

REVIEW ARTICLE

Immune and myodegenerative pathomechanisms in inclusion body myositis

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Introduction

Inclusion body myositis (IBM) is a progressive slow-onset inflammatory myopathy that is characterized by the concomitant presence of multi-focal myofiber-surrounding lymphocytic infiltrates as well as vacuolar myodegeneration.^{1–4} Together with dermatomyositis (DM), polymyositis (PM), necrotizing autoimmune myositis (NAM) and overlap myositis (OM), IBM belongs to the heterogenous group of inflammatory myopathies and, amongst individuals 50 years of age and older, it is considered as a relatively frequent disorder.^{3,5} The underlying interrelationship between the inflammatory component of the disease and the observed multi-protein aggregation remains elusive and

Abstract

Inclusion Body Myositis (IBM) is a relatively common acquired inflammatory myopathy in patients above 50 years of age. Pathological hallmarks of IBM are intramyofiber protein inclusions and endomysial inflammation, indicating that both myodegenerative and inflammatory mechanisms contribute to its pathogenesis. Impaired protein degradation by the autophagic machinery, which regulates innate and adaptive immune responses, in skeletal muscle fibers has recently been identified as a potential key pathomechanism in IBM. Immunotherapies, which are successfully used for treating other inflammatory myopathies lack efficacy in IBM and so far no effective treatment is available. Thus, a better understanding of the mechanistic pathways underlying progressive muscle weakness and atrophy in IBM is crucial in identifying novel promising targets for therapeutic intervention. Here, we discuss recent insights into the pathomechanistic network of mutually dependent inflammatory and degenerative events during IBM.

subject to vigorous debate.^{3,6–8} Unlike other inflammatory myopathies, IBM presents mainly refractory toward immunosuppressive therapy and at present, there is no effective treatment available.^{5,9,10} In this review, we will focus on the current knowledge about the interrelationship of inflammatory and myodegenerative pathomechanisms in IBM.

Clinical Presentation

The disease commonly commences slowly-progressive, sometimes over decades. The clinical presentation is heterogenous and at times difficult to distinguish from other inflammatory myopathies (muscle weakness and

atrophy), motor-neuron disease (asymmetry), and muscular dystrophies (slowly progressive disease).³ In two large observational studies, the mean age of onset has been reported to be 59 ± 9 and 61 years, respectively.^{9,11} The cardinal symptom of this highly debilitating disease is the late-onset steady acquisition of muscular weakness and atrophy over a long period of time whilst sensory function is completely preserved. The decline of muscle strength ranges between 3.5 and 5.5% per annum.^{9,11} Unlike other myopathies, during which proximal muscles are initially affected, IBM shows early involvement of distal muscles. Classical manifestation patterns frequently include the quadriceps, deep finger flexors, foot extensors, and often presents asymmetrically at the beginning. Frequent falls may be an early clinical sign of IBM. Paraspinal and axial muscles may be affected, resulting in head drop and camptocormia.¹² Depending on the study, oropharyngeal dysphagia is reported in up to 40–86% of IBM cases, mostly due to upper esophageal sphincter dysfunction.^{13–16} It develops insidiously, leading to frequent choking episodes and is, alongside pneumonia as a result of immobility, considered to be a potentially fatal complication of IBM. Importantly, dysphagia may be an isolated, initial manifestation and IBM should be considered by the examining physician as differential diagnosis for new onset of dysphagia in the elderly.^{13,14,17–21} Early in the disease course, tendon reflexes remain unaffected, however, hyporeflexia may occur at later stages of the disease due to significant muscle atrophy.¹⁹ The heart muscle remains usually unaffected and the incidence of cardiac muscle abnormalities does not exceed the expected incidence for the respective age group.²² There is no evidence for increased cancer risk in IBM patients.²³

Epidemiology

IBM affects males more frequently (3:1), shows an overall prevalence of approximately 4-15/1000000 (35-71/1000000 > age 50, respectively), is noticeably frequent in Western Australia, Japan, Norway, Olmsted County (Minnesota, USA), and is especially rare in Turkey and India.^{3,24–31} In Japan, the number of diagnosed IBM cases has steadily increased since 1991, whereas the number of PM cases has remained constant.³² Prolonged life span and concomitant increase of the fraction of elderly people as well as westernization of dietary habits in Japan might be contributing factors for this observation.³³ Although it was suggested that mortality is increased in IBM patients, solid evidence is still insufficient and the matter remains subject to larger studies.^{17,34}

Current data prompt that IBM meets the criteria to be categorized as an orphan disease. However, it is likely that the prevalence of IBM is still underestimated. Although

heavily debated, it is conceivable that a significant number of patients diagnosed with PM might in fact suffer from IBM.^{35–38} Aside from erroneous diagnoses, the slowly progressive nature of the disease course and the heterogeneity in its clinical presentation make the condition prone to delayed diagnosis. Increasing awareness and continuous efforts to optimize diagnostic criteria for IBM are of utmost importance in ensuring ample care to patients.

Diagnosis

The chronic disease progression of IBM makes it challenging to detect the condition at an early time point and on average there is a 5-year delay in diagnosis.^{9,39} Creatine kinase levels in serum can be normal to only mildly elevated and will not exceed 10-fold increase above the upper limit of normal. Muscle biopsies of affected areas typically show CD8⁺ T cells surrounding nonnecrotic, healthy appearing muscle fibers that express major histocompatibility complex (MHC) class I. Additionally, ragged-red-, ragged-blue- and cytochrome oxidase-negative fibers, as well as autophagic vacuoles and congophilic amyloid deposits are regularly observed.³

The original diagnostic criteria according to Griggs et al. strongly relied on histopathological features of the disease.⁴⁰ However, it is now apparent that a given muscle biopsy will rarely show all pathological changes that go along with IBM. Basing a definitive diagnosis on the prerequisite of detecting all formerly described histopathological alterations will likely lead to underdiagnosis of the disease. It has been previously described that some patients that fit clinical categorization of IBM, lack canonical biopsy features of IBM.⁴¹ It has become clear that histopathological abnormalities in IBM are likely to appear scattered and patchy in a spatio-temporal manner. The increasing research efforts over the past 45 years, together with accumulated clinical experience, allows physicians today to reliably diagnose the disease not exclusively due to histopathological changes in muscle biopsies but rather through an integrated approach, using clinical and histological observations alike. Therefore, more recently defined diagnostic criteria do not call for the presence of all typical pathological hallmarks but employ the presence of defined patterns of clinical, laboratory, and histological features to categorize the diagnosis into either *clinicopathologically defined IBM*, *clinically defined IBM* or *probable IBM*.^{12,42} One study applying machine learning algorithms to construct data-derived IBM diagnostic criteria claims that the combinational approach of finger flexor or quadriceps weakness, endomysial inflammation, and either invasion of nonnecrotic muscle fibers or rimmed vacuoles, performed with a 90% sensitivity and 96% specificity among 371 patients.⁴³

Pathomechanisms in IBM

Histopathological hallmarks of IBM muscle feature both myodegenerative multi-protein aggregates as well as endomysial lymphocytic infiltrates.²⁻⁴ Several lines of evidence suggest that inflammatory mechanisms precede myodegeneration,⁸ but so far a precise answer and sound evidence is lacking as discussed below in detail. Currently, it remains unresolved and controversially discussed if the inflammatory changes observed in IBM muscle are a direct result of primary myodegeneration or if protein aggregation is secondary to initial inflammatory events. The solution of this conundrum is key to identify an appropriate remedy for this debilitating disease.

Immunopathomechanisms in IBM

Endomysial lymphocytic infiltrations in IBM muscle are usually found at perivascular sites and appear scattered. Similar to PM, the mononuclear infiltrates in IBM predominantly consist of CD8⁺ cytotoxic T cells (CTLs) surrounding nonnecrotic muscle fibers. More than 30% of all invading cells and around 50% of invading CD8⁺ T cells depict activation marker positivity.⁴⁴ Unlike in healthy individuals, scattered clusters of nonnecrotic muscle fibers ectopically express MHC class I molecules in a moderate to strong degree on their surface^{3,45} and infiltrating CD8⁺ T cells form close contacts with these MHC class I expressing fibers (Fig. 1). While a considerable amount of muscle fibers with cytoplasmic abnormalities (such as lined vacuoles) do not express MHC class I, regenerating muscle fibers in IBM muscle do show sarcolemmal expression of this molecule.⁴⁵ Although macrophages constitute only a minor fraction of the mononuclear infiltrates invading nonnecrotic muscle fibers, they account for up to 80% of the infiltrates surrounding necrotic fibers.⁴⁴ Distinct from DM but consistent with lymphocytic infiltrates observed in PM, muscle-invading CD8⁺ T cells stain positive for pore-forming and cytolytic molecules such as perforin, granzyme A, and granulysin.⁴⁶⁻⁴⁸ It has been demonstrated that perforin-polarization within endomysial CD8⁺ T cells occurs toward target myofibers indicative of immunosynapse formation and arguing strongly for a possible recognition of specific antigens presented via MHC class I expressing myofibers.⁴⁹ In line with this, muscle fibers in IBM patients express co-stimulatory molecules such as ICOS-L, CD276, and BB1 on their surface.^{48,50,51}

Immunohistochemical and RT-PCR analyses revealed preferential usage of certain CD8⁺ T cell receptor (TCR) variable segments in endomysial infiltrates compared to peripheral CD8⁺ T cell-TCR profiles in IBM patients.^{52,53} Although conclusive proof is still lacking, this myo-

peripheral discrepancy of TCR restriction suggests that CD8⁺ T cells patrol the muscle in a stochastic manner and only upon recognition of their cognate antigen clonally expand in situ. In line with this, endomysial T cells depict expression of proliferation marker Ki-67 suggestive of a pervasive antigen-driven response within the muscle compartment.⁵⁴ However, specific recruitment to the muscle compartment remains a possibility. Using the combination of RT-PCR, immunohistochemistry and TCR V β chain CDR3 spectratyping in three sequential muscle biopsies of three IBM patients, Amemiya et al. found clonal persistence of CD8⁺ T cells in subsequent muscle biopsies. This is supportive of earlier studies and suggests that IBM might be maintained by a continuous antigen-driven T cell response.^{55,56} Additionally, a more recent CDR3 spectratyping study of CD8⁺ T cell-TCR V β chains in 12 IBM patients identified V β 9, 10, 11, 16, 18, 23, and 24 as subfamilies with the strongest degree in myo-restriction. Indicative of determinant spreading, follow-up muscle biopsies (after 12 months) confirmed persisting CD8⁺ T cell clonality, while the pattern of expanded V β subfamilies had changed.⁵⁷

Viruses

By analogy to numerous autoimmune diseases, a viral contribution to the etiology of IBM has been discussed for as long as the condition has been identified.^{58,59} Presently, it is not ultimately clear by which exact mechanism(s) viruses may trigger autoimmunity. Host-inherent anti-viral responses comprise a meticulously regulated mounting of the immune system. Erroneous and faulty progression of such antiviral responses may lead to subsequent break-down of self-tolerance with concomitant epitope spreading and recognition of auto-antigens⁶⁰ (Fig. 2). Other potential virus-mediated mechanisms include bystander activation and immortalization of low-affinity autoaggressive effector cells due to unphysiological exposure and subsequent presentation of self-antigens in the context of a strong antiviral response.⁶⁰ Despite considerable effort, so far no virus could be isolated and amplified from affected muscle tissue of IBM patients and no conclusive evidence for a viral trigger of this myopathy exists.^{3,61} However, an association with human immunodeficiency virus (HIV)⁶² and human T lymphotropic virus (HTLV)^{63,64} seropositivity has been clearly demonstrated and in the case of HIV as many as 10% of infiltrating CD8⁺ T cells showed specificity for human leukocyte antigen-A* 0201-HIV-gag. Worthy of note, in both cases HIV- and HTLV-derived viral antigens could not be detected in muscle fibers but exclusively on endomysial macrophages.^{62,63} Furthermore, an association between hepatitis C virus (HCV) infection and IBM was recently

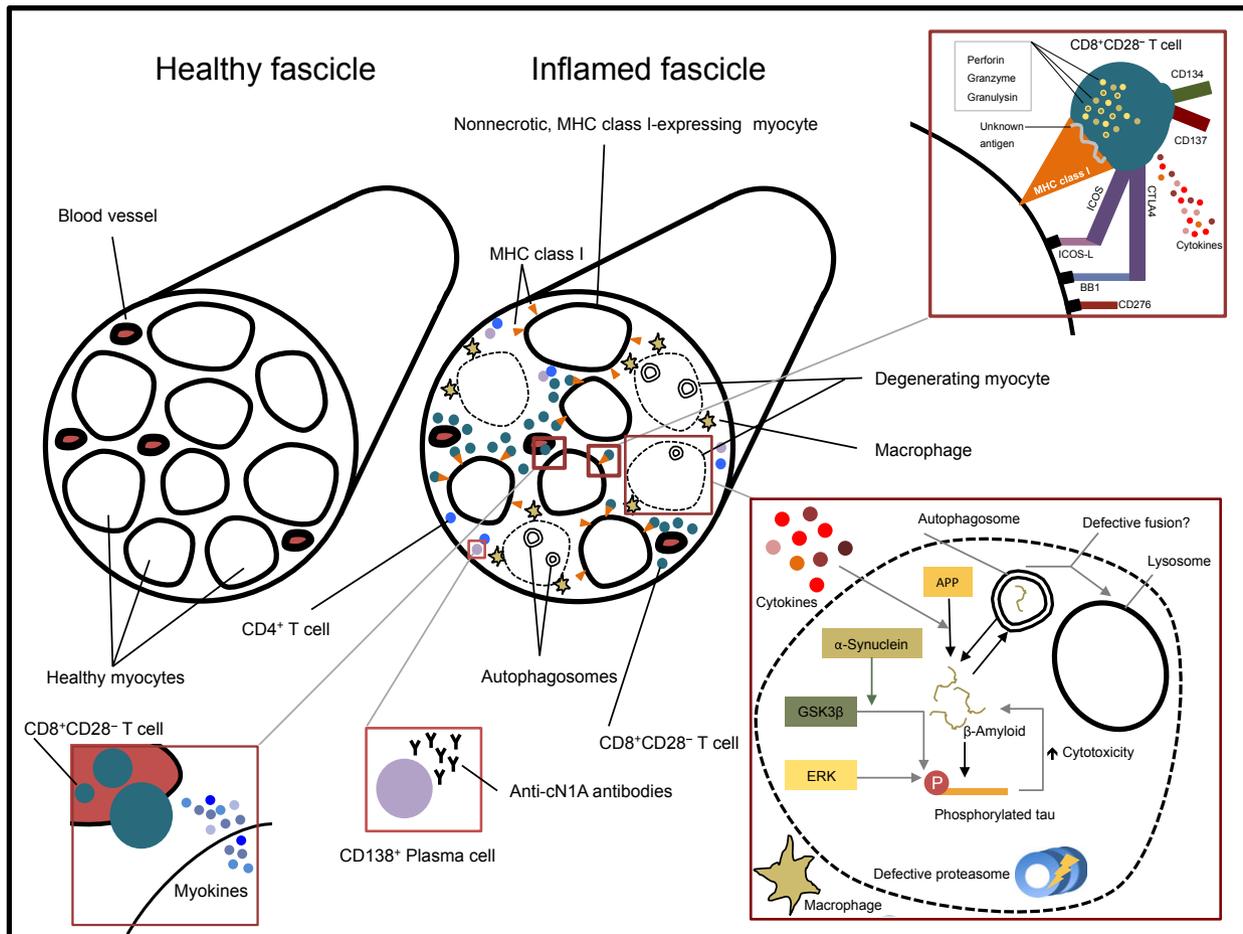


Figure 1. Scheme of the pathological changes in inclusion body myositis compared to healthy muscle. Mainly nonnecrotic, MHC class I-expressing myofibers are surrounded by invading $CD8^+CD28^-$ T cells, which is the predominant immune subset in the endomysial infiltrates. These $CD8^+CD28^-$ T cells form immunological synapses with MHC class I bearing myofibers, contain cytolytic proteins, release proinflammatory cytokines, and express costimulatory molecules corresponding to complementary molecules on the surface of myofibers. Additionally, myofibers themselves are immunologically active via releasing myokines. Albeit they are found less frequently, also $CD4^+$ T cells and $CD138^+$ plasma cells are present in the endomysium and may contribute to the myoinflammatory environment. Degenerating myofibers are mainly surrounded by macrophages and contain APP-derived β -amyloid and phosphorylated tau. If the disruption of proteostasis by virtue of impaired macroautophagy and defective proteasomal degradation is an upstream event in the pathomechanism of IBM or if it follows the increasing aggregation of aberrant proteins, remains a matter of debate.

reported in a Japanese case-control study that included 114 IBM and 44 PM patients.⁶⁵ While the frequency of PM patients that also carried anti-HCV antibodies was comparable to the general population that of IBM patients was significantly increased.⁶⁵ However, these data need to be interpreted carefully and it appears to be unlikely that HCV is a key determinant in the development of IBM. The increasing incidence of IBM in Japan is in strong contrast to the decreasing incidence in HCV infections.⁶⁶⁻⁶⁸ Moreover, countries with relatively high incidence rates of HCV infections belong to the regions that are stricken the least by IBM.^{26,30,61,69-71} It is conceivable that nonpersistent contact with a pathogen suffices to

trigger autoimmunity.⁷² At this point, a viral contribution to the etiopathogenesis of IBM cannot be ruled out, yet more conclusive evidence is clearly needed.

CD8⁺ T cells

With regards to differentiation, surface marker expression and functionality, the $CD8^+$ T cell compartment displays considerable heterogeneity. Short-telomer-bearing $CD8^+CD28^-$ T cells are thought to comprise a highly differentiated oligoclonal subset arising from chronic antigen exposure as hypothesized for IBM^{19,49,73-77} and several autoimmune conditions are accompanied by increased

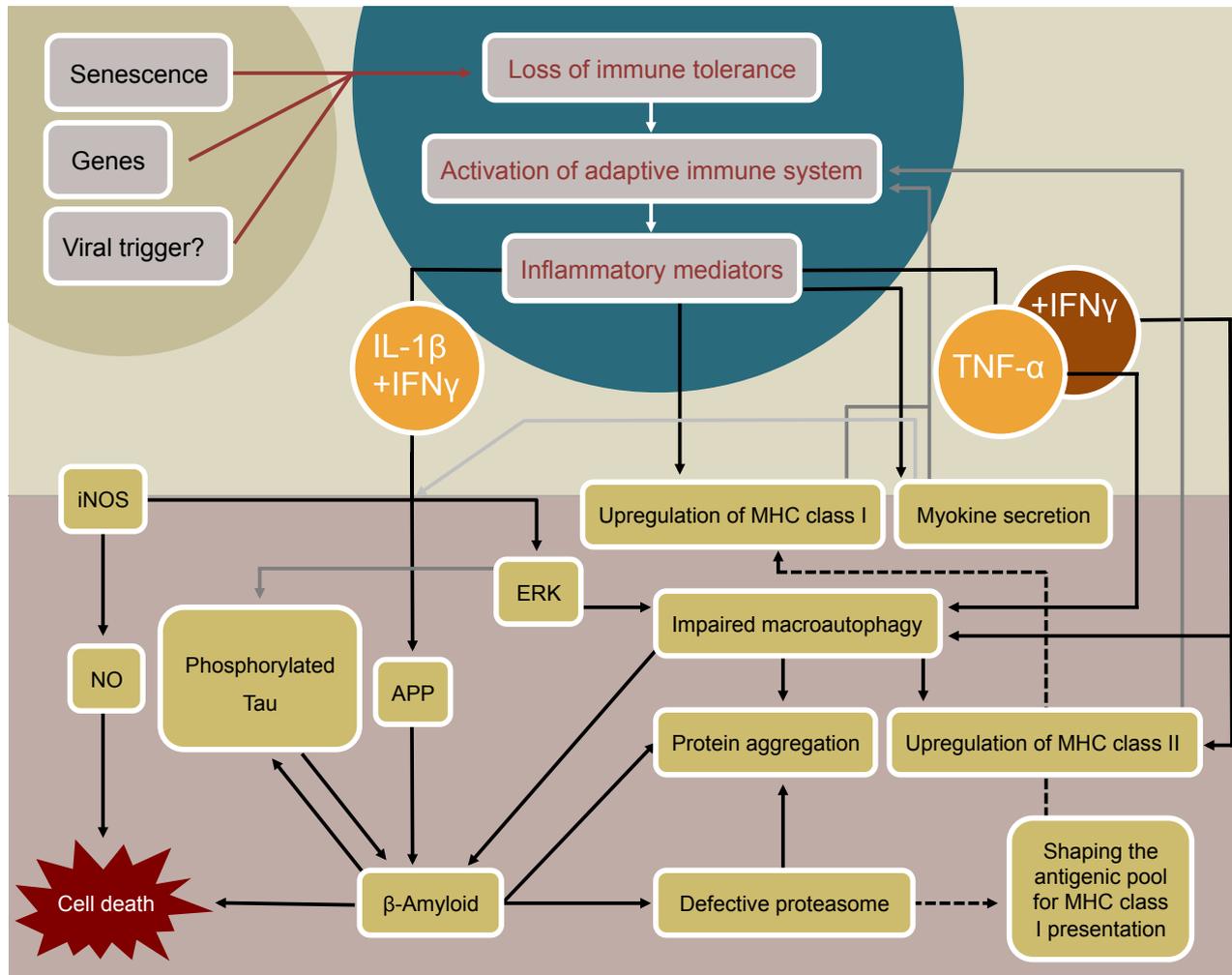


Figure 2. Schematic overview of a possible crosstalk between key pathological mechanisms during IBM. Genetic predisposition, aging, and exposure to a yet unidentified viral trigger may each individually or in combination lead to breakdown of immune tolerance with subsequent activation of the adaptive immune system. Invasion of myoantigen-specific T cells into the endomysium could establish and maintain a pro-inflammatory environment in the muscle. Upregulation of MHC class I and II molecules on myofibers and release of myokines in response to inflammation may serve as a feedback loop that helps to perpetuate disease. Disturbed proteostasis may result in response to specific pro-inflammatory mediators. Conversely, it is argued, that a primary event within myofibers leads to degenerative changes that entail inflammation as a secondary event, yet evidence for this latter scenario is lacking.

frequencies of CD8⁺CD28⁻ T cells.^{78–80} Furthermore, muscle-infiltrating CD8⁺ T cells in patients suffering from PM and DM have been reported to be mainly CD28⁻.⁸¹ Two recent studies showed that frequencies of highly cytotoxic CD8⁺CD28⁻ T cells in inflamed muscle and in peripheral blood of IBM patients are significantly increased and their capability to secrete IFN γ was superior compared to healthy controls.^{82,83} CD8⁺CD28⁻ T cells are devoid of costimulatory interaction between CD80: CD28, however, it is reported that CD8⁺CD28⁻ T cells after CD3 ligation in turn upregulate alternative costimulatory molecules such as inducible costimulator (ICOS), CD134 and CD137⁸⁴ which could facilitate T cell: muscle

fiber interaction and is in keeping with the observed upregulation of ICOS-L on muscle fibers during IBM.⁴⁸

Aside from lacking CD28-expression, expression of the terminally sulfated glycan carbohydrate CD57 is generally regarded as a marker for terminal differentiation and clonal exhaustion on CD8⁺ T cells and this T cell subset is commonly oligoclonally expanded during conditions of chronic immune activation.^{85–87} CD8⁺CD57⁺ T cell frequencies are especially increased in the elderly, they have strong cytotoxic potential, high expression of adhesion molecules, strong migratory potential toward nonlymphoid organs and – indicative of a cytotoxic effector memory phenotype – they depict expression of CX3CR1.

It is believed that CD8⁺CD57⁺ T cells are highly differentiated antigen-driven effector cells in a state of replicative senescence with limited capacities to proliferate.^{88–92} Recent reports, however, suggest that these CD8⁺CD57⁺CD28⁻ T cells might comprise a rather heterogeneous group of highly antigen-experienced cells that, depending on the immunobiological context and stimuli, differ in their susceptibility to apoptosis and their capability to proliferate and expand.^{84,93–95}

During T cell-large granular lymphocytic leukemia (T-LGL leukemia) clonally expanded large granular CD8⁺CD57⁺ CTLs can be found in peripheral blood, spleen and bone marrow.⁹⁶ Interestingly, a recent study describes a previously undiscovered association between IBM and T-LGL leukemia.⁹⁷ T-LGL leukemia is a rare condition within the spectrum of lymphoproliferative disorders on the interface between neoplasia and extensive antigen-driven CTL response and is frequently associated with autoimmune disorders.^{98–100} According to this new study, the clinical criteria for an expanded LGL population in association to an autoimmune disorder¹⁰¹ were met in more than half of the 38 investigated IBM patients. Greenberg and colleagues controversially argue that, at least in some cases of IBM, an initial autoimmune process might transform into a neoplasia-like condition with extensive clonal expansion of large granular CTLs resembling those of T-LGL leukemia.⁹⁷ This is especially intriguing in light of the fact that IBM is refractory to common immunotherapies. Frequently, this lack of efficacy in targeting the immune system has given rise to the assumption that IBM might be primarily a myodegenerative disorder and the inflammatory component little more than an etiopathogenetic epiphenomenon.^{7,102,103} Although further investigation into the matter is needed, this recent study offers a different narrative, which would have substantial implications toward both the diagnosis and therapy of IBM.¹⁰⁴

Other infiltrating immune cells

Aside from the previously described cytotoxic CD8⁺ T cells, inflammatory infiltrates in IBM additionally harbor myeloid cells,¹⁰⁵ plasma cells,¹⁰⁶ and CD4⁺ T cells.^{44,48,82,83,107,108} Early studies have previously revealed that the antigen-presenting properties of human myocytes exceed the mere bearing of MHC class I molecules. In fact, muscle fibers can be categorized as facultative antigen-presenting cells, that in a proinflammatory milieu can upregulate MHC class II molecules, express intercellular adhesion molecule (ICAM)-1 and ICOS ligand (ICOS-L).^{108–110} In line with this, MHC class II expressing muscle fibers are found in IBM.¹⁰⁸ In fact, up to 66.7% of muscle fibers in IBM show high positivity for MHC class II as opposed to lower counts in other IIMs (PM: 23.7%;

DM: 20%).¹¹¹ Interestingly, microdissection studies revealed that HLA-DR, HLA-DB, and CIITA are predominantly upregulated in infiltrated but not in healthy appearing muscle fibers.¹¹² Comparable to their CD8⁺ counterpart, CD4⁺ T cells in IBM are mostly devoid of CD28-expression, display a striking TCR V β restriction, and are expanded in the peripheral blood as well as in inflamed muscle tissue.⁸³ Similar to CTLs, they depict a strong proinflammatory phenotype and cytotoxic properties which might be executed toward MHC class II-bearing muscle fibers.⁸³ Additionally, local presentation of antigen via professional antigen presenting cells (APCs) or MHC class II bearing muscle fibers has been suggested.^{105,108} The pathological role of CD4⁺CD28⁻ T cells during IBM, therefore, might have been underappreciated so far.

Tregs

FOXP3⁺CD4⁺ T regulatory cells (Tregs) constitute a unique lymphocyte subset that holds the capacity to control and limit immune responses mounted against self- and foreign antigens in order to retain immune homeostasis and self-tolerance.^{113,114} It has become evident that distinct tissue-specific Treg populations with unique phenotypical and functional properties exist. In skeletal muscle, they arise from a small pool of resident Tregs and strongly accumulate following muscle damage.^{115,116} Under the control of interleukin (IL)-33, these myophil Tregs execute essential functions in promoting and orchestrating local regeneration upon muscle injury.^{117,118} Importantly, they are significantly diminished in aged mice, leading to insufficient muscle repair upon injury.¹¹⁸ A critical role for Tregs during myositis had already been postulated in an experimental autoimmune myositis model during which antibody-mediated depletion of Tregs leads to significant increase of the histopathological disease score and a more diffuse muscle inflammation pattern.¹¹⁹ On the contrary, *in vitro* expanded adoptively transferred polyclonal Tregs are able to decrease the severity in this model. These findings are extended by a study that, employing a new model, adoptively transferred FOXP3/synaptotagmin VII double mutant-derived lymphocytes into RAG-1^{-/-} mice together with muscle antigens. This entails strong myositis reflected by myofiber infiltrating CD4⁺ and CD8⁺ T cells and macrophages. Coadministration of functional Tregs fully protects animals from developing myositis.¹²⁰ Furthermore, the capacity of Tregs to dampen CD8⁺ T cell cytotoxicity directed against human myoblasts has been confirmed *in vitro*¹²¹ and immunohistochemical studies in IBM muscle revealed presence of Tregs in close spatial association to other infiltrating mononuclear cells. The

amount of Tregs positively correlated with the amount of total CD3⁺ cells. However, these results are not specific for IBM but could be obtained in PM and DM muscle as well.¹²¹ A more recent report shows a significant decrease in circulating Tregs in IBM patients compared to non-myositic controls.⁸² Functionality of the remainder of peripheral Tregs with regard to proliferation-suppression of autologous T cells, however, was unaffected.⁸² This study also confirmed the previous finding that Tregs are indeed present in inflamed muscle of IBM patients.⁸² To which degree this report is specific to IBM or if similar results were to be obtained in other inflammatory myopathies remains to be investigated. The physiological role of muscle-resident Tregs and their contribution during myositis has only begun to unravel. It becomes apparent that few studies so far addressed the presence and subcategorization of CD4⁺ T cells, including Tregs (which constitute up to 60% of CD4⁺ T cells in muscle upon injury¹¹⁶), in muscle infiltrates of IBM. Such work could help to better understand the role of these cells.

B cells, plasma cells, and autoantibodies

Despite the often-proclaimed predominant role of CD8⁺ T cells in IBM, several reports suggest an underrated humoral component in the immunopathology of inflammatory myopathies. Sera from IBM patients contain increased amounts of muscle antigen-reactive monoclonal antibodies¹²² and although CD20⁺ B cells are scarce, substantial numbers of transcriptionally active CD138⁺ plasma cells can be detected in inflamed muscle of IBM patients.¹⁰⁶ Immunoglobulin heavy chain gene transcript analyses in IBM, PM, and DM revealed that these cells undergo isotype switching, oligoclonal expansion and somatic hypermutation which suggests local affinity maturation of antibodies,¹²³ a process that usually occurs in germinal centers under the aid of follicular dendritic cells (fDCs) and follicular B helper T cells.^{124–126} In fact, an early study characterized nodular lymphocytic accumulations in inflamed muscle and found microanatomical organization patterns as well as adhesion molecule expression reminiscent of those in secondary lymphoid organs.¹²⁷ Others, however, have reported that these nodular accumulations lack B cell follicles and presence of DRC⁺ fDCs characteristic for lymphoid germinal centers.¹²⁸

B cell maturation is highly dependent on bidirectional interactions with cognate CD4⁺ T cells.^{124–126} Amongst others, ICOS:B7RP-1 ligation is essential for the successful execution of this crosstalk.¹²⁹ In line with this, ICOS⁺CD4⁺ T cells have been reported to be present in IBM infiltrates.⁴⁸ B cell activating factor of the TNF superfamily (Baff) is a cytokine crucial for B cell survival and has been implicated in autoantibody formation in

patients suffering from autoimmune diseases.¹³⁰ Serum levels of Baff are elevated in some patients suffering from IIMs including IBM patients.¹³¹ Furthermore, Baff transcripts are markedly increased in muscle tissue from IIM patients compared to nonmyositic controls (IBM>PM>DM).¹²⁸ However, serum Baff levels were highest in IIM patients that also had detectable levels of anti-histidyl-tRNA-synthetase antibody Jo-1, an autoantibody that is extremely rare in IBM.^{132–134} Additionally, Baff serum levels seem to positively correlate with serum CK levels, which can be normal to only moderately elevated in IBM.¹³¹

The presence of specific autoantibodies is not only relevant with regard to possible therapeutic options but has immediate implications for diagnosis. Autoantibodies associated with myositis have been identified in more than half of the patients suffering from myositis.^{3,135,136} In 2011, a previously undetected circulating antibody against a muscle-derived protein was found in 52% of IBM patients (13/25) but was absent in control individuals (PM, DM, healthy volunteers).¹³⁷ Shortly thereafter, the group and others identified the target of these antibodies to be cytosolic 5'-nucleotidase 1A (the antibody is now commonly referred to as anti-cN1A antibody).^{138,139} Moderate reactivity of anti-cN1A antibodies was reported to be 70% sensitive and 92% specific for the diagnosis of IBM.¹³⁶ Another study reported similar numbers, detecting anti-cN1A antibodies in 61% of IBM patients but on the other hand also in 5% of PM, 23% of Sjögren's syndrome patients (SS), and 14% of systemic lupus erythematosus (SLE) patients, even in absence of any muscular symptoms.¹⁴⁰ In a subsequent report however, the frequency of seropositive IBM patients was only 34.8% (24/69).¹⁴¹ Circulating anti-cN1A antibodies may aid in distinguishing IBM (37%) from PM and DM (<5%), however, the picture becomes less clear when acknowledging that these antibodies are also detected in autoimmune conditions such as SS (36%) and SLE (20%).¹⁴² The presence of anti-cN1A antibodies is neither associated with gender nor malignancy and appears to be independent of specific HLA-DR alleles.¹⁴¹ Two recent reports also found anti-cN1A antibodies in 33% (102/311)¹⁴³ and 35.8% (24/67)¹⁴⁴ of IBM patients, respectively. The anti-cN1A positive IBM patients showed a higher adjusted mortality risk and depicted more cytochrome oxidase deficient muscle fibers as compared to sero-negative patients.¹⁴³ Moreover, passive immunization with purified IgG fractions derived from either anti-cN1A-positive or anti-cN1A-negative IBM patients in *in vitro* and *in vivo* models, led to myodegenerative changes (such as p62 protein aggregation), resembling those observed in IBM muscle.¹⁴⁴ Whether pathogenicity is directly transferred via anti-cN1A antibodies or if presence of these

autoantibodies is simply indicative of other, yet unidentified mechanisms is so far unclear.¹⁴⁵

Taken together, the above-mentioned findings argue for B cell activation with subsequent production of autoantibodies against muscle epitopes in IBM. However, the pathogenetic role of B cells, their specificity and relevance needs further investigation.⁶

Inflammatory mediators – cytokines, chemokines, and myokines

Signal peptides secreted by invading leukocytes and resident myofibers alike are an integral part of the inflammatory milieu in muscle and are believed to directly contribute to the pathology of IBM via induction of surface molecules (on myofibers and invading leukocytes), chemotaxis of myoaggressive immune cells and subsequent muscle injury. The interplay of soluble factors and expression patterns of their respective surface receptors is complex and dynamic in its nature and a plethora of key suspects have been suggested.¹⁴⁶

An early immunocytochemistry study that evaluated expression of inflammatory mediators in myositis, found predominant presence of IL-1 α (in endothelial cells), IL-1 β and TGF- β (both in inflammatory cells), albeit no apparent difference in the expression pattern was observed between DM, PM and IBM.¹⁴⁷ Similarly, De Bleeker and colleagues detected TNF- α in macrophages, endothelial cells, and central myonuclei in IBM, DM, and PM muscle but not in that of nonmyositic controls.¹⁴⁸ Chronic administration of TNF- α via osmotic minipumps has shown to be already sufficient to attract neutrophils and macrophages to the muscle compartment.¹⁴⁹ A more recent study found mRNA levels of GM-CSF, IL-4, IL-10, IL-12, IL-13, IL-23, IL-1 β and TNF- α to be significantly increased in IBM muscle compared to healthy controls. Although this was also true for PM muscle, DM muscle did not present with increased levels of these cytokines and TNF- α showed the highest values in IBM patients.¹⁵⁰

Thrombospondin-1 (TSP-1) has been reported to function as a chemoattractant for leukocytes to sites of inflammation and interaction with its ligands activates and perpetuates autoaggressive T cell expansion.^{151,152} Furthermore, expression of TSP-1 and its binding partners CD36 and CD47 is upregulated on mRNA and protein level in IBM¹⁵³ and TNF- α can induce TSP-1 and CD47 expression on human myoblasts *in vitro*.¹⁵³

A crucial role for IFN γ in the pathoetiology of IBM has been proposed as well.¹³⁶ During IBM, muscle fibers ubiquitously express MHC class I on their surface and to a higher degree than DM or PM muscle.¹⁵⁴ However, not all MHC class I bearing myofibers depict presence of

immune infiltrates and although MHC class I expression seems to sustain CD8⁺ T cell myoinfiltration in the case of IBM, MHC class I expression by itself seems not to be sufficient to entail infiltration of cytotoxic T cells (as demonstrated during DM, where muscle fibers express MHC class I but no CD8⁺ T cell infiltrates can be detected).^{45,155} Ivanidze *et al.* reported segmental upregulation of IFNGR2, exclusively on attacked MHC class I-expressing myofibers *vs.* MHC class I bearing myofibers that did not have infiltrates (nonattacked myofibers). The expression of IFNGR2 positively correlated with the amount of infiltrating CD8⁺ T cells.¹¹²

This strongly argues for MHC class I upregulation upstream and independent of IFN γ signaling during IBM. It is possible that, following this ubiquitous expression of MHC class I on myofibers upon a so far unknown trigger, CD8⁺ T cells might recognize cognate antigen in a stochastic manner, become activated, expand and secrete proinflammatory cytokines which, in turn, may induce IFNGR expression and perpetuate susceptibility toward further myocytotoxicity.¹¹² In line with this, CD8⁺CD28⁻ T cells found in the peripheral blood of IBM patients are more prone to produce IFN γ and IFN γ -inducible chemoattractant mediators such as CXCL-9, CXCL-10 and IL-12 are increased in serum of IBM patients compared to nonmyositic controls.⁸² Aside from infiltrating leukocytes, myofibers themselves might also actively participate in secreting proinflammatory mediators. Upregulated mRNA expression levels of CXCL-9 and CXCL-10 in muscle biopsies of IBM patients had been reported before and the same study demonstrated synthesis of CXCL-9 and CXCL-10 by human muscle fibers after IFN γ incubation *in vitro*.¹⁵⁶ However, as with other cytokines discussed previously, CXCL-9 and CXCL-10 regulation is confirmed for other IIMs as well and these changes seem to reflect a general inflammatory milieu and maintenance of such within the muscle compartment.¹⁵⁷

One conceivable alternative mechanism responsible for initial upregulation of MHC class I (upstream of IFN γ signalling) includes viral genesis (as discussed above)^{62–64,158} or other proinflammatory cytokines like TNF- α and IL-1 β .^{112,159} Interestingly, in addition to IFNGR2 expression, transcripts of RANTES and Stat3 are reported to be increased in attacked myofibers *vs.* nonattacked myofibers as well.¹¹² RANTES is produced in response to TNF- α and synergistic effects between TNF- α and IFN γ with regards to RANTES synthesis are reported.^{160,161} Additionally, pro-inflammatory cytokines like IL-1 β and, in particular, TNF- α might hamper myoregeneration in IBM (and other IIMs) by suppressing myogenic microRNAs such as miR-1, miR-133a and miR-133b.¹⁵⁰ TNF-like weak inducer of apoptosis (TWEAK) is a recently described member of the TNF superfamily. The proinflammatory cytokine signals

through binding to its receptor Fn14 and activates NF κ B in a TGF β -activated kinase 1-(TAK1-) dependent manner.¹⁶² TWEAK is expressed in a wide variety of cell types including monocytes and macrophages, dendritic cells and T cells^{163–167} and its implications in controlling muscle tissue repair and regeneration have reaffirmed its role as a key regulator of myogenesis.^{164,168} As opposed to DM, PM and healthy mesoangioblasts, IBM mesoangioblasts fail to fully differentiate into skeletal myotubes.¹⁶⁹ A recent study found increased TWEAK-Fn14-expression in IBM muscle compared to DM and PM muscle. Moreover, culture media from IBM-derived differentiating mesoangioblasts show significantly higher levels of TWEAK as compared to nonmyositic or DM controls and IBM-derived mesoangioblasts depict higher Fn14-expression than those derived from other IIMs.¹⁷⁰ During chronic inflammatory conditions, TWEAK has been shown to mediate proliferation of precursor cells while prohibiting their terminal differentiation.¹⁷¹ Furthermore, a critical role for TWEAK/Fn14 in fostering muscle atrophy has been proposed.^{172,173} Therefore, disbalance of the TWEAK/Fn14 axis may, similarly to what has been reported for TNF- α and IL-1 β , block myogenic differentiation through NF κ B-signalling^{174,175} and, additionally, promote progressive muscle wasting and atrophy during IBM. Interestingly, in a colitis model, TWEAK, IL-13, and TNF- α act in concert and synergistically promote intestinal epithelial cell injury and induction of fibroblast proliferation.^{176,177} Although a possible role for IL-13 in the pathomechanism of IBM has not been addressed thus far, mRNA levels in muscle derived from nonmyositic controls and different IIMs depicted the highest and most consistent levels of IL-13 in IBM samples.¹⁵⁰

Finally, potential regulatory roles in the pathomechanism of IIM have been ascribed to IL-17A and IL-15.^{178,179} However, most studies have so far focused on PM and DM and little data are currently available in IBM. Given the similarities in the inflammatory muscle milieu, especially that of PM, IL-15 and IL-17A should be further investigated for their involvement in IBM.

The differential interaction of selected cytokines with degenerative pathomechanisms during IBM will be discussed further below.

Degenerative pathomechanisms in IBM

Aside from the previously discussed inflammatory component of IBM, its pathoetiology includes distinct myodegenerative changes including, but not limited to, vacuolization, abnormal posttranslational modifications of proteins with subsequent congophilic misfolded multi-protein aggregates and dysfunctional mitochondrial activity.^{7,103} The observation that IBM behaves largely

refractory to anti-inflammatory treatment gave rise to the proposition that inflammatory myofiber infiltrates are largely an epiphenomenon to age-related primary myodegenerative events similar to neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD).^{7,103} In the following section, we will discuss the degenerative changes observed in IBM muscle and evaluate the possible pathomechanistic interrelationship with inflammatory processes.

Amyloid- β , α -Synuclein, Presenilin, Tau

Detection of intracellular accumulation of amyloid precursor protein (APP)-derived amyloid- β (A β) peptides as congophilic inclusions was amongst the first evidence for defective myoproteostasis in IBM muscle.^{180–182} A β is usually generated as an either 40 or 42 amino acid-long peptide. The more hydrophobic 42 amino acid long isoform exhibits a stronger tendency to self-associate into insoluble fibrils, oligomerize and cluster into aggregates than A β _{1–40}.^{183–185} It is therefore considered to be more cytotoxic and the predominant isoform to be accumulated as oligomers in IBM muscle.^{186,187} Congophilic A β is detected in up to 70% of IBM muscle fibers and mostly found in nonvacuolated areas.¹⁸⁸ A β peptides are generated via the sequential cleavage of the transmembrane glycoprotein APP by the protease β -site of the APP cleaving enzyme 1 (BACE1) and the γ -secretase complex.^{189–191} Components of the sequential cleavage machinery of APP, such as BACE1, are upregulated in IBM muscle.^{192,193} Recently, a γ -secretase activating protein (GSAP) has been characterized, which selectively mediates A β generation via facilitating the interaction between γ -secretase complex members and APP-CTF.¹⁹⁴ IBM muscle fibers depict increased protein and mRNA expression of GSAP in comparison to nonmyositic controls.¹⁹³ Consequently, the members of the γ -secretase complex that catalyzes the final step of the A β generation, such as nicastrin, presenilin-1 (PS-1) and presenilin enhancer 2 are increased on protein and mRNA level in IBM muscle.¹⁹³ Phosphorylation of APP by glycogen synthase kinase 3 β (GSK3 β) facilitates increased generation of cytotoxic A β .^{195,196} In line with these findings, GSK3 β is activated and APP is found to be highly phosphorylated in IBM muscle.¹⁹⁷

One study evaluated plasma levels of A β in IBM patients compared to myositic and nonmyositic controls and although A β plasma levels were increased in IBM compared to PM, levels were also elevated in DM disqualifying the assay as an appropriate diagnostic tool.¹⁹⁸ A more recent report, however, found that plasma levels of BACE1, PS-1 and soluble APP are increased in IBM patients compared to healthy controls and patients diagnosed with PM and DM.¹⁹⁹

Aside from extracellular amyloid plaques, also intraneuronal neurofibrillary tangles mainly comprised of the microtubule-associated protein tau, are a morphological feature of AD brains.^{200–202} There is evidence that A β partly executes its cytotoxicity upstream of tau hyperphosphorylation and subsequent self-assembly.^{203,204} Aspects of A β cytotoxicity are tau-dependent, indicating a reciprocal, self-enhancing component during the interaction of the two.²⁰⁵ Cytoplasmic hyperphosphorylated tau tangles in AD brains consist predominantly of 15–21 nm long paired helical filaments (PHF).²⁰¹ Similarly, in IBM muscle, accumulations of hyperphosphorylated tau-containing PHF are observed and kinases such as extracellular signal-regulated kinase (ERK) or GSK3 β , which have been reported to phosphorylate tau, are increased and colocalized with tau in IBM muscle fibers.^{206–210}

α -Synuclein, another aberrant protein that is present as insoluble cytoplasmic aggregates in neurodegenerative brain disorders also abnormally accumulates in IBM muscle fibers.^{211–215} Expression and toxicity of the small protein is increased under conditions of oxidative stress but is negatively regulated by the activity of heat shock proteins.^{216–218} Interestingly, α -synuclein has been reported to facilitate phosphorylation of tau by the above mentioned kinase GSK3 β .²¹⁹

In addition to A β , hyperphosphorylated tau and α -synuclein also ApoE,^{220,221} p62/SQSTM1²²² and prion protein^{223–225} are found to be aggregated in IBM muscle fibers, all indicative of protein dyshomeostasis being a distinctive feature of IBM. In support of this, a recent study found that treatment with arimoclomol, a coinducer of heat shock responses, significantly ameliorated IBM-like phenotype in vitro and in vivo and appeared to be safe in a proof-of-concept study with IBM patients.²²⁶ The clinical efficacy of arimoclomol is currently tested in a clinical trial (NCT02753530).

Faulty protein disposal: the proteasome & autophagy

Although there is now a plethora of convincing evidence for severe defects in myoproteostasis, the individual specificity of the aforementioned aggregated proteins in IBM pathology remains to be further evaluated. The pathoetiology of IBM appears to follow a dynamic pattern and a given muscle biopsy at a given stage of the disease may greatly differ from those taken at different time points or even locations. In fact, the paradox is not limited to IBM. In AD, which regularly serves skeptics as a paramount example for a *bona fide* amyloid-disorder, more than one-third of ApoE noncarriers that clinically present with mild to moderate cognitive deficit, do not show significant cerebral amyloidosis in positron emission tomography.^{227–229}

The underlying causative event that promotes and propagates self-aggregation of aberrant proteins in IBM myofibers has yet to be elucidated. A delicately regulated surveillance of protein turnover is especially crucial in postmitotic cells such as neurons and myocytes.^{230–234} Eukaryotic cells employ two predominant molecular systems to keep a tight balance between translation and degradation of cellular proteins, namely the proteasomal system and autophagy.^{235–237} Dysfunction in either of these two proteolytic systems, and subsequent imbalance of protein homeostasis, is one of nine defining characteristics of cellular aging.²³⁸ However, severity, time of onset, and acceleration of these pathomechanisms determine to which degree these changes will meet the pathological spectrum.^{235,237,238}

The proteasome

In the ubiquitin-proteasomal system (UPS), sequentially polyubiquitylated proteins are targeted toward the barrel-shaped multipartite 26S proteasome which executes caseinase-like, trypsin-like, and chymotrypsin-like proteolytic activities located on its β -subunits (β 1, β 2, and β 5, respectively).²³⁹ The proteasomal system is tightly controlled by regulatory molecules and executes ubiquitin-dependent and -independent proteolytic degradation.²⁴⁰ Polyubiquitination-independent recognition and subsequent degradation of oxidized substrates by the core particle 20S proteasome is especially relevant in the context of aging cells.^{235,241}

Fratta *et al.* have reported that the 26S proteasome co-stains with A β , phosphorylated tau, ubiquitin, and heat shock protein 70 (Hsp70) in muscle biopsies from IBM patients.²⁴² While protein expression of proteasomal subunits 19S, 20S α , and 20S β is greatly increased in IBM muscle compared to age-matched controls, proteolytic activity of the proteasomal machinery is significantly impaired in IBM muscle. In accordance with this, inhibition of the proteasome in human myofibers in vitro leads to formation of aberrant multiprotein aggregates.²⁴²

Particularly cells of the hematopoietic system harbor a unique form of the proteasome, termed the immunoproteasome, in which the catalytic β subunits β 1, β 2, β 5 have been replaced by β 1i, β 2i, and β 5i resulting in increased enzymatic cleavage following hydrophobic residues and decreased cleavage following acidic residues, respectively.^{243–245} Exchange of constitutive proteasome subunits with immunoproteasome sub-units distinctively shapes the pool of MHC class I ligands and can be facilitated via exposure to proinflammatory cytokines like IFN γ and TNF- α .^{243,244} A recent study found that the immunoproteasome sub-units β 1i and β 5i are upregulated and colocalized with MHC class I molecules in IBM

muscle.²⁴⁶ In vitro experiments show that exposure to TNF- α and IFN γ increases replacement toward immuno-proteasomal sub-units in primary human myoblasts and the selective inhibition of proteasomal subunit β 5i in myoblasts results in increased expression of TNF- α and IFN γ -dependent myokines like IL-1 β , IL-6, CXCL-9, and CXCL-10.²⁴⁶ However, these results were also obtained in other IIM such as DM and immune-mediated necrotizing myopathy, indicating a downstream effect of preceding myoinflammatory events. It remains unclear if proteasomal dysfunction is a primary event in IBM pathology, or if soluble intermediates of aggregation-prone proteins facilitate proteasomal inhibition.

Autophagy

Autophagy comprises a set of intracellular catabolic pathways that degrade cytoplasmic content by means of the lysosomal system.^{247,248} While occupying pivotal roles during host defense against microbes, induction of tolerance, antigen-presentation, and tissue differentiation, a key function of autophagy pathways is to maintain a well-balanced proteostasis and provision of metabolic building blocks and energy sources in response to nutrient deprivation and other cellular stressors^{248–252} As opposed to the proteasomal system, autophagy, in addition to removing aberrant proteins, aids in the removal of defective or excess mitochondria, lysosomes and peroxisomes and keeps, thereby, homeostasis on the level of macromolecules and whole organelles alike.^{253–256} Consequently, defective autophagy pathways have been ascribed a pathological role in degenerative diseases of the brain^{236,237,257} and emerging evidence implicates autophagy in the pathoetiology of IBM. Initial hints about malregulated autophagy in IBM were introduced in an early study in 1980.²⁵⁸ However, it took another 24 years for evidence that members of the autophagy machinery (*Atg5* and *Atg12*) are upregulated on mRNA level in IBM muscle as compared to healthy and amyotrophic lateral sclerosis muscle.²⁵⁹ We demonstrated that accumulated APP and its proteolytic fragment A β in skeletal muscle fibers are targeted for lysosomal degradation via macroautophagy.²⁶⁰ We observed APP/A β -containing autophagosomes at increased frequency in muscle fibers of IBM muscle biopsies, but not in nonmyopathic muscle or nonvacuolated myopathic controls. Moreover, A β -containing autophagosomes were almost exclusively observed in degenerating muscle fibers of the type II (fast-twitching) and in part associated with overexpression of MHC class I and II on myofibers and invasion by CD4⁺ and CD8⁺ cells.²⁶⁰ A more recent immunohistochemistry study reports overexpression of the autophagy proteins ATG5, microtubule-associated protein light chain 3

(LC3) and Beclin-1 in IBM muscle biopsies. Interestingly, lymphocytic infiltrates were predominantly found surrounding Beclin-1⁺ myofibers.²⁶¹ Recently, components of chaperone-mediated autophagy were identified to be increased in IBM as well.²⁶² Güttsches et al. identified an overrepresentation of rare missense coding variants of an autophagic adaptor protein facilitating autophagosome trafficking, *FYCO1*, in IBM patients and suggested that a failure in autophagosome/endosome trafficking may underlie IBM pathogenesis.²⁶³ In addition to *FYCO1*, missense pathogenic variants responsible for autophagosome maturation and degradation (VCP and p62/SQSTM1) have been found in patients with IBM.^{264,265} We could previously show that autophagy is constitutively active in human myocytes and can be upregulated via the proinflammatory cytokines TNF- α ¹⁰⁸ or IFN γ together with IL-1 β .²⁶⁶ Interestingly, composite exposure to TNF- α and IFN γ leads to significant autophagy-dependent translocation of intracellular MHC class II to the cell surface in myocytes and more than 40% of muscle fibers in IBM that costain for autophagosomes and MHC class II have contact to CD4⁺ and CD8⁺ infiltrating T cells.¹⁰⁸ Dengjel and colleagues reported that upregulation of autophagic activity, by means of altered lysosomal processing, significantly increases the fraction of intracellular source protein-derived peptides presented on MHC class II.²⁶⁷ These findings suggest that the proinflammatory environment in IBM muscle promotes induction of autophagy in myofibers and subsequently enhances surface MHC class II, thereby maintaining CD4⁺ T cell infiltrates via the presentation of yet unknown self-peptides. Intracellular antigen presentation via MHC class I molecules is also regulated by the autophagy machinery, because autophagy-related proteins enhance MHC class I internalization for degradation and thereby diminish antigen display on the cell surface.²⁶⁸ Indeed, in vivo studies have primarily found enhanced CD8⁺ T cell responses in mice with defective autophagy in antigen-presenting cells^{268,269,270} A potential mechanism leading to such increased CD8⁺ T cell expansions is that, in the absence of autophagy, more substrate becomes available for canonical MHC class I loading²⁷¹ or by decreased endocytosis and degradation of cell surface MHC class I molecules.²⁶⁸ Thus, defective autophagy in skeletal muscle fibers could drive increased MHC class I expression and CD8⁺ T cell accumulation.

Brain biopsies of early (preclinical) and moderate stage AD patients exhibit impaired neuronal autophagic activity represented by increased numbers of LC3⁺ autophagosomes and diminished fusion of these vesicles with lysosomes into autolysosomes.²⁷² Moreover, autophagosomes purified from an APP₆₉₅-transfected murine fibroblast-like cell line, contain copious amounts of APP, PS1, nicastrin,

and γ -secretase complex with functional amyloidogenesis at the site of the autophagosome, resulting in $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides.²⁷² Importantly, these generated peptides were not instable intermediates as they did not seem to undergo additional cleavage after further 24 h incubation in autophagosomes as opposed to their $A\beta$ -specific cleavage in lysosomal fractions.²⁷² In line with this, *in vitro* exposure of human muscle cells to autophagy inducers TNF- α or rapamycin leads to marked increase of intracellular APP and $A\beta$ oligomers. Specific siRNA-mediated knockdown of the essential autophagy gene *Atg12* prevents the assembly of autophagosomes and abrogates TNF- α -mediated accumulation of $A\beta$ in muscle cells.²⁷³ These findings appear to be in contrast to a subsequent study in which Nogalska *et al.* report increased accumulation of $A\beta$ oligomers upon inhibition of autophagy in cultured human myofibers.¹⁸⁷ However, these results should be carefully interpreted since inhibition of autophagy was carried out by using chloroquine and bafilomycin A1 (a specific inhibitor of the V-ATPase), both of which lack specificity and are believed to inhibit lysosomal acidification and thereby subsequent fusion of the autophagosome with lysosomes rather than the assembly of the autophagosome,²⁷⁴ which results in impaired autophagosome maturation and accumulation of autophagosomes. In the case of bafilomycin A1, the actual inhibitory potential with regard to blocking autophagosome-lysosome fusion has been doubted previously and bafilomycin A1 increases LC3 lipidation to a similar degree as autophagy-inducer rapamycin.^{275,276} Conversely, others have confirmed increased $A\beta$ generation associated with preceding autophagosome accumulation in different mammalian cell types.^{277–279}

Autophagy and the proteasomal system are unequivocally colligated in their endeavor to keep proteostasis. Selective transport of target molecules toward degrading vesicles or macromolecular structures like the proteasome requires multifunctional adaptor molecules. Polyubiquitylated proteins can be targeted via p62/SQSTM1 or neighbor of BRCA1 gene 1 (NBR1) for degradation via the proteasomal or the autophagy/lysosomal system.^{280–283} In IBM muscle, both p62/SQSTM1 and NBR1 are upregulated on protein and mRNA level and colocalize with phosphorylated tau in protein aggregates.^{222,284} The cargo protein p62/SQSTM1 can bind Lys63-linked ubiquitin and phosphorylation at Ser403 of p62 enhances the binding affinity of p62 to ubiquitin.²⁸⁵ A recent study demonstrates that aggregated p62/SQSTM1 is largely phosphorylated at Ser403 in muscles of IBM patients and Lys63-linked ubiquitin colocalized with p62/SQSTM1 aggregates, suggesting impaired initiation of selective autophagy targeting ubiquitinated proteins.²⁸⁶

Generally, as a result of active cellular synthesis processes, an unpreventable fraction of misfolded proteins,

the so-called defective ribosomal products (DRiPs) arise and need to be subsequently cleared from the cytosol in order to avoid cell stress and cytotoxicity. In one proposed model, DRiPs are polyubiquitylated and subsequently subjected to proteasomal degradation. The resulting peptides are fed into the MHC class I presenting pathway and will be surveilled by CD8⁺ T cells.²⁸⁷ Upon impairment of autophagy in HeLa cells, its substrates accumulate in p62/SQSTM1-positive aggresome-like induced structures and are fed into the proteasomal pathway with subsequent presentation via MHC class I.²⁷¹ Differential activity of autophagy might, therefore, shape the peptide pool presented on MHC class I and it is tempting to speculate that impairment of this control mechanism in IBM muscle abets invasion of myoaggressive immune cells.

Differential diagnosis can be challenging facing the PM-IBM spectrum of T cell-rich inflammatory myopathies. The recent advances in identifying autophagy as a relevant malregulated process in IBM has already yielded practical application in that using a combination of LC3 (sensitive) and transactive response DNA-binding protein 43 kDa (TDP-43) (specific) stainings was found to be effective in discriminating IBM from PM.²⁸⁸

Interrelationship between inflammation, cell stress and myodegeneration

It has been proposed that muscle invasion of peripheral immune cells and progressive myodegeneration are closely linked in the development of IBM.²⁸⁹ However, the precise sequence of events remains incompletely understood. In IBM muscle, but not in PM or DM, IL-1 β is spatially associated with $A\beta$ and degenerative changes positively correlate with the degree of inflammation in IBM patients.²⁹⁰ Furthermore, exposure of human myotubes to IL-1 β leads to upregulation of APP and accumulation of $A\beta$ *in vitro* and this effect can be synergistically promoted by composite exposure with IFN γ .²⁹⁰ Pro-inflammatory stimuli, such as IL-1 β , TNF- α , and IFN γ augment expression of inducible nitric oxide synthase (iNOS), and composite exposure of murine muscle cells with IFN γ and $A\beta$ peptides provoke robust nitric oxide (NO) production.^{291–293} Expression of iNOS in IBM muscle has been reported several years ago²⁹⁴ and more recently confirmed and extended: iNOS expression and concomitant NO production was enhanced in IBM muscle compared to DM and PM muscle.²⁹⁵ More importantly, nitrotyrosine, the product of tyrosine nitration in the presence of metabolically active NO, colocalized with $A\beta$ in IBM muscle fibers. *In vitro* assays revealed that exposure of primary human muscle cells to IL-1 β together with IFN γ

elicits strong NO production, followed by necrotic cell death.²⁹⁵ Conversely, the pharmacological inhibition of iNOS prevented cytokine-mediated accumulation of A β and necrotic cell death indicative of iNOS being at the interface of proinflammatory stress and degenerative changes in IBM muscle. Taken together, these data revealed a crucial role for IBM-relevant pro-inflammatory mediators in the promotion of amyloidogenesis in the muscle. In double-transgenic MCK-APP/PS1 mice, an animal model for IBM, chronic exposure to inflammatory stimuli significantly increases deposition of the insoluble and cytotoxic A β ₁₋₄₂ in myofibers whereas A β ₁₋₄₀ levels remain unchanged. In addition, chronic inflammation by virtue of TNF- α , IL-6, and IL-1 β , facilitated and enhanced GSK3 β -mediated phosphorylation of tau in myofibers resulting in pronounced motor impairment.²¹⁰

The small heat shock protein α B crystallin is constitutively expressed in human skeletal muscle cells, binds misfolded proteins in order to avert their aggregation and its expression is sensitive to TNF- α -mediated induction.²⁹⁶⁻²⁹⁸ During AD pathology expression of α B crystallin is increased in CNS resident glial cells that are found in close spatial proximity to extracellular amyloid and neurofibrillary tangles.²⁹⁹ Human myotubes exposed to the combination of IL-1 β and IFN γ show marked induction of α B crystallin and APP.³⁰⁰ More importantly, expression of α B crystallin is increased in IBM muscle (and to a lower degree in PM and DM) not only in muscle fibers with structural abnormalities but also in normal appearing myofibers, suggesting an early event in IBM pathogenesis that links pro-inflammatory cell stress to accumulation of aberrant proteins.^{300,301}

The neuronal receptor for advanced glycation endproduct (RAGE) has been implicated in a pro-inflammatory pathway of AD pathology in that binding of its ligand A β facilitates NF κ B-dependent M-CSF production and subsequent chemotaxis of myeloid cells.³⁰² High mobility group box 1 (HMGB1), another ligand for RAGE, is a nuclear DNA-binding protein that can be actively secreted or passively released upon necrotic cell death (but not upon apoptosis) and exert proinflammatory effects by triggering myeloid cells to secrete substantial amounts of TNF- α , IL-1 β , IL-6, IL-8, macrophage inflammatory protein (MIP)-1 α , MIP-1 β .³⁰³⁻³⁰⁵ HMGB1 is expressed and released by human skeletal muscle cells upon muscle injury and via binding to RAGE expressed on the surface of myoblasts facilitates myogenesis and muscle regeneration.^{306,307}

RAGE, in association with reactive oxygen species- and NF κ B-dependent pathways and HMGB1 are overexpressed in myositis.³⁰⁸⁻³¹⁰ In IBM muscle, RAGE and HMGB1 colocalize with A β and neurofilament/tau and composite exposure of human muscle cells with IFN γ

and IL-1 β leads to cytoplasmic translocation and subsequent release of HMGB1 into the extracellular space.³¹⁰ Furthermore, exposure of human muscle cells to exogenous HMGB1 is equally potent in triggering A β accumulation as IFN γ /IL-1 β .³¹⁰ These findings strongly suggest a facilitator role for the HMGB1-RAGE-A β -axis in interconnecting inflammatory and degenerative events during IBM.³¹⁰

In a possible pathological setting, necrosis-undergoing myofibers might release vast amounts of HMGB1. Excessive presence of this mediator might overwrite its pro-myoregenerative function and rather promote protein aggregation in RAGE-expressing muscle cells as well as release of proinflammatory cytokines by infiltrating immune cells. This cascade perpetuates and amplifies the myoaggressive microenvironment in IBM.

Collectively, mounting evidence from in vitro studies, animal models, and human muscle samples suggests that inflammation in IBM can trigger and sustain cell stress in skeletal muscle with subsequent accumulation of unwanted proteins and irreversible muscle fiber damage.

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Conflict of Interest

The authors declare that there is no financial or other relationships that might lead to a perceived conflict of interest.

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