translation. (Mutations in the other RNA-binding proteins in the same family—TDP-43, FUS, and TIA1—have not yet been shown to cause the pleiotropic MSP phenotype, but they have been associated with individual diseases in the MSP spectrum: Amyotrophic lateral sclerosis and frontotemporal dementia can be caused by mutations in *TARDBP* (TDP-43) and *FUS*, while one form of distal myopathy with rimmed vacuoles is caused by mutations

in TIA1.) Intriguingly, clearance of stress granules is mediated by autophagy and requires VCP (67); thus, it is likely that abnormal granulophagy ultimately leads to cellular dysfunction and injury in different degenerative disorders that are part of the MSP spectrum (62). Further supporting this mechanism, a recent study showed that expression of the muscle phenotype in MS4 patients is based on the synergism between *SQSTM1* and *TIA1* mutations that results in impaired stress granule clearance (68). Interestingly, an IBM-like muscle phenotype also develops in mice with a muscle-specific deficiency of Ulk1 and Ulk2, two Atg1-like kinases that play a role in autophagy induction and also promote the resolution of stress granules in a VCP-dependent, autophagy-independent manner (69); this finding supports the central role of abnormal stress granule metabolism in the pathogenesis of MSPs.

Defect of Chaperone-Assisted Selective Autophagy: Myofibrillar Myopathies

MFMs are a group of etiologically diverse muscle disorders that show similar muscle pathology: (*a*) central rubbed-out (core-like) regions seen on oxidative enzyme histochemistry; (*b*) sarcoplasmic inclusions or protein aggregates that are often LC3- and p62-positive and are ultrastructurally composed of granulofilamentous material (similar accumulations can sometimes be seen in colchicine myopathy) (**Figure 4*f***); (*c*) progressive disorganization of the myofibrillar architecture that starts at the Z-disc (a protein assembly that anchors actin myofilaments and forms the sarcomeric boundary) and usually manifests as Z-band streaming and disorganization of the myofibrillar apparatus; and (*d*) rimmed vacuoles, which are often, but not always, present (reviewed in References 70 and 71; see also Reference 72). Clinical presentation varies depending on the mutated gene, but most often starts with weakness in distal muscles and then over time spreads into the proximal and sometimes axial muscles; cardiac involvement is common and can manifest as hypertrophic cardiomyopathy, dilated cardiomyopathy, conduction abnormalities, or a combination of these (71). The age of onset varies from early childhood to late adulthood, but it occurs most commonly in middle adulthood (71).

The MFM phenotype can be caused by mutations in many different genes, all of which encode proteins that are required for Z-disc formation and stability. One large group of MFM proteins, which includes desmin, myotilin, ZASP, and filamin C, has a structural role in the maintenance of Z-disc and sarcomere integrity; the other large group includes the chaperones αB-crystallin (HSPB5), BAG3, DNAJB6, and HSPB8, many of which play a key role in the chaperone-assisted selective autophagy (CASA) pathway that is critical for degradation of damaged Z-disc proteins (71, 73–75). (Although CASA requires chaperone function, it involves autophagosome formation and is distinct from chaperone-mediated autophagy that directly targets specific protein cargo to the lysosome.) The CASA machinery includes the chaperones HSPA8 (Hsp70) and HSPB8, the cochaperones BAG3 and STUB1 (CHIP), and the autophagic receptor p62; together, these proteins form a tension-induced autophagy pathway that is essential for the homeostasis of tissues that are exposed to marked mechanical stress (72–74). In muscle tissues, the CASA complex primarily acts on filamin C, which mediates actin–actin and actin–integrin interactions and thereby senses both internal and external mechanical forces. When CASA chaperones detect tension-induced unfolding of this mechanosensitive protein, they target it for autophagic degradation; if the CASA pathway is not fully functional, misfolded filamin C and other Z-disc proteins form protein aggregates, while the Z-disc itself loses structural integrity, leading to the disorganization of the myofibrillar apparatus that is seen in MFMs (74, 76, 77).

Interestingly, mutations in some MFM genes can also cause non-MFM disease phenotypes that overlap with other autophagic disorders discussed in this review. For example, mutations in *DES* (desmin), *MYOT* (myotilin), and *DNAJB6* can cause limb girdle muscular dystrophy with

rimmed vacuoles (71, 78), while mutations in *HSPB8* can cause Charcot–Marie–Tooth disease type 2 or a distal myopathy with rimmed vacuoles (79). In addition, mutations in *DES* can rarely cause a muscle phenotype indistinguishable from the classic AVMs that are caused by a defect in the general autophagic flux (80).

MYOPATHIES WITH DEFECTS OF AUTOPHAGY INDUCTION

When there is a failure of autophagy induction, the autophagosomes do not form (**Figure 1**) and, therefore, cannot accumulate; consequently, myopathies with defects in autophagy induction do not show typical autophagic features (an increase in the number of autophagic vacuoles on electron microscopy and the accumulation of LC3-positive sarcoplasmic structures on light microscopy), are not classified as autophagic myopathies, and may be more prevalent than currently recognized (the conclusive evidence of an autophagy induction defect requires experimental manipulation of the autophagic flux in the animal or cellular models of disease, which are not always available).

The first demonstration of diminished autophagy induction in a muscle disease was made in collagen VI knockout mice, a model of congenital muscular dystrophy (81). Muscle from these mice (as well as muscle from humans with congenital muscular dystrophy) shows an accumulation of abnormal mitochondria and a malformed sarcoplasmic reticulum in addition to chronic dystrophic features. Somewhat similar changes were seen in mice with muscle-specific deletion of the *ATG7* gene, which is required for autophagy induction: While the ATG7-deficient muscles did not show chronic myopathic features suggestive of muscular dystrophy, they did show fiber atrophy, the accumulation of abnormal mitochondria, distension of the sarcoplasmic reticulum, and an increase in the number of coarse, p62-positive sarcoplasmic aggregates upon denervation (which under normal conditions leads to autophagy induction) (82). (Targeted deletion of *ATG7* was necessary because constitutive deletion of genes that regulate autophagy induction, such as *ATG7*, is lethal in the early postnatal period due to a metabolic failure associated with the transition to an intermittent feeding schedule.) The similarities between the $ATG7^{-/-}$ and $ColVI^{-/-}$ muscles prompted an investigation of the autophagic flux in $ColVI^{-/-}$ mice; the results showed a decrease in the autophagy on-rate (i.e., an autophagy induction defect) that resulted in abnormal clearance of damaged cellular organelles (including mitochondria), with oxidative stress, fiber atrophy, and fiber apoptosis and degeneration as the end result (81). Subsequently, a partial failure of autophagy induction was also demonstrated in *mdx* mutant mice (a mouse model of Duchenne muscular dystrophy) (83), raising the possibility that autophagy induction defects play a role in the pathogenesis of a wide spectrum of muscular dystrophies. Importantly, forced autophagy induction (by starvation or a low-protein diet) led to anatomic and functional improvement in both dystrophy models (81, 83), indicating that autophagy induction defects in muscular dystrophies are biologically significant.

Alterations in autophagy induction have also been detected in centronuclear myopathies, which are a heterogeneous group of inherited muscle disorders that are characterized pathologically by an abnormal position of muscle fiber nuclei and other intracellular organelles; in spite of the profound structural abnormalities of the muscle fibers present in these disorders, muscle weakness is thought mainly to be due to inappropriate formation and maintenance of the T-tubules, sarcoplasmic reticulum, and triads (the specialized sites of excitation–contraction coupling in skeletal muscle) (84). The three genes exclusively associated with the centronuclear myopathy phenotype are involved in regulation of the phosphoinositide metabolism and intracellular vesicular transport: Myotubularin 1 (*MTM1*) is a gene encoding phosphoinositide phosphatase, which dephosphorylates phosphatidylinositol 3-phosphate [PI(3)P] and phosphatidylinositol 3,5-phosphate,

while dynamin 2 (*DYN2*) and amphiphysin 2 (*BIN1*) encode phosphoinositide-binding proteins that regulate intracellular membrane trafficking (84). Given that nucleation of the phagophore is triggered by activation of the class III PI3 kinase complex I (PI3KC3-C1) and localized production of PI(3)P (5), it seemed likely that mutations in the centronuclear myopathy–associated genes could affect autophagy regulation. Indeed, deletion of *MTMR14* (Jumpy) (which encodes an *MTM1*-related phosphoinositide phosphatase that can produce a centronuclear myopathy phenotype when combined with another mutation) (84) was shown to cause overactive autophagy in a cell culture model system (85). However, this effect was not fully replicated in mouse models of the *MTM1*-associated centronuclear myopathy: While *MTM1*-deficient muscle does show evidence of overactive autophagy in the early stages of the disease (86), in the later stages (which more closely mimic human disease), there is a defect in autophagy induction that is associated with fiber atrophy and the accumulation of abnormal mitochondria and ubiquitinated protein aggregates (86, 87). Importantly, the muscle phenotype in these mice was improved by pharmacologic inhibition of the mechanistic target of rapamycin (mTOR) kinase, which activates autophagy (87). Similar abnormalities of mitochondria and the sarcoplasmic reticulum were also seen in heterozygous mice that express the most frequent human *DYN2* mutation (88), while the inhibition of autophagy induction was demonstrated in their homozygous littermates who died in the perinatal period with defects similar to those seen in mice with a constitutive deletion of ATG genes (89). Taken together, these studies suggest that a defect in autophagy induction may play a role in the pathogenesis of many nonautophagic skeletal myopathies; however, the relevance of this pathway (and, therefore, the likelihood of clinical benefit from its therapeutic manipulation) is likely to differ among different muscle diseases and will have to be separately investigated for each condition.

MYOPATHIES WITH UNCLEAR AND/OR COMPLEX AUTOPHAGY DEFECTS

GNE Myopathy

GNE myopathy was first described in the early 1980s in Japan, where it was termed distal myopathy with rimmed vacuoles (90), and Israel, where the term quadriceps-sparing myopathy was initially used (91). In the early 2000s, it was discovered that both disorders are caused by mutations in GNE, a bifunctional enzyme that plays a key role in sialic acid synthesis (92, 93); thenceforth, this disease entity has been known as GNE myopathy. The disease presents in early adulthood with foot drop caused by weakness of the tibialis anterior muscle; this is followed by a progressive involvement of other muscle groups, including the paraspinal and respiratory musculature, but typically (although not always) excluding the quadriceps (reviewed in Reference 94). Cardiac involvement is rare. Muscle biopsies from patients with GNE myopathy show clusters of small angulated fibers with rimmed vacuoles; on electron microscopy, these vacuoles are surrounded by abundant autophagic material and contain tubulofilamentous inclusions similar to those seen in sIBM (95). Interestingly, the GNE enzyme is expressed at a lower level in skeletal muscle than in many other tissues (such as the liver, kidney, and lung); thus, it is not clear why the disease preferentially targets skeletal muscle (96).

Although the etiology of GNE myopathy has been known for almost 20 years, the pathogenesis of this disease is still relatively poorly understood. The experimental evidence suggests a causal link between glycoprotein hyposialylation (which can be demonstrated in both murine and human muscle) and autophagy impairment: prophylactic treatment of GNE model mice with sialic acid compounds leads to an increase in sialic acid levels in skeletal muscle and an almost complete rescue of the disease phenotype, with muscles from treated mice showing a decrease in the number of rimmed vacuoles, a decrease in the size of the LC3-II-positive autophagosomal compartment,

and an increase in fiber size compared with untreated control mice (97). However, clinical trials of sialic acid supplementation failed to detect similar improvements in humans (94), raising the possibility that the mouse model of GNE myopathy does not fully recapitulate human disease. Moreover, it is not clear how glycoprotein hyposialylation causes autophagy impairment. One possible explanation is that hyposialylation leads to an increase in the cellular load of misfolded proteins that, over time, overwhelms the autophagic apparatus in skeletal muscle; however, muscle from patients with GNE myopathy does not show activation of the unfolded protein response (98). Interestingly, in *GNE* mutant murine muscle, sialic acid deficiency leads to oxidative stress that can be ameliorated by oral antioxidant treatment; however, it has not yet been established whether this treatment also attenuates the autophagic abnormalities that are a characteristic feature of GNE myopathy (99). Thus, the sequence of events between sialic acid deficiency and autophagy impairment remains to be elucidated.

Sporadic Inclusion Body Myositis

sIBM is the most common skeletal myopathy in people over the age of 50; it is more common in men than women, typically presents with a weakness of the quadriceps and deep finger flexor muscles (although the paraspinal and oropharyngeal musculature can also be affected), has a slowly progressive but inexorable course leading to a loss of mobility within 10–15 years, and is currently without effective therapy (reviewed in References 100 and 101). The heart is usually not involved. On histology (**Figure 5*a–d***), sIBM shows both inflammatory features (prominent endomysial inflammatory infiltrates that consist of macrophages and CD8-positive T lymphocytes, muscle fiber necrosis and regeneration, invasion of nonnecrotic muscle fibers by inflammatory cells, and strong, diffuse upregulation of MHC-1 in muscle fibers) and features of a myodegenerative disease with impairment of selective autophagy (chronic myopathic findings reminiscent of a muscular dystrophy, rimmed vacuoles, and the presence of LC3-, p62-, and TDP-43-positive sarcoplasmic aggregates). In addition, mitochondrial abnormalities (ragged red fibers and cytochrome c oxidase–negative fibers) are often present (102), suggesting that mitophagy (selective autophagy of mitochondria) is also affected. Ultrastructurally, rimmed vacuoles in sIBM (as in other rimmed vacuole myopathies) contain protein inclusions that consist of 15–18-nm tubulofilaments and are surrounded by a rim composed of autophagic material (**Figure 5*e,f***).

The etiology of sIBM is not known, but the unique combination of inflammatory and autophagic features seen in this disease, both of which are required for histologic diagnosis (102–104), has prompted a vigorous debate about the relationship between these two pathologic processes. Although sIBM is refractory to immunosuppression, several lines of evidence suggest that inflammation is the inciting event that drives the secondary myodegenerative process (reviewed in References 105 and 106): (*a*) Muscle samples from patients with hereditary inclusion body myopathies (such as MSP1 or GNE myopathy) and from mouse models of those diseases do not show inflammation, as would be expected if inflammation was a consequence of the primary myodegenerative process; (*b*) sIBM has an earlier age of onset when it develops in the context of HIV infection (107) and is associated with a systemic inflammatory process (such as sarcoidosis, rheumatoid arthritis, and systemic lupus erythematosus) in ~15% of cases; (*c*) autoantibodies against cytosolic 5′-nucleotidase 1A are detected in 50–60% of sIBM patients, a significantly higher frequency than in healthy controls or in patients with other inflammatory conditions; and (*d*) in a significant subset of sIBM patients, highly aggressive T cells that share some features with T cell large granulocytic leukemia were detectable in the circulation and in affected skeletal muscles. Thus, sIBM is conceptually similar to the progressive form of multiple sclerosis, which (unlike relapsing–remitting

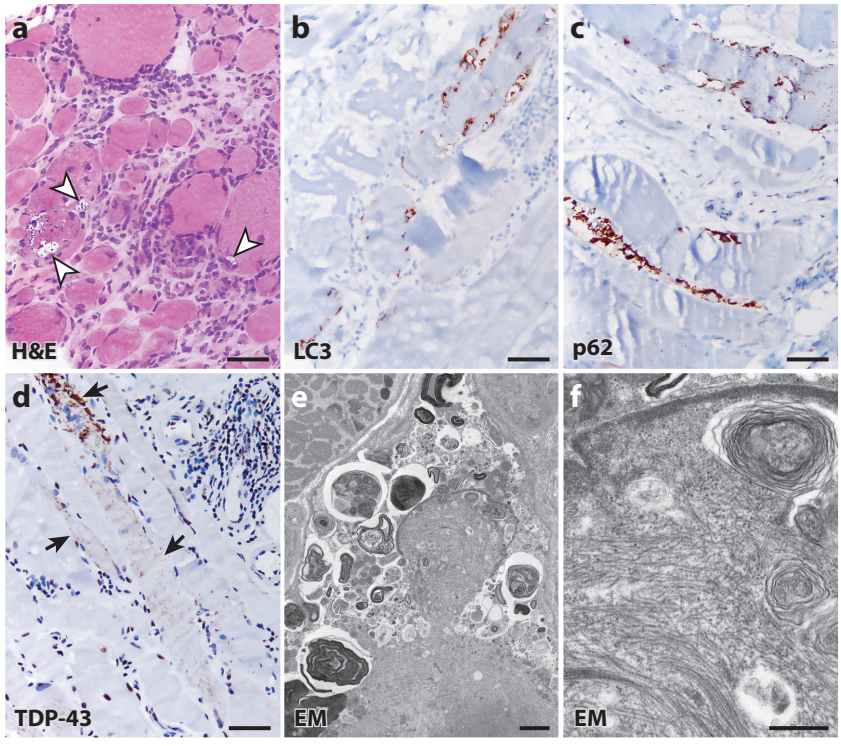

Figure 5

Sporadic inclusion body myositis. (*a*) A representative hematoxylin and eosin (H&E)–stained cryosection shows chronic myopathic features, endomysial inflammation, invasion of nonnecrotic fibers with inflammatory cells, and rimmed vacuoles (*arrowheads*). (*b*,*c*) Rimmed vacuoles and the accumulation of coarse protein aggregates are highlighted by both (*b*) LC3 and (*c*) p62 immunohistochemistry, while (*d*) TDP-43 immunohistochemistry highlights sarcoplasmic puncta and coarse aggregates in scattered fibers (*arrows*). (*e*,*f*) Electron microscopy (EM) shows rimmed vacuoles consisting of autophagic material in the rim (best seen in *e*) and an inclusion body composed of 15–18-nm tubular filaments in the center (best seen in *f*). Scale bars *a–d*: 50 μm; scale bar *e*: 2 μm; scale bar *f*: 0.5 μm.

multiple sclerosis) is resistant to immunosuppressive therapy and has a progressive course reminiscent of a neurodegenerative disease (108). Interestingly, sIBM shows a strong genetic association with multiple risk alleles within the HLA locus, further supporting an immune-mediated process as the inciting event in its pathogenesis (109); however, genetic evidence also supports a role for autophagic dysfunction in the pathogenesis of this disease. Specifically, rare missense variants in (*a*) the MSP-associated genes *VCP*, *SQSTM1*, and *HNRPA2B1* and (*b*) the MFM-associated genes *FLNC*, *ZASP*, and *BAG3* occur at a higher frequency in sIBM patients than in control populations, suggesting that they raise the risk of sIBM development (109). In addition, a recent study (110) demonstrated that missense variants in *FYCO1*—which encodes for an LC3-binding protein present in rimmed vacuoles—were detected in 11.6% of sIBM patients studied; interestingly, FYCO1 is required for kinesin-mediated centrifugal transport of autophagic membranes, a process that seems to be critical for the redistribution of phagophores (which are generated in the perinuclear region) to the sites of autophagosome formation at the cell periphery (111).

Taking everything together, a preponderance of evidence suggests that in sIBM, an inflammatory disease process of unknown etiology elicits chronic autophagic dysfunction; over time,

the resulting dysfunction of basal autophagy leads to the development of a myodegenerative, treatment-resistant clinical phenotype. Although the mechanistic link that connects chronic inflammation to autophagic dysregulation remains unclear, the overall histopathologic and genetic findings suggest that autophagic dysfunction in sIBM affects at least two distinct processes: (*a*) the FYCO1-mediated centrifugal microtubular transport of the phagophore to the sites of autophagosome formation and (*b*) VCP- and p62-dependent selective autophagy.

DOWNSTREAM MECHANISMS AND IMPLICATIONS FOR TREATMENT

While upstream mechanisms that lead to autophagic defects in skeletal muscle are fairly well understood, the downstream mechanisms—that is, the effects of those autophagic defects on skeletal muscle function and viability—remain to be fully elucidated.

In myopathies with autophagy induction defects (such as muscular dystrophies and centronuclear myopathies), the main downstream effect of the resulting autophagic impairment is the accumulation of damaged cellular organelles. Interestingly, these malfunctioning organelles do not seem to be directly detrimental to skeletal muscle health; rather, they elicit a signaling cascade that ultimately results in sarcopenia (muscle atrophy) and muscle weakness (82) (**Figure 6**). Confirming that autophagy deficiency plays an important role in the pathogenesis of these disorders, the treatments that stimulate autophagy induction (such as starvation, a low-protein diet, or pharmacologic inhibition of mTOR kinase) have proven beneficial in animal models of congenital and Duchenne muscular dystrophies (81, 83) and have showed promising results in a limited proof-of-principle clinical trial of a low-protein diet in eight adult patients with collagen VI–related congenital muscular dystrophies (112).

In contrast, a massive buildup of mature autophagosomes and undigested cargo seems to be the main downstream effect of the autophagic flux defects that cause AVMs; however, it is not entirely clear how this autophagic buildup leads to muscle weakness and muscle fiber necrosis. At least in part, muscle damage in AVMs is thought to be due to the mechanical disruption of the myofibrillar apparatus caused by an abnormal accumulation of autophagic material (113); consequently, stimulating autophagy induction in the setting of an autophagic flux defect would be expected to worsen the fiber injury by increasing the volume of accumulated autophagosomes and autophagic debris. Indeed, concomitant treatment with colchicine and an autophagy inducer (either the mTOR kinase inhibitor rapamycin or a statin) has been shown to worsen the observed muscle pathology in a mouse model of colchicine AVM (58). However, the overexpression of Vps15 and Vps34 (the regulatory and catalytic subunits of PI3KC3, the phosphoinositide kinase that is required for autophagy induction) in myoblasts derived from patients with Danon disease alleviates some aspects of this disease, such as LC3-II and glycogen accumulation (114). Intriguingly, the effect of Vps15 and Vps34 overexpression may not be due to the role of PI3KC3 in autophagy induction: *Vps15*-null muscles show a buildup of autophagosomes rather than an autophagy-deficient phenotype reminiscent of the one seen in *ATG7*-null muscles, suggesting that the main role of PI3KC3 in skeletal muscle is to regulate one or more of the later steps in the autophagic cascade (114). In agreement with this model, the overexpression of TFEB (a transcription factor that drives lysosome biogenesis) also decreases autophagic buildup and glycogen accumulation in Pompe disease muscle (115), suggesting that a late block in the autophagic flux can be attenuated by increasing the number of lysosomes that are available for autophagosome–lysosome fusion. Importantly, mechanical disruption by the accumulated autophagic debris is only one part of the AVM story: Using different strains of *GAA* knockout mice, two recent studies have conclusively demonstrated that a failure of satellite cell–mediated muscle regeneration plays a key role in the pathogenesis

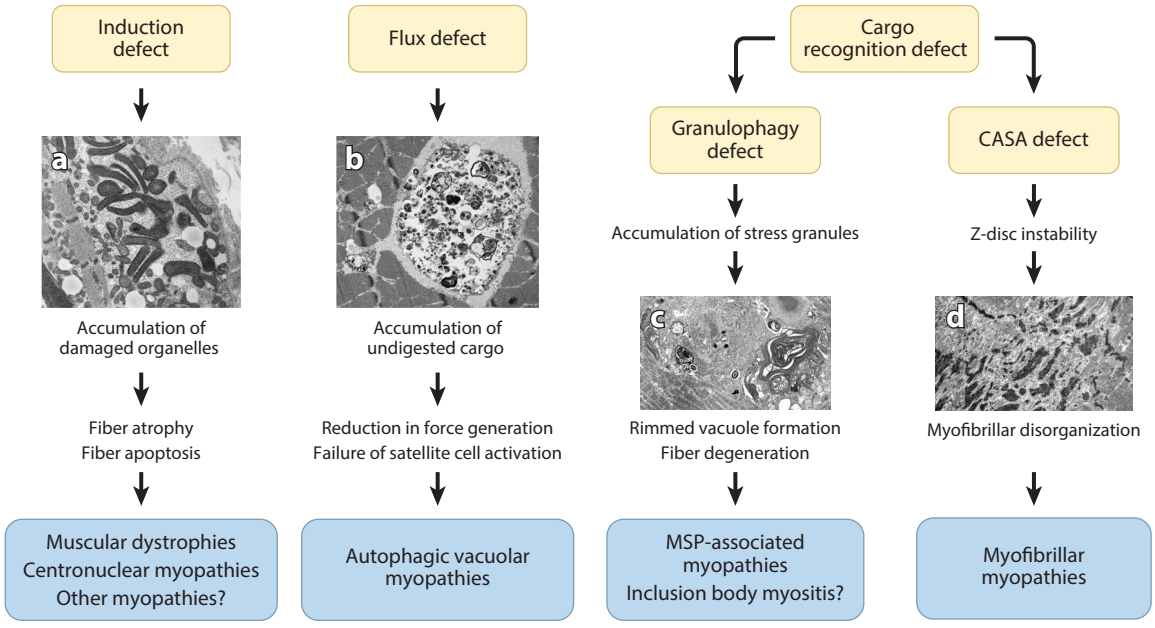

Figure 6

The role of autophagic defects in the pathogenesis of skeletal myopathy. In myopathies with autophagy induction defects (such as muscular dystrophies and centronuclear myopathies), the accumulation of damaged cellular organelles activates signaling cascades that contribute to fiber atrophy and apoptosis; however, there is no accumulation of autophagosomes. In contrast, autophagic vacuolar myopathies are caused by defects in the general autophagic flux that lead to a massive accumulation of autophagosomes and undigested cargo, ultimately resulting in a reduction of the mechanical force generated during muscle contraction (i.e., muscle weakness) and a failure of muscle regeneration. Finally, cargo recognition defects lead to two different, defect-specific disease phenotypes: A granulophagy defect results in rimmed vacuole formation and inclusion body myopathy, while a CASA defect causes myofibrillar myopathy by impairing Z-disc stability. The electron micrographs illustrate (*a*) subsarcolemmal accumulation of damaged mitochondria that show marked variation in their size and shape; (*b*) an autophagic vacuole with an accumulation of undigested, electron-dense autophagic cargo; (*c*) a rimmed vacuole with a rim of autophagic material and a center that consists of aggregated proteins; and (*d*) myofibrillar disorganization with streaming of the Z-disc material. Abbreviations: CASA, chaperone-assisted selective autophagy; MSP, multiple system proteinopathy.

of Pompe myopathy (42, 43) (**Figure 6**). Because functional autophagy is important for effective satellite cell activation (116), both groups have postulated that the failure of satellite cell activation in Pompe disease is secondary to autophagic defects that underlie this disorder; however, this hypothesis has yet to be experimentally tested. In addition, it remains to be seen whether similar satellite cell failure occurs with other types of autophagic dysfunction or whether it is specific to Pompe disease and other AVMs.

In myopathies caused by defects of selective autophagy (the MFMs, MSPs, and possibly sIBM), the downstream mechanisms seem to reflect cargo that is not appropriately degraded; consequently, the ultimate disease phenotypes tend to be similar regardless of whether the disease-causing mutations affect the autophagic machinery or the cargo itself. For example, the MFM phenotype can be caused by mutations either in the Z-disc structural proteins or in the chaperones that are responsible for the degradation of these proteins through the CASA pathway (71); under conditions of chronic mechanical stress, a problem in either element ultimately leads to Z-disc unraveling, myofibrillar disorganization, and muscle weakness (76, 77) (**Figure 6**). Similarly, the MSP phenotype can be caused by mutations either in the RNA-binding proteins that

form stress granules or in the components of the machinery that are required for clearance of these granules (62); ultimately, a defect in either component results in an excess of stress granule proteins, which contain prion-like low-complexity domains that are essential for their normal function (liquid–liquid phase separation is required for stress granule formation) and also make them prone to misfolding and aggregation (117) (**Figure 6**). Therefore, one would expect that forced autophagy induction would be beneficial in both MFMs and MSPs; however, studies in animal models in which such manipulations were attempted have shown inconsistent results (76, 77, 118–120). These inconsistencies may reflect the challenges inherent in attempts to enhance a specific subtype of selective autophagy without affecting the rest of the autophagic cascade; as such, therapies aimed at direct cargo modulation may ultimately prove more fruitful in treating disorders of selective autophagy. Indeed, treatment with arimoclomol (CytRx; an inducer of the heat shock response) improved muscle strength and IBM-like pathology in the mouse model of MSP1 and has shown promise in a small, proof-of-principle clinical trial of sIBM patients (121). Similarly, treatment with geranylgeranylacetone (another activator of the heat shock response) led to improved cardiac function in a heart-specific mouse model of αB-crystallin-associated MFM (122).

In summary, autophagy defects play major roles in the pathogenesis of a diverse group of skeletal myopathies; however, the downstream consequences of autophagy impairment—and, therefore, the benefits of any autophagy-modifying therapies—depend on the specific mechanism of autophagic dysfunction that underlies each disease. As a result, optimal treatment strategies need to be based on a detailed understanding of individual disease mechanisms and will have to be developed and evaluated on a disease-by-disease basis.

SUMMARY POINTS

1. Autophagy is an evolutionarily conserved catabolic process by which various types of cytoplasmic cargo are targeted for lysosomal degradation; under basal conditions, the cargo includes damaged cellular organelles and protein aggregates (selective autophagy), while under stress conditions, such as starvation, the cargo consists of entire cytosolic droplets that are sent to the lysosome for nutrient recycling (bulk autophagy).
2. Skeletal muscle is a postmitotic tissue that has high metabolic activity and little cellular turnover, but it is constantly exposed to mechanical stress; as such, it is highly dependent on autophagy for its homeostasis and often shows abnormalities in the setting of defective autophagy.
3. Autophagic vacuolar myopathies (which can have either a genetic or toxic etiology) are caused by defects in the late stages of autophagy that produce a block in overall autophagic flux, causing a buildup of mature autophagosomes and undigested cargo.
4. Multiple system proteinopathies—age-dependent, multisystem degenerative disorders that often involve skeletal muscle, but can also affect the brain, spinal cord, peripheral nerves, and bone—are due to the abnormal metabolism of stress granules and can be caused by genetic defects in granulophagy (selective autophagy of stress granules).
5. Myofibrillar myopathies are due to dysfunction of chaperone-assisted selective autophagy, which is required for maintenance of the myofibrillar apparatus under conditions of mechanical stress.

6. Defects of autophagy induction (which lead to an accumulation of damaged cellular organelles and muscle fiber atrophy, but lack the autophagosomal buildup typical of classic autophagic myopathies) have thus far been implicated in the pathogenesis of muscular dystrophies and centronuclear myopathies, but may be more common than is currently recognized.
7. Autophagic defects in sporadic inclusion body myositis (the most common skeletal myopathy occurring after age 50) are complex and likely multifactorial, but they seem to be triggered by an inciting inflammatory process of an unknown etiology.
8. Given the wide spectrum of autophagic defects that are observed in different skeletal myopathies, the benefits of autophagy-modifying therapies will vary and need to be evaluated on a disease-by-disease basis.

DISCLOSURE STATEMENT

The author is a consultant for Audentes Therapeutics.

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