Review

The role of protein aggregation in the pathogenesis of inclusion body myositis

A.M. Snedden[1,2], K.A.B. Kellett[3], J.B. Lilleker[1,2], N.M. Hooper[3,4], H. Chinoy[1,5,6]

[1]Centre for Musculoskeletal Research, Faculty of Biology, Medicine and Health, The University of Manchester, UK;
[2]Manchester Centre for Clinical Neurosciences, Salford Royal Hospital, Northern Care Alliance NHS Foundation Trust, Manchester Academic Health Science Centre, Salford, UK;
[3]Division of Neuro-science and Experimental Psychology, School of Biological Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, UK;
[4]Geoffrey Jefferson Brain Research Centre, Manchester Academic Health Science Centre, Northern Care Alliance and University of Manchester, UK;
[5]National Institute for Health Research Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, The University of Manchester, UK;
[6]Department of Rheumatology, Salford Royal Hospital, Northern Care Alliance NHS Foundation Trust, Manchester Academic Health Science Centre, Salford, UK.

Andrew M. Snedden, MRCP, MSc
Katherine A.B. Kellett, PhD
James B. Lilleker, MRCP, PhD
Nigel M. Hooper, PhD
H.ector Chinoy, FRCP, PhD

Please address correspondence to:
Andrew Snedden,
Centre for Musculoskeletal Research, Faculty of Biology, Medicine and Health, The University of Manchester,
2nd floor, Stopford Building,
Oxford Road,
M13 9PT Manchester, United Kingdom.
E-mail: andrew.snedden@manchester.ac.uk

Received on January 4, 2022; accepted in revised form on January 31, 2022.

Clin Exp Rheumatol 2022; 40: 414-424.

Key words: inclusion body myositis, pathological protein aggregation, endoplasmic reticulum stress, neurodegenerative diseases, TDP-43 proteinopathies, biomarkers

Funding and competing interests: page 421.

ABSTRACT

Inclusion body myositis (IBM) is characterised by infiltration of CD8⁺ T-cells and signs of protein aggregation such as rimmed vacuoles and inclusion bodies. Aggregated proteins include those present in neurodegenerative diseases, and also those involved in protein homeostasis. The aim of this review is to discuss the pathological effects of protein aggregates and the process of aggregation following immune attack in IBM.

Immune attack is likely to cause protein aggregation by impairing endoplasmic reticulum (ER) and mitochondrial function. Apoptotic and necrotic pathways are activated, possibly leading to nucleo-cytoplasmic coagulation. Overexpression of nuclear and ribosomal proteins in rimmed vacuoles suggests that the vacuoles develop from the collapse of myonuclei and the surrounding ER.

Aggregated proteins can activate the NLR family pyrin domain containing 3 (NLRP3) inflammasome or provoke a humoral immune response. Heat shock proteins, ribosomal proteins and protein fragments may provoke interferon-gamma and cytotoxic T-cell responses in a similar manner to Mycobacterium tuberculosis antigens. Persistent provocation can lead to T-cell large granular lymphocytic leukaemia which is resistant to immunosuppression, and would explain the progression from polymyositis to IBM. Protein aggregates may impair the cellular machinery, and proteins may propagate along a myocyte in a prion-like manner. These pathological mechanisms may prevent myocyte regeneration following damage from eccentric muscle contraction, causing weakness and atrophy in a characteristic pattern.

Further understanding of the mechanisms of protein aggregation in IBM may lead to additional therapies as well as novel muscle and blood biomarkers. Earlier diagnosis and treatment may result in improved outcomes when effective therapies are available.

Introduction

Inclusion body myositis (IBM) is an idiopathic inflammatory myopathy (IIM) characterised by the infiltration of mainly cytotoxic CD8⁺ T-cells into the endomysium of myocytes, which does not improve clinically with immunosuppressive or non-immunosuppressive therapies. Myopathic features are also found, such as rimmed vacuoles, inclusion bodies and ragged red fibres (1) which reflect protein aggregation and mitochondrial dysfunction. Many proteins have been detected in IBM aggregates that are key proteins in the pathogenesis of neurodegenerative diseases, such as beta amyloid (Aβ) (2), microtubule-associated protein tau (tau) (3), α-synuclein (4), TAR-DNA binding protein-43 (TDP-43) (5) and the prion protein (6). Some authors therefore reasoned that IBM was unresponsive to immunosuppression as it was a predominantly myodegenerative disease with protein aggregation provoking a secondary immune response (7, 8). Others reasoned that the tendency of polymyositis to progress to IBM, as well as associations with a major histocompatibility complex (MHC) haplotype (9), suggested that IBM is primarily an inflammatory myositis causing impaired protein homeostasis (1, 10).

Recent work has shown that highly differentiated cytotoxic CD8⁺ T-cells expressing killer cell lectin-like receptor subfamily G1 (KLRG1) aggressively invade IBM muscle, with upregulation of perforins, granzymes and cytokines, including interferon-gamma (IFN-γ) (11). These T-cells also had a similar pattern of cell surface markers and cytotoxicity to that seen in T-cell large granular lymphocytic (T-LGL) leukae-

mia, which was previously shown to be associated with IBM (12). T-LGL leukaemia is a clonal expansion that is triggered by chronic antigenic stimulation, and treatment with immunotherapies have so far had a limited effect (13). This finding helps explain the treatment-resistant nature of IBM, and a monoclonal antibody that specifically targets these KLRG1$^+$ T-cells is in clinical trials (14). Whilst development of a chronic leukaemia is likely to be a key factor in IBM pathology, it is unclear how this T-cell attack leads to protein aggregation, and the role that protein aggregates have in myocyte dysfunction and inflammation. By understanding these mechanisms, we may therefore determine which proteins have a pathological role or can be used as biomarkers, thus improving the diagnosis and treatment of IBM.

In this review we will discuss possible mechanisms that lead to protein aggregation following T-lymphocyte cytotoxicity, and discuss the role of these proteins in triggering further immune attack and dysfunction of the myocyte. We also discuss how identification and characterisation of these proteins may assist diagnosis of IBM from muscle and blood samples.

Immune attack and possible mechanisms of protein aggregation in IBM

IBM muscle is infiltrated by CD8$^+$ T-cells, but cytotoxic CD4$^+$ CD28^{null} T-cells, dendritic cells and plasma cells can also be found (1). The only known antigen for this immune response is Cytosolic 5'-nucleotidase 1A (cN1A) protein, with antibodies against this protein found in 33–76% of patients with IBM (15, 16). Antigen presentation to T-cells results from cleavage of proteins by the proteasome, immunoproteasome (17) (Fig. 1) and/or other proteases into peptides, which are then loaded onto MHC Class I and/or MHC Class II for transportation to the cell membrane (18, 19). If the antigen is recognised, then T-cell attack may occur concurrently in three ways:

1. The cell releases cytokines such as IFN-γ (20) and tumour necrosis factor-alpha (TNF-α) (21), resulting in responses including upregulation of MHC Class I and II (22), inducible nitric oxide synthase induction (23) as well as initiation of apoptosis (24) and necroptosis (see "Cell death and protein aggregation") (25).
2. Cytotoxic T-cells release perforins, which transiently form a pore in the sarcolemma (Fig. 2) (26, 27). This allows an influx of Ca^{2+} (28) and granzymes into the myocyte, and granzymes A, B, K and H are known to be upregulated in IBM (11). Granzymes are serine proteases that can initiate apoptosis through either the caspase cascade or caspase independent pathways, leading to nicking of DNA, generation of reactive oxygen species, mitochondrial dysfunction, cytochrome C release from mitochondria and apoptosis (29).
3. The CD95L /Fas ligand (FasL) binds to the CD95/Fas receptor on the target cell surface, which activates the caspase cascade leading to apoptosis (30). It can also lead to necroptosis in a caspase independent fashion (25). Fas is strongly expressed in atrophic IBM fibres (31).

The precise mechanism by which prolonged immune attack leads to protein aggregation in IBM is not clearly described but may be due to several factors. Prolonged rupture of the sarcolemma leads to Ca^{2+} influx, electrolyte and reduction/oxidation (redox) potential changes in the cytosol (29), which might then impair endoplasmic reticulum (ER) function and mitochondrial electron transport chain function. Dysregulated redox status, reactive oxygen species (ROS) as well as activation of Fas, TNF-Receptors (TNF-R), Toll-like receptors 3 and 4 (TLR3/TLR4) can then lead to apoptosis, necroptosis and pyroptosis (25). Protein aggregation may ensue in the following ways:

ER dysfunction and protein aggregation

Changes in the redox potential of the ER affects oxidative folding of proteins, leading to exposure of their hydrophobic residues which are aggregate-prone and thus toxic to the cell (32). In normal cell function, proteins are accurately folded and modified inside the ER following translation by ribosomes (33). Misfolding of proteins alters ER protein homeostasis causing ER stress, which is detected by signalling peptides and leads to ER-associated degradation (ERAD). This involves translocation of proteins to the cytoplasm for degradation by the proteasome and activation of the unfolded protein response (UPR), with upregulation of ER chaperones and folding proteins (34). ER stress has been induced in yeast cells using specific inhibitors or mutations impairing ERAD and UPR, leading to widespread aggregation of hydrophobic and aggregate-prone proteins (35).

A similar process generating aggregate-prone proteins may therefore occur following ER stress in IBM muscle. In contrast to yeast cells, the ER in myocytes appears to be continuous with the sarcoplasmic reticulum (SR) (36), but there is evidence of compartmentalisation of regions and protein transport (37, 38). Should the compartmentalisation be disturbed by T-cell attack then presumably the influx of calcium would also affect oxidative folding and generate hydrophobic and aggregate-prone proteins (Fig. 2, Point 2). SR and ER disruption may also impair transport of proteins from ribosomes to the export sites (37), thus causing accumulation of protein in the ER.

Mitochondrial dysfunction and protein aggregation

Mitochondrial electron transport chain dysfunction due to changes in cell redox potential can lead to protein aggregation in cell culture experiments. The addition of mitochondrially-targeted redox agents or an inner membrane uncoupling agent to breast cancer cell cultures led to formation of aggregates >450kDa containing tumour protein P53, and particles 1–5μm across (39). The aggregates were sequestered in the autophagosome and resistant to lysosomal degradation. Similarly, in yeast cell cultures with mutations that impaired mitochondrial protein translation, protein homeostasis was impaired and multiple cytoplasmic protein aggregates containing heat shock protein 104 were formed (40).

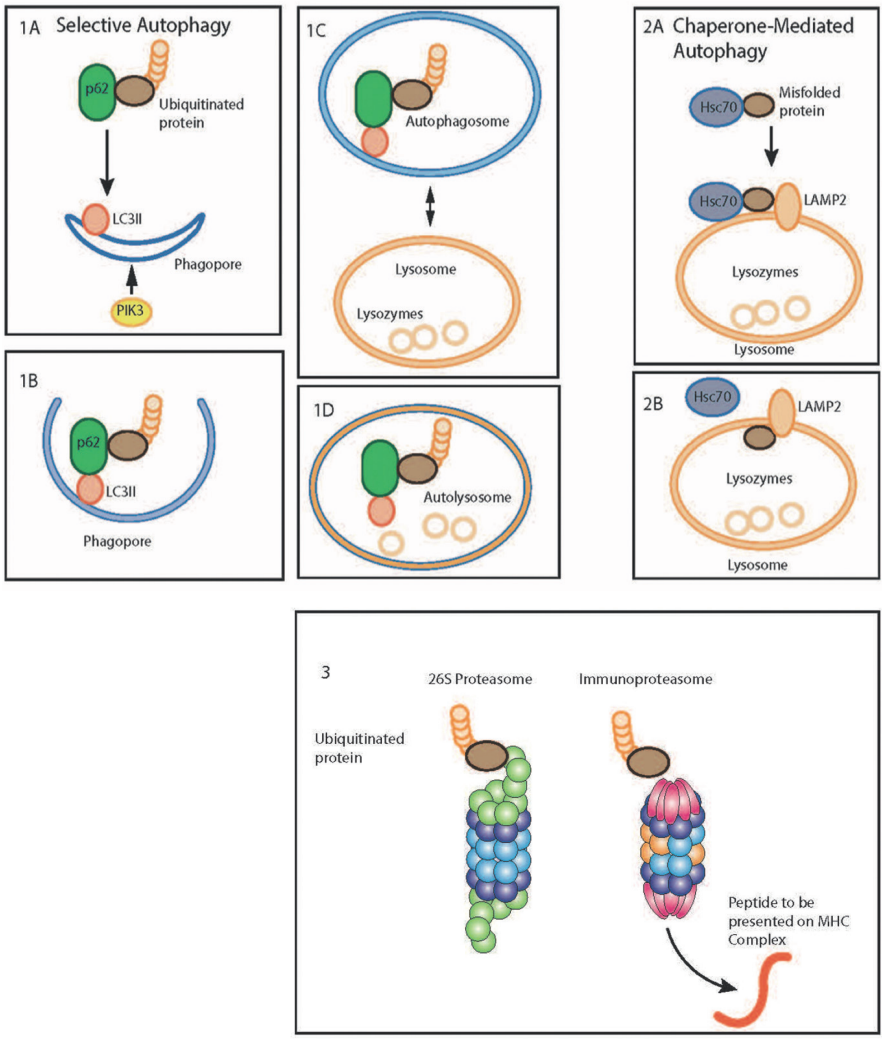

Fig. 1. Autophagy and the ubiquitin-proteasome system.
Proteins are cleared from the cell by autophagy or the ubiquitin-proteasome system (UPS). In selective autophagy (51), inhibition of the mechanistic Target of Rapamycin (mTOR) pathway leads to activation of the phosphatidylinositol 3-kinase (PI3K) complex which assists with membrane nucleation and phagopore formation (1A). The adaptor protein sequestosome-1/p62 binds to ubiquitinated proteins and then binds to LC3 on the phagopore surface. The phagopore membrane gradually expands to envelop the cargo proteins (1B) until it does so completely to form the autophagosome (1C). This fuses with a lysosome to form an autolysosome (1D), where proteins are degraded by lysozymes. In chaperone-mediated autophagy, Heat shock cognate 71kDa protein (Hsc70) binds to misfolded proteins, then binds to LAMP2 on the lysosome surface (2A). The protein translocates across and is degraded (2B). Whilst LAMP2, p62 and LC3 and can used as biomarkers (52) for IBM, p62 appears to aggregate in other myopathic conditions and so is not specific for IBM (53). In the Ubiquitin-Proteasome system (17) ubiquitinated proteins can be degraded by the 26S proteasome or immunoproteasome. The immunoproteasome is expressed in response to cytokines such as IFN-γ, has different subunits and cleavage sites compared to the 26S Proteasome, and generates peptides which can be presented by MHC Class I. The immunoproteasome is upregulated in IBM, with *PSMB8* (encoding the β5i subunit) in particular being highly expressed in IBM muscle (Table II) (54). The β5i subunit has also been found in IBM muscle on immunostaining, and one group found its enzymatic activity was increased in IBM muscle (55), although another group found it to be reduced (56). If the activity is increased then immunoproteasome-cleaved protein fragments might have a role as IBM biomarkers.

Cell death and protein aggregation

Whilst granzyme cleavage activates caspases and induces apoptosis (41), activation of Fas, TNFR and other death receptors leads to the formation of the ripoptosome (42), with the subunits determining whether cell survival, apoptosis or necroptosis occurs. Apoptosis is a caspase-dependent process where the cell structure is demolished, and the cell is phagocytosed before the cell contents are released (43). Necrosis is uncontrolled cell death leading to rupture of the cell and release of pro-inflammatory molecules, and when activated by the ripoptosome it is called necroptosis (44). A small study showed that partial infiltration of IBM muscle fibres by $CD8^+$ T-cells led to apoptosis, with no apoptosis in the fully invaded fibres, and very little apoptosis in dermatomyositis muscle fibres (45). If apoptosis is upregulated in IBM, this may lead to an increase in truncated BH3-interacting domain death agonist (tBID) and other mediators of apoptosis in the muscle and blood of these patients, which could serve as biomarkers.

Pyroptosis, a caspase medicated form of necrosis, occurs in polymyositis and dermatomyositis following activation of the NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome promoted by glycolysis (46). The NLRP3 inflammasome is also activated in Valosin-containing protein (VCP) myopathy (47), a genetic myopathy which has pathological features similar to IBM (48). No studies have investigated its role in IBM, but given the similarities between IBM, PM and VCP myopathy it is possible that NLRP3 activation and pyroptosis also occur in IBM.

Necrotic cells are frequently seen in IBM and they may occur due to necroptosis, pyroptosis, or secondary necrosis if apoptotic cells are not cleared by macrophages. In cell cultures secondary necrosis following apoptosis led to aggregation of proteins by a mechanism known as nucleo-cytoplasmic coagulation (NCC), where disulfide bridges formed between proteins (49). The authors argued that this is a protective mechanism to prevent exposure of epitopes to the immune system and mitigate autoimmunity, although some immunogenic proteins were able to escape this. As NCC tended to occur in similar types of proteins to those found to aggregate in IBM (*e.g.* GTPases, heat shock proteins (Hsps), and nuclear proteins (50)) then it is possible this type of protein aggregation also occurs in IBM muscle.

Which proteins aggregate in IBM?

Proteins involved in neurodegenerative diseases

Early studies of IBM found cytoplasmic microtubular filaments on electron

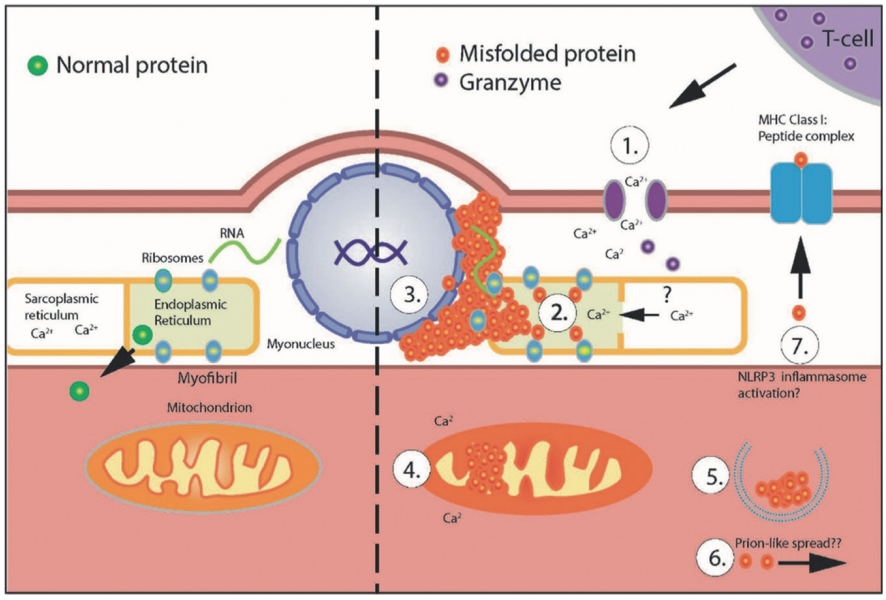

Fig. 2. Possible pathogenic roles of protein aggregation in IBM muscle.
1. T-cells release perforins and granzymes, as well as inflammatory cytokines. They also express death receptor ligands such as FasL (see "Immune attack and mechanisms of protein aggregation in IBM") **2.** Redox changes in the cytosol and disruption of the SR/ER lead to ER stress, protein misfolding and failure of protein clearance from the ER. This leads to further protein aggregation. **3.** Nuclear and ER proteins aggregate around the nucleus, impairing nuclear function. RVs may be formed after nuclear and ER collapse. **4.** Cytosolic aggregation of protein and changes in the redox potential in the cell may disrupt mitochondrial function **5.** Proteins are not cleared due to failure of autophagy and the UPS-proteasome system. **6**. The prion-like spread of proteins in neurodegenerative diseases raises the possibility of localised prion-like propagation in muscle. **7.** Presentation of peptides by MHC Class I is likely to trigger further immune attack. Aggregated proteins may also activate the NLRP3 inflammasome.

microscopy (EM) (57), which are still included in current diagnostic criteria (58). Vacuolated fibres were then stained with Congo Red dye, which showed amyloid deposits with either wispy or plaque-like morphology (59). Askanas *et al*. found that in IBM muscle amorphous deposits were stained with antibodies targeting Aβ in sporadic IBM (2). Other studies have had mixed results, with some studies confirming the presence of Amyloid Precursor Protein (APP) and Aβ (a toxic fragment following cleavage of APP by β- and γ-secretase) on immunostaining (60, 61), and other studies finding no evidence of Aβ aggregation (62, 63). Our group has used [18F] Florbetapir, a positron emission tomography (PET) tracer targeting Aβ, and increased uptake was seen in IBM patient muscles compared to controls (64). Although in pre-clinical studies [18F] Florbetapir bound avidly to Aβ plaques in post-mortem brain tissue (65), it is possible that there is some non-specific binding in muscle. It is plausible that APP and Aβ may be found in protein aggregates in IBM, but there is no definite evidence that they have a major role in IBM pathology.

Further studies specifically sought other proteins involved in neurodegenerative diseases, such as tau (66), prion protein (67), α-synuclein (68), TDP-43 (69) as well as proteins involved in maintaining protein homeostasis and markers of oxidative stress (7). These have mostly been detected with immunohistochemistry, Western blotting and in some cases EM immunohistochemistry, and detection of proteins depends on the antibodies used. Proteomic studies have provided a more systematic assessment of the proteome in IBM muscle.

Mass spectrometry studies of the proteome in IBM

One of the first attempts to identify proteins upregulated in IBM by mass spectrometry (MS) was performed by Li *et al*. where they found upregulation of 16 proteins including APP, transthyretin [accumulates in tissues in amyloidosis (70)], superoxide dismutase 1 [mutations in this protein can cause amyotrophic lateral sclerosis (ALS) (71)] and various Hsps. Another study using MS and microarray data found a reduction in fast twitch sarcomeric and glycolytic enzyme proteins and mRNA compared to IIM and normal controls (72), which is in keeping with the Type II muscle fibre atrophy seen in IBM (73). In contrast, Myosin heavy chains 3 and 8 (MYH3 and MYH8) protein and mRNA were upregulated in IBM, and these isoforms are associated with muscle regeneration (74, 75).

Laser microdissection has also been used to assess different features in IBM muscle. One study performed tandem mass spectrometry on myofibres surrounded by inflammatory cells which had been cut out by laser dissection, and compared to normal controls (76). This showed 337 proteins which were exclusively found in IBM muscle. The biopsies were immunostained with antibodies for extracellular matrix (ECM) and basement membrane proteins. The ECM protein biglycan was deposited on myofibres that were surrounded or invaded by inflammatory cells. Biglycan is known to be pro-inflammatory (77) and so may provoke further immune attack.

Another study used laser microdissection to cut out rimmed vacuoles and cytoplasmic controls from the same biopsy, and the relative abundance of proteins that were detected with mass spectrometry from each group were compared (50). This resulted in a list of 243 proteins that were expressed 1.5 times more in rimmed vacuoles than cytoplasm. FYVE and coiled-coil domain-containing protein 1 (FYCO1) and p62 (Table I) were the most overexpressed. TDP-43, a heterogeneous nuclear ribonucleoprotein (hnRNP) which aggregates in ALS, was also markedly overexpressed, as was 60S ribosomal protein L8 and heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1) (50). These findings are consistent with other studies (5, 78). The high abundance of nuclear and ribosomal proteins suggests that rimmed vacuoles are from collapsed nuclei and surrounding ER.

In the list of proteins overexpressed in RVs in this study, there are notable

Table I. Relative abundance of proteins in RVs compared to cytoplasm on laser dissection and mass spectrometric analysis of IBM muscle.

Protein	Ratio of abundance in RVs: Cytoplasm	Protein	Ratio of abundance in RVs: Cytoplasm
FYVE and coiled-coil domain-containing protein 1 (FYCO1)	**n/a**	TAR DNA-binding protein 43 (TDP-43)	**47.23**
sequestosome-1/ p62	**n/a**	60S ribosomal protein L8	**43.78**
acid ceramidase	**n/a**	myosin-14	**30.89**
syncoilin	**n/a**	moesin	**30.77**
transaldolase	**n/a**	clathrin heavy chain 1	**29.06**
collagen alpha-1(XV) chain	**n/a**	spectrin beta chain, erythrocytic	**20.84**
heterogeneous nuclear ribonucleoprotein U	**n/a**	plastin-2	**19.31**
peroxisomal multifunctional enzyme type 2	**n/a**	40S ribosomal protein S3a	**19.14**
LIM domain only protein 7	**n/a**	muscle-related coiled-coil protein	**18.72**
lysosome membrane protein 2	**n/a**	heterogeneous nuclear ribonucleoproteins A2/B1	**17.05**
macrophage-capping protein	**n/a**	fibrinogen gamma chain	**15.6**
myosin-7B	**n/a**	fibulin-5	**15.49**
protein cordon-bleu	**n/a**	protein disulfide-isomerase A6	**14.79**
protein ATP1B4	**n/a**	transforming growth factor-beta-induced protein ig-h3	**14.42**
ezrin	**n/a**	60S ribosomal protein L17	**13.23**
ferritin light chain	**n/a**	T-complex protein 1 subunit alpha (TCP-1-alpha)	**12.87**
contactin-associated protein-like 4	**n/a**	xin actin-binding repeat-containing protein 2 (XIRP-2)	**12.82**

Abundance of proteins in RVs compared to normal cytoplasm in IBM muscle expressed as a ratio. **n/a** - denotes a protein that was detected in RVs but not in the cytoplasm. Less abundant proteins detected in RVs that were not detected in cytoplasm are omitted from this Table. Adapted from supplementary data from (50) Guttsches *et al*.: Proteomics of rimmed vacuoles define new risk allele in inclusion body myositis. *Ann Neurol* 2017; 81(2): 227-239. Reprinted with permission.

absences of APP, tau, α-synuclein and the prion protein (50). This may be because these proteins do not have a major role in IBM, or may be because they aggregate in the cytoplasm rather than the ER and nucleus, and so do not accumulate in RVs. It is possible they might be found in other protein aggregates in IBM muscle, such as cytoplasmic inclusions which stain with Congo Red and occasionally Aβ stains, and "squiggly inclusions" which stain with anti-tau antibodies (79).

Possible pathogenic roles of proteins which aggregate in IBM

We have discussed how immune attack can lead to impaired cellular and protein homeostasis and protein aggregation, but the opposite is also likely to occur. Here we discuss how protein aggregation may impair cellular function and provoke further immune attack.

Antigens for immune response

Aggregates of proteins in IBM muscle could trigger an immune response mediated by the NLRP3 inflammasome. Activation of the NLRP3 inflammasome occurs in two steps (85). The inflammasome is first primed by Interleukin1 (IL-1) Receptors, TNFRs or TLRs following stimulation by IL-1β, TNF-α or pathogen associated molecular patterns (PAMPs) such as double stranded RNA. NLRP3 activation can then be triggered by diverse stimuli including hypercalcaemia, ROS, particulate matter, bacterial and viral proteins and toxins, which then leads to pyroptosis (see "Cell death and protein aggregation") as well as IL-1β and Interleukin-18 (IL-18) release.

Activation of the NLRP3 inflammasome by TDP-43, a-synuclein, phosphorylated tau and paired helical filaments has been demonstrated *in vitro* and in neurodegenerative disease models (86-88), therefore their aggregation in IBM muscle may also activate the NLRP3 inflammasome. Similarly, biglycan (discussed in "Mass spectrometry studies"), aggregates on the myocyte membranes in IBM (76) and is known to activate macrophages (77) and the NLRP3 inflammasome (89). It is possible that cN1A, which dephosphorylates DNA, may mimic a viral protein, thus activating TLRs and the NLRP3 inflammasome in IBM muscle as well as provoking a humoral immune response. Other possible protein antigens in IBM which could cause a humoral immune response and activate the NLRP3 inflammasome include histone H4. This protein binds to DNA and is prominently expressed in IBM RVs (50). Antibodies specific to a histone H3:H4 tetramer are present in hydralazine-induced lupus (90), but so far these antibodies have not been reported in IBM patients. Desmin aggregates are prominent in RVs (50) and may also provoke a humoral immune response, as antibodies derived from a single plasma cell from an IBM patient have been found to be specific to desmin (91). The significance of this is unclear as only a single plasma cell was found, and anti-desmin antibodies in IBM patient blood have not been reported either.

As well as mimicking viral proteins, partially degraded proteins may mimic intracellular bacterial pathogens, leading to a further cytotoxic T-cell response. In *Mycobacterium tuberculosis* (*M. tuberculosis*) infection, the bacteria survives in phagosomes after being phagocytosed by macrophages, before escaping to the cytoplasm (92). If *M. tuberculosis* protein antigens are presented to $CD4^+$ T-cells then this leads to a release of IFN-γ and a cytotoxic T-cell response (93). In IBM muscle, misfolded and aggregated proteins may be only partially degraded by defec-

tive autophagy or proteasome degradation, and released back into the cytosol. These misfolded proteins and their fragments can then be presented to circulating macrophages and lymphocytes, and if they mimic a *M. tuberculosis* protein then this would provoke IFN-γ release and a cytotoxic T-cell response. Proteins that aggregate in IBM and might mimic intracellular bacterial antigens include Hsps (94) and ribosomal proteins (50), as DnaK (a bacterial member the Hsp90 family) (84) and 50S ribosomal protein L7/L12 are found in all purified protein derivatives used in tuberculin skin tests that diagnose *M. tuberculosis* infection (95). Protein fragments may also mimic 6kDa early secretory antigen target (ESAT-6), a small protein used to stimulate lymphocytes that have been exposed to *M. tuberculosis* in IFN-γ release assays (96). If such proteins provided mild but chronic antigenic stimulation to circulating lymphocytes then this could lead to the development and infiltration of $KLRG1^+$ T-cells with a T-LGL leukaemia phenotype as described by Greenberg *et al*. (11, 12). This could be a key step in the progression of polymyositis, responsive to immunosuppression, to treatment-resistant IBM.

Impaired nuclear function and protein translation

If protein clearance by autophagy and the UPS system is insufficient, the cell can sequester ubiquitinated proteins by transporting them along microtubules to the perinuclear microtubule organising centre (MTOC) to form the aggresome (97). Whilst the aggresome is thought to be protective (98), an *in vitro* study in cultured cells found that long-term aggresome accumulation caused abnormal nuclear morphology, double stranded DNA breaks, cell cycle arrest and disruption of mitotic spindle apparatus (99). In myocytes abnormal aggresome formation has been described in genetic myopathies including reducing body diseases (100) as well as in IBM (56), but both occur at cytoplasmic locations and are probably inclusion bodies. However myonuclei are surrounded by ribosomes and ER (36), and so perinuclear protein aggregation from ER dysfunction may still cause similar nuclear disruption in IBM myocytes and satellite cells (Fig. 2, Point 3). TDP-43 is a nuclear protein with functions including regulation of transcription, alternative splicing and mRNA stabilisation, as well as shuttling mRNA into the cytoplasm (101). TDP-43 is also required for regeneration of skeletal muscle *in vivo* (102), therefore any loss of normal TDP-43 function in IBM due to its aggregation may lead to impaired nuclear function and impaired regeneration of damaged myocytes. As well as aggregating in IBM (5), TDP-43 also aggregates in neurodegenerative diseases including some forms of Frontotemporal dementia (FTD), ALS (103, 104) and Limbic-predominant age-related TDP-43 encephalopathy (LATE) (105) by initially forming stress granules (106, 107). In comparison, cytoplasmic *myogranules* of TDP-43 that were bound to sarcomeric protein mRNA were found during regeneration of chemically injured skeletal muscle, and localised back to the nuclei several days after the injury (102). Myogranules from cultured myocytes did not express markers of stress granules found in ALS and FTD (106, 107) and were considered distinct entities. However, they did form urea-insoluble TDP-43 fibrils in regenerating myocytes in *VCP* mutant mice, and when isolated myogranules were incubated together they formed aggregates (102). Therefore, any impairment of protein clearance could allow myogranules containing TDP-43 to aggregate in a myocyte, impairing mRNA processing and protein translation.

Cytoplasmic TDP-43 aggregates in muscle biopsies are considered to be a more reliable biomarker for IBM (108), and phosphorylated TDP-43 is also more abundant in IBM muscle (109). Phosphorylation of TDP-43 (110) (as well tau (111) and Aβ (112)) increases its propensity to aggregate, and so phosphorylated TDP-43 has been assessed as a cerebro-spinal fluid and blood biomarker of ALS (113). Therefore, phosphorylated and unphosphorylated TDP-43 may also have a role as a blood biomarker in IBM.

The interferon induced guanylate-binding protein 2 (GBP2) protein was five times more abundant in RVs than healthy cytoplasm in IBM muscle (50), and is translated from *GBP2*, the most upregulated mRNA in IBM muscle compared to normal controls (54) (Table II). It has multiple functions including defence against viral and intracellular pathogens, including *M. tuberculosiss* (80). In the case of RNA virus infection, viral replication was inhibited *in vitro* by LC3 targeting GBPs (particularly guanylate-binding protein 1 (GBP1) and GBP2) to form oligomers on the cytoplasmic viral replication complex membrane following IFN-γ stimulation (114). It is possible that the damaged myonuclei or satellite cell nuclei in IBM may also trigger this anti-viral response, thus inhibiting nuclear function. Alternatively, GBP2 has a role in quality control of mRNA splicing (81), and this may be upregulated if mRNA splicing is impaired in the myonucleus due to protein aggregation.

Prion-like propagation of protein aggregates

In ALS and FTD there is evidence for a prion-like spread of TDP-43 as it appears to propagate to different parts of the central nervous system (CNS) depending on the stage of these diseases (115). This is in keeping with the structure of TDP-43, which contains a nuclear localisation signal (NLS) and a large prion-like domain at the C-terminal end (84). The prion protein can be misfolded in the CNS so that it adopts a more aggregation-prone structure, which in turn causes other prion proteins to fold into the same structure (116). These form toxic aggregates which rapidly seed to neighbouring cells in the CNS (116). Similarly, TDP-43 can propagate across synapses in neuronal culture (117), and TDP-43 from exosomes taken up into neighbouring cells can seed TDP-43 aggregates (117).

This prion-like spread of TDP-43 in ALS and FTD raises the possibility that TDP-43 aggregates could spread between myonuclei in myocytes, or even between myocytes (Fig. 2, Point 6). Protein propagation in myotubes was investigated using a mosaic transfection model (118), where multiple myoblasts were fused to form a myotube. One my-

Table II. Top ten genes upregulated in IBM muscle compared to normal controls.

Rank	Genes upregulated in IBM muscle compared to normal controls	Function
1	***GBP2***- Guanylate-binding protein 2	Defends against viral, bacterial and parasite infections. Quality control for alternative splicing (80, 81)
2	***BIRC3***- Baculoviral IAP repeat-containing protein	Regulates caspases and apoptosis (82), increases nuclear factor kappa light chain enhancer of activated B cells (NF-κB) pathway activity (83).
3	***PSMB8***- Proteasome subunit beta type-8	Cleaves proteins as part of the immunoproteasome to generate peptides that bind to MHC Class I (19).
4	***GBP1***- Guanylate-binding protein 1	Defends against viral, bacterial and parasitic infections. Involved in oxidative killing and delivers microbial peptides to autolysosomes (80).
5	***CCL13***- C-C motif chemokine 13	Chemokine that attracts monocytes, lymphocytes and basophils (84).
6	***ITGAL***- Integrin alpha-L	Involved in leukocyte adhesion and transmigration, cytotoxic T-cell and antibody mediated killing by granulocytes and monocytes (84).
7	***GBP5***- Guanylate-binding protein 5	Activator of NOD- LRR and pyrin domain containing protein 3 (NRLP3) inflammasome (80, 84).
8	***HLA-DQA1***- Human leukocyte antigen class II histocompatibility antigen, DQ alpha 1 chain	MHC Class II protein (84).
9	***CD8A***- T-cell surface glycoprotein CD8 alpha chain	Co-receptor for MHC Class I: peptide complex (84).
10	***HLA-DOA***- HLA class II histocompatibility antigen, DO alpha chain	Modulator of MHC Class II restricted antigen presentation (84).

mRNA sequencing of IBM muscle biopsies, with the 10 most upregulated genes compared to normal controls. Adapted from (54) Pinal-Fernandez *et al*.: Machine learning algorithms reveal unique expression profiles in muscle biopsies from patients with different types of myositis. *Ann Rheum Dis* 2020; 79(9): 1234-42. Reprinted with permission.

onucleus expressed inducible red fluorescent protein genes of different sizes fused to a classical nuclear localisation signal (cNLS) in an expression vector. Following induction, the tagged proteins did propagate between myonuclei, with smaller proteins spreading further, although larger proteins could do so if the classical nuclear import pathway was inhibited. This finding may be relevant, as cleavage of TDP-43 creates smaller fragments which could propagate down the myocyte, with some fragments presumably missing a TDP-43 Nuclear Localization Signal. Interestingly, 35kDa and 25kDa TDP-43 fragments are found in both IBM and ALS, suggesting cleavage by the same proteases in both conditions (109). If there is propagation of TDP-43 or other proteins in IBM, then it is unlikely to be progressive in a similar manner to neurodegenerative diseases, as a longitudinal study shows that weakness in IBM can plateau or even improve (119).

Mitochondrial dysfunction

Whilst mitochondrial dysfunction induces protein aggregation (discussed in section "Immune attack and mechanisms of protein aggregation in IBM"), the reverse may also be true in IBM (Fig. 2, Point 4). In ALS and FTD aggregation of TDP-43 results in mitochondrial pathology *in vivo* and *in vitro*, and many possible mechanisms have been implicated (120). This may be due to importation of TDP-43 into the mitochondrial inner membrane, regulating oxidative phosphorylation components and thus increasing ROS and decreasing ATP levels (120). Aβ and phosphorylated tau are also known to be toxic to mitochondria in Alzheimer's disease (121, 122). In contrast, aggregation of p62 and FYCO1 have not been shown to cause mitochondrial dysfunction, but these proteins are involved in mitophagy (123, 124). There is some evidence linking TDP-43 aggregation and mitochondrial dysfunction in IBM, as TDP-43, phosphorylated TDP-43, p62 and mitochondrial respiratory transport chain enzymes stained the same damaged myofibres in IBM muscle (109). Mitochondrial pathology is likely to precede nuclear collapse in IBM, as it has been seen in some forms of polymyositis without vacuoles, termed PM-Mito (69), which may be a milder form or precursor of IBM.

Impaired myocyte regeneration

Impaired myocyte regeneration due to protein aggregation and organelle dysfunction may explain the pattern of atrophy in IBM, with marked atrophy of finger flexors, knee extensors and gastrocnemius in clinical and imaging studies (1, 125, 126). It has been proposed that eccentric muscle contraction causing micro-trauma may be the initial inflammatory event causing myositis (10). Although no formal studies have been performed assessing types of muscle contraction in different muscle groups during daily activity, it is plausible that there is more eccentric contraction in these muscles, such as when gradually releasing grip from a heavy object or sitting down in a chair. When this eccentric contraction occurs muscle length increases and myofilament overlap decreases until there is a sharp decrease in tension (127), which allows a muscle fibre to be overstretched and damaged (128, 129). Reduced regen-

eration has been seen in IBM muscle (79), and so after repeated fibre injury these muscle groups would become atrophied over time.

Conclusions

In this review we have discussed possible mechanisms of protein aggregation in IBM following cytotoxic T-cell attack, and myocyte dysfunction. We have also discussed possible roles of aggregated proteins as antigens, disruptors of nuclear and mitochondrial function and prions propagating further protein aggregation in myocytes. However, a lot of the mechanisms of protein aggregation and dysfunction in IBM remain unknown. This review has focused on the roles of proteins which accumulate in RVs, but RVs are only found in a small proportion of myocytes in IBM muscle. Further work needs to be performed on cytoplasmic proteins with more sensitive proteomic techniques to identify less abundant proteins but to also characterise their post-translational modifications. This may lead to novel therapeutic targets, and may identify biomarkers for the diagnosis of IBM, disease activity and progression.

The recent discovery of invading KLRG1$^+$ T- cells in IBM muscle (11) that meet the criteria for T-LGL leukaemia (12) helps explain the treatment-resistant nature of the disease. A monoclonal antibody specific for KLRG1 has been synthesised, and has been used in a phase I study (14). Preliminary results show early signs of efficacy, so we may be on the cusp of having an effective treatment for IBM. This antibody may be effective as a monotherapy for IBM, but drugs which assist in protein homeostasis and regeneration such as arimoclomol or bimagrumab may be useful adjunctive therapies.

However, therapies may have a limited effect on advanced IBM where there is extensive atrophy and fatty infiltration (1), and so early diagnosis is important. A muscle biopsy is invasive and cannot be performed routinely, but serum protein biomarkers would allow early diagnosis and monitoring of a clinical response, and could even be used as a routine screening test. For this to occur, further investigation of protein aggregation in IBM muscle is necessary, focusing on characterisation of post-translational modifications as well as identification of proteins which may be released from myocytes.

Funding

H. Chinoy is supported by the National Institution for Health Research Manchester Biomedical Research Centre Funding Scheme. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health.

Competing interests

H. Chinoy has received grant support from UCB and Eli Lilly, consulting fees from Astra Zeneca, Eli Lilly, Orphazyme and Novartis, and has been a speaker for Biogen and UCB. He is supported by the National Institution for Health Research, Manchester Biomedical Research Centre Funding Scheme. The other authors have declared no competing interests.

References

1. GREENBERG SA: Inclusion body myositis: clinical features and pathogenesis. *Nat Rev Rheumatol* 2019; 15: 257-72.
2. ASKANAS V, ENGEL WK, ALVAREZ RB: Light and electron microscopic localization of beta-amyloid protein in muscle biopsies of patients with inclusion-body myositis. *Am J Pathol* 1992; 141: 31-6.
3. ASKANAS V, ENGEL WK, BILAK M, ALVAREZ RB, SELKOE DJ: Twisted tubulofilaments of inclusion body myositis muscle resemble paired helical filaments of Alzheimer brain and contain hyperphosphorylated tau. *Am J Pathol* 1994; 144: 177-87.
4. PACIELLO O, WÓJOK S, ENGEL WK, MCFERRIN J, ASKANAS V: Parkin and its association with α-synuclein and AβPP in inclusion-body myositis and AβPP-overexpressing cultured human muscle fibers. *Acta Myol* 2006; 25: 13-22.
5. WEIHL CC, TEMIZ P, MILLER SE *et al*.: TDP43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *J Neurol Neurosurg Psychiatry* 2008; 79: 1186-9.
6. KOVÁCS GG, KALEV O, GELPI E *et al*.: The prion protein in human neuromuscular diseases. *J Pathol* 2004; 204: 241-7.
7. ASKANAS V, ENGEL WK, NOGALSKA A: Sporadic inclusion-body myositis: A degenerative muscle disease associated with aging, impaired muscle protein homeostasis and abnormal mitophagy. *Biochim Biophys Acta* 2015; 1852: 633-43.
8. ASKANAS V, ENGEL WK, NOGALSKA A: Pathogenic considerations in sporadic inclusion-body myositis, a degenerative muscle disease associated with aging and abnormalities of myoproteostasis. *J Neuropathol Exp Neurol* 2012; 71: 680-93.
9. ROTHWELL S, COOPER RG, LUNDBERG IE *et al*.: Immune-array analysis in sporadic inclusion body myositis reveals HLA-DRB1 amino acid heterogeneity across the myositis spectrum. *Arthritis Rheumatol* 2017; 69: 1090-9.
10. BENVENISTE O, STENZEL W, HILTON-JONES D, SANDRI M, BOYER O, VAN ENGELEN BGM: Amyloid deposits and inflammatory infiltrates in sporadic inclusion body myositis: the inflammatory egg comes before the degenerative chicken. *Acta Neuropathol* 2015; 129: 611-24.
11. GREENBERG SA, PINKUS JL, KONG SW, BAECHER-ALLAN C, AMATO AA, DORFMAN DM: Highly differentiated cytotoxic T cells in inclusion body myositis. *Brain* 2019; 142: 2590-604.
12. GREENBERG SA, PINKUS JL, AMATO AA, KRISTENSEN T, DORFMAN DM: Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. *Brain* 2016; 139: 1348-60.
13. LAMY T, MOIGNET A, LOUGHRAN TP: LGL leukemia: From pathogenesis to treatment. *Blood* 2017; 129: 1082-94.
14. SOLER-FERRAN D, COUTREAU M, ESCOBAR J, COURTEMANCHE K, NEEDHAM M, GREENBERG S: Depletion of KLRG1+ T cells in a first-in-human clinical trial of ABC008 in inclusion body myositis (IBM). *Arthitis Rheumatol* 2021; 73.
15. PLUK H, VAN HOEVE BJA, VAN DOOREN SHJ *et al*.: Autoantibodies to cytosolic 5´-nucleotidase 1A in inclusion body myositis. *Ann Neurol* 2013; 73: 397-407.
16. GREENBERG SA: Cytoplasmic 5´-nucleotidase autoantibodies in inclusion body myositis: Isotypes and diagnostic utility. *Muscle Nerve* 2014; 50: 488-92.
17. BARD JAM, GOODALL EA, GREENE ER, JONSSON E, DONG KC, MARTIN A: Structure and Function of the 26S Proteasome. *Annu Rev Biochem* 2018; 87: 697-724.
18. LÁZARO S, GAMARRA D, DEL VAL M: Proteolytic enzymes involved in MHC class I antigen processing: A guerrilla army that partners with the proteasome. *Mol Immunol* 2015; 68: 72-6.
19. SADEGH-NASSERI S, KIM A: Exogenous antigens bind MHC class II first, and are processed by cathepsins later. *Mol Immunol* 2015; 68: 81-4.
20. DE ARAÚJO-SOUZA PS, HANSCHKE SCH, VIOLA JPB: Epigenetic control of interferon-gamma expression in CD8 T cells. *J Immunol Res* 2015; 2015: 1-7.
21. RATNER A, CLARK WR: Role of TNF-α in CD8$^+$ cytotoxic T lymphocyte-mediated lysis. *J Immunol* 1993; 150: 4303-14.
22. BOEHM U, KLAMP T, GROOT M, HOWARD JC: Cellular responses to Interferon-gamma. *Annu Rev Immunol* 1997; 8: 749-95.
23. SONG W, LU X, FENG Q: Tumor necrosis factor-α induces apoptosis via inducible nitric oxide synthase in neonatal mouse

cardiomyocytes. *Cardiovasc Res* 2000; 45: 595-602.
24. EL JAMAL SM, TAYLOR EB, ABD ELMAGEED ZY *et al*.: Interferon gamma-induced apoptosis of head and neck squamous cell carcinoma is connected to indoleamine-2,3-dioxygenase via mitochondrial and ER stress-associated pathways. *Cell Div* 2016; 11: 11.
25. VANDEN BERGHE T, LINKERMANN A, JOUAN-LANHOUET S, WALCZAK H, VANDENABEELE P: Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nat Rev Mol Cell Biol* 2014; 15: 135-47.
26. KRAWCZYK PA, LAUB M, KOZIK P: To kill but not be killed: controlling the activity of mammalian pore-forming proteins. *Front Immunol* 2020; 11: 601405.
27. BINAH O, KLINE R, BERKE G, HOFFMAN B: Mechanisms whereby lytic granules from cytotoxic T lymphocytes damage guinea pig ventricular myocytes. *Scand J Immunol* 1993; 37: 53-7.
28. JONES J, FRITH S, PIDDLESDEN S *et al*.: Imaging Ca2+ changes in individual oligodendrocytes attacked by T-cell perforin. Immunology. 1991; 74: 572-7.
29. KISELEVSKY DB: Granzymes and mitochondria. *Biochem* 2020; 85: 131-9.
30. SUDA T, TAKAHASHI T, GOLSTEIN P, NAGATA S: Molecular cloning and expression of the fas ligand, a novel member of the tumor necrosis factor family. *Cell* 1993; 75: 1169-78.
31. DE BLEECKER JL, MEIRE VI, VAN WALLEGHEM IE, GROESSENS IM, SCHRÖDER MJ: Immunolocalization of FAS and FAS ligand in inflammatory myopathies. *Acta Neuropathol* 2001; 101: 572-8.
32. CAMPIONI S, MANNINI B, ZAMPAGNI M *et al*.: A causative link between the structure of aberrant protein oligomers and their toxicity. *Nat Chem Biol* 2010; 6: 140-7.
33. WALTER P, RON D: The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 2011; 334: 1081-6.
34. HAZE K, YOSHIDA H, YANAGI H, YURA T, MORI K: Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* 1999; 10: 3787-99.
35. HAMDAN N, KRITSILIGKOU P, GRANT CM: ER stress causes widespread protein aggregation and prion formation. *J Cell Biol* 2017; 216: 2295-304.
36. KAISTO T, METSIKKÖ K: Distribution of the endoplasmic reticulum and its relationship with the sarcoplasmic reticulum in skeletal myofibers. *Exp Cell Res* 2003; 289: 47-57.
37. RAHKILA P, VÄÄNÄNEN K, SARASTE J, METSIKKÖ K: Endoplasmic reticulum to Golgi trafficking in multinucleated skeletal muscle fibers. *Exp Cell Res* 1997; 234: 452-64.
38. ROSSI D, BARONE V, GIACOMELLO E, CUSIMANO V, SORRENTINO V: The sarcoplasmic reticulum: an organized patchwork of specialized domains. *Traffic* 2008; 9: 1044-9.
39. BIEL TG, ARYAL B, GERBER MH, TREVINO JG, MIZUNO N, RAO VA: Mitochondrial dysfunction generates aggregates that resist lysosomal degradation in human breast cancer cells. *Cell Death Dis* 2020; 11: 460.
40. SUHM T, KAIMAL JM, DAWITZ H *et al*.: Mitochondrial translation efficiency controls cytoplasmic protein homeostasis. *Cell Metab* 2018; 27: 1309-22.e6.
41. ROUSALOVA I, KREPELA E: Granzyme B-induced apoptosis in cancer cells and its regulation. *Int J Oncol* 2010; 37: 1361-78.
42. SCHILLING R, GESERICK P, LEVERKUS M: Characterization of the ripoptosome and its components. *In* ASHKENAZI A, WELLS JA, YUAN J (Eds): Methods in Enzymology. New York (USA), Associated Press, 2014; 545: 83-102.
43. TAYLOR RC, CULLEN SP, MARTIN SJ: Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008; 9: 231-41.
44. DAVIDOVICH P, KEARNEY CJ, MARTIN SJ: Inflammatory outcomes of apoptosis, necrosis and necroptosis. *Biol Chem* 2014; 395: 1163-71.
45. DANIELSSON O, HÄGGQVIST B, GRÖNTOFT L, ÖLLINGER K, ERNERUDH J: Apoptosis in idiopathic inflammatory myopathies with partial invasion; a role for $CD8^+$ cytotoxic T cells? *PLoS One* 2020; 15: e0239176.
46. LIU D, XIAO Y, ZHOU B *et al*.: PKM2-dependent glycolysis promotes skeletal muscle cell pyroptosis by activating the NLRP3 inflammasome in dermatomyositis/polymyositis. *Rheumatology* 2021; 60: 2177-89.
47. NALBANDIAN A, KHAN AA, SRIVASTAVA R *et al*.: Activation of the NLRP3 inflammasome is associated with valosin-containing protein myopathy. *Inflammation* 2017; 40: 21-41.
48. WATTS G, THOMASOVA D, RAMDEEN S *et al*.: Novel VCP mutations in inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. *Clin Genet* 2007; 72: 420-6.
49. SAMSON AL, HO B, AU AE *et al*.: Physicochemical properties that control protein aggregation also determine whether a protein is retained or released from necrotic cells. *Open Biol* 2016; 6: 160098.
50. GÜTTSCHES A-K, BRADY S, KRAUSE K *et al*.: Proteomics of rimmed vacuoles define new risk allele in inclusion body myositis. *Ann Neurol* 2017; 81: 227-39.
51. KHANDIA R, DADAR M, MUNJAL A *et al*.: A comprehensive review of autophagy and its various roles in infectious, non-infectious, and lifestyle diseases: current knowledge and prospects for disease prevention, novel drug design, and therapy. *Cells* 2019; 8: 674.
52. VITTONATTO E, BOSCHI S, CHIADÒ-PIAT L *et al*.: Differential diagnosis of vacuolar muscle biopsies: use of p62, LC3 and LAMP2 immunohistochemistry. *Acta Myol* 2017; 36: 191-8.
53. MILISENDA JC, PINAL-FERNANDEZ I, LLOYD TE *et al*.: Accumulation of autophagosome cargo protein p62 is common in idiopathic inflammatory myopathies. *Clin Exp Rheumatol* 2021; 39: 351-6.
54. PINAL-FERNANDEZ IAGO, CASAL-DOMINGUEZ M, DERFOUL A *et al*.: Machine learning algorithms reveal unique gene expression profiles in muscle biopsies from patients with different types of myositis. *Ann Rheum Dis* 2020; 79: 1234-42.
55. BHATTARAI S, GHANNAM K, KRAUSE S *et al*.: The immunoproteasomes are key to regulate myokines and MHC class I expression in idiopathic inflammatory myopathies. *J Autoimmun* 2016; 75: 118-29.
56. FRATTA P, ENGEL WK, MCFERRIN J, DAVIES KJA, LIN SW, ASKANAS V: 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: 517-26.
57. CALABRESE LH, MITSUMOTO H, CHOU SM: Inclusion body myositis presenting as treatment-resistant polymyositis. *Arthritis Rheum* 1987; 30: 397-403.
58. ROSE MR: 188th ENMC International Workshop: Inclusion Body Myositis, 2–4 December 2011, Naarden, The Netherlands. *Neuromuscul Disord* 2013; 23: 1044-55.
59. MENDELL JR, SAHENK Z, GALES T, PAUL L: Amyloid filaments in inclusion body myositis: Novel findings provide insight into nature of filaments. *Arch Neurol* 1991; 48: 1229-34.
60. LECLERC A, TOMÉ FMS, FARDEAU M: Ubiquitin and β-amyloid-protein in inclusion body myositis (IBM), familial IBM-like disorder and oculopharyngeal muscular dystrophy: An immunocytochemical study. *Neuromuscul Disord* 1993; 3: 283-91.
61. VILLANOVA M, KAWAI M, LÜBKE U *et al*.: Rimmed vacuoles of inclusion body myositis and oculopharyngeal muscular dystrophy contain amyloid precursor protein and lysosomal markers. *Brain Res* 1993; 603 :343-7.
62. SHERRIFF FE, JOACHIM CL, SQUIER MV, ESIRI MM: Ubiquitinated inclusions in inclusion-body myositis patients are immunoreactive for cathepsin D but not β-amyloid. *Neurosci Lett* 1995; 194: 37-40.
63. SALAJEGHEH M, PINKUS JL, TAYLOR JP *et al*.: Sarcoplasmic redistribution of nuclear TDP-43 in inclusion body myositis. *Muscle Nerve* 2009; 40: 19-31.
64. LILLEKER JB, HODGSON R, ROBERTS M *et al*.: Florbetapir positron emission tomography: Identification of muscle amyloid in inclusion body myositis and differentiation from polymyositis. *Ann Rheum Dis* 2019; 78: 657-62.
65. CHOI SR, GOLDING G, ZHUANG Z *et al*.: Preclinical Properties of 18 F-AV-45: A PET Agent for Aβ Plaques in the Brain. *J Nucl Med* 2009; 50: 1887-94.
66. NOGALSKA A, D'AGOSTINO C, ENGEL WK, ASKANAS V: Novel demonstration of conformationally modified tau in sporadic inclusion-body myositis muscle fibers. *Neurosci Lett* 2011; 503: 229-33.
67. ASKANAS V, BILAK M, ENGEL WK, ALVAREZ RB, TOMÉ F, LECLERC A: Prion protein is abnormally accumulated in inclusion-body myositis. *Neuroreport* 1993; 5: 25-8.
68. ASKANAS V, ENGEL WK, ALVAREZ RB, MCFERRIN J, BROCCOLINI A: Novel immunolocalization of α-synuclein in human muscle of inclusion- body myositis, regenerating and necrotic muscle fibers, and at neuromus-

cular junctions. J *Neuropathol Exp Neurol* 2000; 59: 592-8.
69. TEMIZ P, WEIHL CC, PESTRONK A: Inflammatory myopathies with mitochondrial pathology and protein aggregates. *J Neurol Sci* 2008; 278: 25-9.
70. SEKIJIMA Y: Hereditary transthyretin amyloidosis. *In* ADAM MP, ARDINGER HH, PAGON RA *et al*. (Eds.): GeneReviews®. University of Washington, Seattle, 2001 (Updated 2021): 1993-2022.
71. ROSEN DR, SIDDIQUE T, PATTERSON D *et al*.: Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993; 362: 59-62.
72. PARKER KC, KONG SW, WALSH RJ *et al*.: Fast-twitch sarcomeric and glycolytic enzyme protein loss in inclusion body myositis. *Muscle Nerve* 2009; 39: 739-53.
73. ARNARDOTTIR S: Sporadic inclusion body myositis: morphology, regeneration, and cytoskeletal structure of muscle fibres. *J Neurol Neurosurg Psychiatry* 2004; 75: 917-20.
74. CARRARO U, DALLA LIBERA L, CATANI C: Myosin light and heavy chains in muscle regenerating in absence of the nerve: Transient appearance of the embryonic light chain. *Exp Neurol* 1983; 79: 106-17.
75. MARÉCHAL G, SCHWARTZ K, BECKERS-BLEUKX G, GHINS E: Isozymes of myosin in growing and regenerating rat muscles. *Eur J Biochem* 1984; 138: 421-8.
76. DOPPLER K, LINDNER A, SCHÜTZ W, SCHÜTZ M, BORNEMANN A: Gain and loss of extracellular molecules in sporadic inclusion body myositis and polymyositis-a proteomics-based study. *Brain Pathol* 2012; 22: 32-40.
77. IOZZO RV, SCHAEFER L: Proteoglycans in health and disease: novel regulatory signaling mechanisms evoked by the small leucine-rich proteoglycans. *FEBS J* 2010; 277: 3864-75.
78. PINKUS JL, AMATO AA, TAYLOR JP, GREENBERG SA: Abnormal distribution of heterogeneous nuclear ribonucleoproteins in sporadic inclusion body myositis. *Neuromuscul Disord* 2014; 24: 611-6.
79. ASKANAS V, ENGEL WK: Inclusion-body myositis: Muscle-fiber molecular pathology and possible pathogenic significance of its similarity to Alzheimer's and Parkinson's disease brains. *Acta Neuropathol* 2008; 116: 583-95.
80. TRETINA K, PARK ES, MAMINSKA A, MACMICKING JD: Interferon-induced guanylate-binding proteins: Guardians of host defense in health and disease. *J Exp Med* 2019; 216: 482-500.
81. LU YY, KREBBER H: Nuclear mRNA quality control and cytoplasmic NMD are linked by the guard proteins Gbp2 and Hrb1. *Int J Mol Sci* 2021; 22: 11275.
82. WANG C-Y, MAYO MW, KORNELUK RG, GOEDDEL DV, BALDWIN AS: NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 1998; 281: 1680-3.
83. VARFOLOMEEV E, GONCHAROV T, FEDOROVA AV *et al*.: c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor α (TNF-α)-induced NF-κB activation. *J Biol Chem* 2008; 283: 24295-9.
84. BATEMAN A, MARTIN M-J, ORCHARD S *et al*.: UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res* 2021; 49: 480-9.
85. KELLEY N, JELTEMA D, DUAN Y, HE Y: The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int J Mol Sci* 2019; 20: 3328.
86. ZHAO W, BEERS DR, BELL S *et al*.: TDP-43 activates microglia through NF-κB and NLRP3 inflammasome. *Exp Neurol* 2015; 273: 24-35.
87. YAN Y-Q, FANG Y, ZHENG R, PU J-L, ZHANG B-R: NLRP3 inflammasomes in Parkinson's disease and their regulation by Parkin. *Neuroscience* 2020; 446: 323-34.
88. JIANG S, MAPHIS NM, BINDER J *et al*.: Proteopathic tau primes and activates interleukin-1β via myeloid-cell-specific MyD88- and NLRP3-ASC-inflammasome pathway. *Cell Rep* 2021; 36: 109720.
89. BABELOVA A, MORETH K, TSALASTRA-GREUL W *et al*.: Biglycan, a danger signal that activates the NLRP3 inflammasome via Toll-like and P2X receptors. *J Biol Chem* 2009; 284: 24035-48.
90. RUBIN RL: Autoantibody specificity in drug-induced lupus and neutrophil-mediated metabolism of lupus-inducing drugs. *Clin Biochem* 1992; 25: 223-34.
91. RAY A, AMATO AA, BRADSHAW EM *et al*.: Autoantibodies produced at the site of tissue damage provide evidence of humoral autoimmunity in inclusion body myositis. *PLoS One* 2012; 7: e46709.
92. SIMEONE R, SAYES F, LAWARÉE E, BROSCH R: Breaching the phagosome, the case of the tuberculosis agent. *Cell Microbiol* 2021; 23: e13344.
93. MUSTAFA AS, SHABAN FA, AL-ATTIYAH R *et al*.: Human Th1 cell lines recognize the Mycobacterium tuberculosis ESAT-6 antigen and its peptides in association with frequently expressed HLA class II molecules. *Scand J Immunol* 2003; 57: 125-34.
94. DE PAEPE B, CREUS KK, WEIS J, DE BLEECKER JL: Heat shock protein families 70 and 90 in Duchenne muscular dystrophy and inflammatory myopathy: Balancing muscle protection and destruction. *Neuromuscul Disord* 2012; 22: 26-33.
95. PRASAD TSK, VERMA R, KUMAR S *et al*.: Proteomic analysis of purified protein derivative of Mycobacterium tuberculosis. *Clin Proteomics* 2013; 10: 8.
96. PATHAN AA, WILKINSON KA, WILKINSON RJ *et al*.: High frequencies of circulating IFN-γ-secreting CD8 cytotoxic T cells specific for a novel MHC class I-restricted Mycobacterium tuberculosis epitope in M. tuberculosis-infected subjects without disease. *Eur J Immunol* 2000; 30: 2713-21.
97. TYEDMERS J, MOGK A, BUKAU B: Cellular strategies for controlling protein aggregation. *Nat Rev Mol Cell Biol* 2010; 11: 777-88.
98. TANAKA M, KIM YM, LEE G, JUNN E, IWATSUBO T, MOURADIAN MM: Aggresomes formed by α-Synuclein and Synphilin-1 are cytoprotective. *J Biol Chem* 2004; 279: 4625-31.
99. LU M, BOSCHETTI C, TUNNACLIFFE A: Long term aggresome accumulation leads to DNA damage, p53-dependent cell cycle arrest, and steric interference in mitosis. *J Biol Chem* 2015; 290: 27986-8000.
100. LIEWLUCK T, HAYASHI YK, OHSAWA M *et al*.: Unfolded protein response and aggresome formation in hereditary reducing-body myopathy. *Muscle Nerve* 2007; 35: 322-6.
101. WOOD A, GURFINKEL Y, POLAIN N, LAMONT W, LYN REA S: Molecular mechanisms underlying TDP-43 pathology in cellular and animal models of ALS and FTLD. *Int J Mol Sci* 2021; 22: 4705.
102. VOGLER TO, WHEELER JR, NGUYEN ED *et al*.: TDP-43 and RNA form amyloid-like myo-granules in regenerating muscle. *Nature* 2018; 563: 508-13.
103. NEUMANN M, SAMPATHU DM, KWONG LK *et al*.: Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006; 314: 130-3.
104. ARAI T, HASEGAWA M, AKIYAMA H *et al*.: TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006; 351: 602-11.
105. NELSON PT, DICKSON DW, TROJANOWSKI JQ *et al*.: Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. *Brain* 2019; 142: 1503-27.
106. UDAN-JOHNS M, BENGOECHEA R, BELL S *et al*.: Prion-like nuclear aggregation of TDP-43 during heat shock is regulated by HSP40/70 chaperones. *Hum Mol Genet* 2014; 23: 157-70.
107. RATTI A, GUMINA V, LENZI P *et al*.: Chronic stress induces formation of stress granules and pathological TDP-43 aggregates in human ALS fibroblasts and iPSC-motoneurons. *Neurobiol Dis* 2020; 145: 105051.
108. HINIKER A, DANIELS BH, LEE HS, MARGETA M: Comparative utility of LC3, p62 and TDP-43 immunohistochemistry in differentiation of inclusion body myositis from polymyositis and related inflammatory myopathies. *Acta Neuropathol Commun* 2013; 1: 29.
109. HUNTLEY ML, GAO J, TERMSARASAB P *et al*.: Association between TDP-43 and mitochondria in inclusion body myositis. *Lab Investig* 2019; 99: 1041-8.
110. HASEGAWA M, ARAI T, NONAKA T *et al*.: Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol* 2008; 64: 60-70.
111. ALONSO ADC, ZAIDI T, NOVAK M, GRUNDKE-IQBAL I, IQBAL K: Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci USA* 2001; 98: 6923-8.
112. KUMAR S, REZAEI-GHALEH N, TERWEL D *et al*.: Extracellular phosphorylation of the amyloid β-peptide promotes formation of toxic aggregates during the pathogenesis of Alzheimer's disease. *EMBO J* 2011; 30: 2255-65.
113. REN Y, LI S, CHEN S *et al*.: TDP-43 and phosphorylated TDP-43 levels in paired

plasma and CSF Ssamples in amyotrophic lateral sclerosis. *Front Neurol* 2021;12: 663637.

114. BIERING SB, CHOI J, HALSTROM RA *et al*.: Viral replication complexes are targeted by LC3-guided interferon-inducible GTPases. *Cell Host Microbe* 2017; 22: 74-85.e7.
115. BRETTSCHNEIDER J, DEL TREDICI K, IRWIN DJ *et al*.: Sequential distribution of pTDP-43 pathology in behavioral variant frontotemporal dementia (bvFTD). *Acta Neuropathol* 2014; 127: 423-39.
116. PRUSINER SB: Molecular biology of prion diseases. *Science* 1991; 252: 1515-22.
117. FEILER MS, STROBEL B, FREISCHMIDT A *et al*.: TDP-43 is intercellularly transmitted across axon terminals. *J Cell Biol* 2015; 211: 897-911.
118. TAYLOR-WEINER H, GRIGSBY CL, FERREIRA DMS *et al*.: Modeling the transport of nuclear proteins along single skeletal muscle cells. *Proc Natl Acad Sci USA* 2020; 117: 2978-86.
119. OLDROYD AGS, LILLEKER JB, WILLIAMS J, CHINOY H, MILLER JAL: Long-term strength and functional status in inclusion body myositis and identification of trajectory subgroups. *Muscle Nerve* 2020; 62: 76-82.
120. LUCINI CB, BRAUN RJ: Mitochondrion-dependent cell death in TDP-43 proteinopathies. *Biomedicines* 2021; 9: 376.
121. KANDIMALLA R, MANCZAK M, YIN X, WANG R, REDDY PH: Hippocampal phosphorylated tau induced cognitive decline, dendritic spine loss and mitochondrial abnormalities in a mouse model of Alzheimer's disease. *Hum Mol Genet* 2018; 27: 30-40.
122. LUSTBADER JW, CIRILLI M, LIN C *et al*.: ABAD directly links Aß to mitochondrial toxicity in Alzheimer's disease. *Science* 2004; 304: 448-52.
123. LI W, HE P, HUANG Y *et al*.: Selective autophagy of intracellular organelles: Recent research advances. *Theranostics* 2021; 11: 222-56.
124. COSTELLO MJ, BRENNAN LA, BASU S *et al*.: Autophagy and mitophagy participate in ocular lens organelle degradation. *Exp Eye Res* 2013; 116: 141-50.
125. GUIMARAES JB, ZANOTELI E, LINK TM *et al*.: Sporadic inclusion body myositis: MRI findings and correlation with clinical and functional parameters. *Am J Roentgenol* 2017; 209: 1340-7.
126. DAY JA, BAJIC N, GENTILI S, PATEL S, LIMAYE V: Radiographic patterns of muscle involvement in the idiopathic inflammatory myopathies. *Muscle Nerve* 2019; 60: 549-57.
127. GORDON AM, HUXLEY AF, JULIAN FJ: The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J Physiol* 1966; 184: 170-92.
128. ARMSTRONG RB, WARREN GL, WARREN JA: Mechanisms of exercise-induced muscle fibre injury. *Sport Med* 1991; 12: 184-207.
129. PROSKE U, MORGAN DL: Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol* 2001; 537: 333-45.