Protein Aggregation in Inclusion Body Myositis, a Sporadic Form Among Protein Aggregate Myopathies, and in Myofibrillar Myopathies – a Comparative Study

ALEXANDRA BASTIAN1, H.H. GOEBEL2

1“Colentina” University Hospital, Department of Pathology, Bucharest, Romania
2Johannes Gutenberg University Clinical Hospital, Department of Neuropathology, Mainz, Germany

Protein aggregation has been identified in muscle fibres and, thus, in certain neuromuscular disorders. There are certain similarities between IBM and DRM: midlife or late-onset clinical symptoms, apparently of both sporadic and genetic background, morphologically autophagocytosis by vacuole formation, which is frequent in IBM though rare in DRM, and presence of tubulofilamentous aggregates, which is almost regular in IBM but scantily found in DRM as β-amyloid components have been identified as accruing proteins, both in IBM and DRM.

Previous studies pointed to the hypothesis that clear morphological borders between the two types of diseases – hereditary inclusion body myopathies/myositis and desmin-related myopathies may not exist. Therefore, we analysed and morphologically characterised the spectrum of proteins accumulating in both types of disorders in order to compare them and more clearly define similarities and dissimilarities between these two different groups of protein aggregate myopathies. Previous studies [7] showed that there is an overlap among some of the proteins accruing in these diseases, but there might also be differences in that a large number of proteins found aggregated in desmin-related myopathies had not yet been described in IBM. The aim of describing the comparative protein profiles is to give more insights into the mechanism of protein aggregation within muscle fibres.

Material & Methods. We studied diagnostic muscle biopsies from 10 sIBM patients and 6 MM patients with histological, histochemical, enzyme histochemical, ultrastructural and immunohistochemical techniques using a large number of antibodies.

Results. We noticed a partial overlap of protein expression in the two cohorts of patients for sarcomeric, chaperone and mostly for cytoskeletal proteins. In both of the cohorts, the nuclear proteins were absent in the cytoplasmic bodies. A different pattern of immunolabelling was noted for trans-sarcolemmal proteins, constantly enhanced in the inclusion bodies in MM, but never found in IBM, except for δ-sarcoglycan, dysferlin and caveolin.

Conclusions. The partial overlap among some of the proteins accruing in these diseases raise the hypothesis that clear nosological borders between s-IBM and MM may not always exist. There are also dissimilarities in the pattern of protein aggregation, suggesting that other additional factors are involved in the pathogenesis.

Keywords: myofibrillar myopathies, desmin-related myopathies (DRM), inclusion body myositis (IBM), rimmed vacuoles.

Sporadic inclusion body myositis (s-IBM) is an inflammatory myopathy, morphologically characterized by varying degrees of inflammatory infiltrates and rimmed vacuoles with or without inclusion bodies made by abnormal aggregates of proteins in muscle fibres. Myofibrillar myopathies (MM)/desmin-related myopathies (DRM), a subgroup in the family of protein aggregate myopathies, are a clinically and genetically heterogeneous group of muscular diseases marked by abnormal accumulation of proteins in muscle fibres. We compared the spectrum of proteins accumulating in both types of disorders.

We studied diagnostic muscle biopsy specimens from ten unrelated patients with s-IBM. In all the cases, the diagnosis was established following combined clinical and morphopathological criteria [1–3]. Three patients were women and seven were men. The age of onset varied from 40 to 75 years. Symptoms at the time of diagnosis consisted of slowly progressing proximal and distal muscle weakness and wasting with depressed or absent tendon reflexes especially in the lower limbs. The pattern of muscle weakness was variable, but mostly distal and asymmetric. In all of the cases the skeletal muscle biopsies were characterized by variable numbers of atrophic or normally sized fibers containing one or more rimmed vacuoles, often associated with a nucleus and best seen by modified Gömöri trichrome stain and muscle fibers.

with cytoplasmic inclusions. The vacuolated muscle fibres were ultramorphologically characterised by intracytoplasmic and, though less frequently, also intranuclear, tubulofilaments with diameters of around 16–21 nm which appeared as paired helical filaments, a morphologic hallmark of IBM. Other common findings at electron microscopic level were myelin-like bodies (membranous whorls) of varying size and shape disrupting the myofibrils and aggregates of mitochondria and aggregates of cytoplasmic inclusions. The vacuolated muscle fibres were characterised by changes in the distribution of cells among muscle fibres or as larger collections around muscle fibres, mostly T lymphocytes around vessels and mainly macrophages near and within muscle fibres. Variation in myofiber diameters, endomysial fibrosis, occasional necrotic and regenerating fibers, angulated atrophic, and muscle fibers showing irregular densities within the sarcoplasm, in subsarcolemmal or centrally located areas, as eosinophilic masses on hematoxylin-eosin stain and dark-blue on modified Gömöri trichrome stain, often devoid of oxidative and ATPase enzyme activities. Phagocytosis, necrosis and basophilia of muscle fibres were absent, as well as COX-negative fibres and inflammatory infiltrates. One ragged red fibre was noted in the deltoid muscle specimen. There was no upregulation of MHC-I on muscle fibres. Immunohistochemically, desmin and other proteins were focally expressed within numerous muscle fibres. Electron microscopy of deltoid and gastrocnemius muscle tissues revealed granulofilamentous material beneath the sarcolemma as well as among the muscle fibrils. In both muscles autophagic vacuoles contained some pseudomyelin lamellae and debris as well as – deposited as aggregates – tubulofilaments of the helical-filament type. The brother’s explanted heart muscle showed variation in muscle cell diameters and numerous densities within muscle fibres which, by immunohistochemistry, contained desmin and numerous other proteins. By electron microscopy, these patches within cardiac myocytes were composed of granular and filamentous material or electron dense material, very similar to the granulofilamentous material within skeletal muscle fibres. Molecular analysis based on DNA extracted from nucleated blood cells revealed a heterozygous novel GAG-GAC mutation in exon 3 of the desmin gene at 2q35 chromosome, resulting in amino acid change Glu245Asp in the desmin molecule, confirmed the morphological diagnosis of desminopathy. This case, published by us in 2005 [6], enlarged the spectrum of known mutations in the desmin gene, but also the molecular spectrum of desmin-related myopathies as well as genotype-morphotype correlations.

The third case of MM was a 67-year old man with proximal and distal muscle atrophy of the lower limbs, difficulty climbing stairs and getting out a chair for the past few years, and a cardiomyopathy with an ejection fraction reduced at 25%. He was clinically diagnosed with a distal myopathy of the Markesbery-Griggs-Udd type. His five year-
younger brother had a ten to twelve year history of slowly progressive weakness first in the feet, subsequently involving his distal upper extremities and carried the diagnosis of s-IBM. On the muscle biopsy, he had numerous rimmed vacuoles, but no endomysial inflammation and no invasion of non-necrotic muscle fibres. The muscle biopsies of our patient, from quadriceps and triceps suralis, showed numerous cytoplasmic inclusion bodies containing desmin and other proteins, necrotic and angular atrophic fibres and many rimmed vacuoles.

Patient 4 was a 42 year-old man with a rigid-spine syndrome, muscle weakness when climbing stairs or walking long distances, myopathic EMG and elevated CK level. He had a younger brother also affected. The biopsy from biceps brachialis muscle revealed numerous cytoplasmic bodies and autophagic-rimmed vacuoles with tubulofilamentous aggregates. The cytoplasmic bodies stained positive with anti-desmin antibodies and others. The molecular analysis showed no mutations in desmin, αB-Crystallin or Selenoprotein N1 genes.

The fifth patient was a woman aged 62 years with weakness in her lower limbs for two years, a myopathic EMG and elevated CK level. Her biopsy muscle specimen from triceps suralis muscle showed atrophic and hypertrophic fibres, increased number of internal nuclei and areas of condensation of the sarcoplasm indicating myofibrillary myopathy, reacting with numerous antibodies.

Patient 6 was a 74 year-old woman clinically diagnosed with a proximal myopathy of the lower limbs. Her skeletal muscle biopsy from quadriceps femoris muscle revealed abnormal deposits in the muscle fibres, located mainly subsarcolemmaly, but also in the center of the fibres, as well as lobulated muscle fibres, numerous core-targetoid lesions and rare ragged red fibres. By electron microscopy, she had areas of Z-disk streaming and granulofilamentous material between the myofibrils in her muscle biopsy tissue specimen.

RESULTS

EXPRESSION OF TRANSSARCOLEMML PROTEINS

In eight of the ten patients with s-IBM we found normal subsarcolemmal staining for dystrophin, only two cases showing mild sarcoplasmic increase as granular deposits. In all the cases with MM we found ectopic cytoplasmic expression of dystrophin in structurally abnormal fibres, that clearly indicate dystrophin as a useful immunocytochemical marker for abnormal regions in MM.

In normal adult muscle, utrophin is located at the neuromuscular synapse and myotendinous junctions, where it participates in post-synaptic membrane maintenance and acetylcholine receptor clustering. In four of our six patients with MM, utrophin was found aggregated within cytoplasmic bodies, as well as along the membrane in some of the regenerated fibres. Only four of our ten s-IBM cases showed areas of increased DRP 2 expression along the sarcolemmal surface, but utrophin was not found aggregated in the cytoplasm of the muscle fibres.

We studied the profiles of four members of the sarcoglycans complex, as part of the dystrophin-based membrane cytoskeleton of muscle fibre. Its normal function being the stabilisation of the transmembrane β-dystroglycan protein with dystrophin, we were interested in their pattern in the two types of diseases. We found variable expression of the four sarcoglycans in the MM cases, more consistent, among them, for δ-sarcoglycan in the sarcoplasmic inclusions and all the cases showed normal sarcolemmal expression. In the s-IBM cohort α, β and γ sarcoglycans were never found accruing in inclusion bodies or elsewhere in the sarcoplasm of vacuolated or normal appearing fibres, but were constantly positive along the sarcolemmal surface. Surprisingly, δ sarcoglycan was expressed not only at the periphery of the fibres, but in seven of the ten cases also in cytoplasmic bodies and at the rim of vacuoles as granular deposits.

Laminin α 2 (merosin), major component of the myofibre basal lamina, interacts in normal muscle fibre with the plasma membrane and mediates interactions between the basal lamina and the endomysial connective tissue. In our patients with s-IBM, the two isoforms of merosin were never found aggregated elsewhere than in the normal location at the basal lamina. On the contrary, we found abnormal accumulation of merosin within the muscle fibres in five of the six cases of MM.

The expression of α and β dystroglycans, that bind dystrophin intracellularly and laminin extracellularly, thus forming a critical link between the extracellular matrix and the cytoskeleton, was found normal in all our studied patients from the s-IBM lot. In five of the six cases of MM, abnormal accumulations of α and β dystroglycans were detected in cytoplasmic bodies within muscle fibres.
Collagen 6, component of the extracellular matrix, with its important role in anchoring basal lamina to the endomysial connective tissue, was normally expressed in all our s-IBM cases, as well as in all cases with MM. In two of the latter group of patients we found several additional foci of immunopositivity in the sarcoplasmic inclusions.

Normal expression of β and γ laminins was found in all s-IBM specimens; two of the six patients with MM showed limited areas of abnormal accumulation in the sarcoplasm.

The study of dysferlin showed in all cases, from both cohorts, apart from normal immunostain on the plasma membrane, constant increased expression. In muscle fibres of patients with s-IBM, dysferlin was expressed in small and large vacuolated fibres, appearing as granular deposits bordering vacuoles, as diffuse accumulation in the sarcoplasm and even inside the vacuoles. Immunoreactivity of dysferlin was observed as positive subsarcomerrial aggregates and intracytoplasmic inclusions in all MM cases.

Caveolin, a major protein of plasmalemmal microdomain caveolae, is a proven intracellular transporter of cholesterol, thus influencing its homeostasis. In IBM muscle fibres, caveolin immunoreactive inclusions were observed in vacuolated fibres as plaque-like deposits or diffuse accumulation, but were also noticed in regenerated and necrotic fibres. In the muscle fibres of the MM cohort, caveolin showed focal increased immunostain under the sarcolemma and in centrally located areas in all the cases.

The expression of n NOS (neural nitric oxide synthase) in the muscle fibres was found increased in all patients from MM cohort as part of the cytoplasmic bodies and in eight of ten patients with IBM as granular or compact foci of immunopositivity in the cytoplasm of vacuolated muscle fibres, more pronounced at the rim of the vacuoles.

Expression of the Sarcomeric Proteins

Actin, the major component of thin myofilaments, has been previously found in lesions of desminopathies by some investigators [14]. In our study, actin was found accumulated in cytoplasmic bodies in all cases with MM and in nine of ten cases with s-IBM as diffuse accumulation in the cytoplasm, but more consistently expressed at the border areas of the vacuoles.

α actinin is a physiological protein component of the Z-bands, cross-linking actin filaments. Electron microscopic findings showed excessive involvement of Z bands and so of α actinin in the hyaline structures of desminopathies, but in another extensive study this was only occasionally expressed by immunohistochemistry [14]. All our MM cases showed consistent expression of α actinin in the sarcoplasmic inclusions, similar to the positive immunostaining found in all ten IBM cases in vacuolated muscle fibres, mostly at the periphery of vacuoles and as patchy cytoplasmic deposits.

In our comparative study we were especially interested in the expression of myotilin as a recently discovered Z-disk-associated key protein localized along the sarcolemmal membrane and within I bands that control sarcomere assembly, cross-links actin filaments and binds to α actinin and γ-filamin. Recently, mutations in myotilin were found in myofibrillar myopathy [4]. An extensive study on 63 patients of MM described, for the first time in the literature, increased myotilin expression in 90% of the fibres that were abnormal in trichrome-stained sections and suggested that myotilin is the most reliable immunocytochemical marker for abnormal fibre regions in MM [4]. In fact, all our muscle biopsy fibres from MM patients expressed myotilin in the sarcoplasmic inclusions, as all the cases with IBM had increased myotilin immunostain in the cytoplasm of vacuolated fibres in the vicinity of vacuoles and even in normal appearing fibres as irregular areas of deposition.
EXPRESSION OF CYTOSKELETAL PROTEINS

Desmin is located in mature skeletal muscle between the subsarcolemmal region and the nuclear membrane, associated with lamin B and around the myofibrillar Z discs, encircling and interconnecting myofibrils at this level, thus aligning myofibrils and linking them to nuclei, to the plasma membrane, especially in the region of the costameres and to cytoplasmic organelles such as mitochondria. In the heart, desmin is increased in Purkinje fibres, as a major component, and at the level of intercalated discs. In our immunohistochemical study we found in each muscle specimen of patients with MM a markedly increased expression of desmin in intrasarcoplasmic deposits, ultrastructurally with a granulofilamentous aspect. The accumulation of desmin in IBM is still controversial in the literature; desmin has been shown to accumulate abnormally, among other proteins [7]. Other study found normal expression of desmin in hypertrophic and normal sized muscle fibres in all patient biopsies [16]. Our study showed in all patients strong expression of desmin, more obvious at the rim of vacuoles, as diffuse or patchy accumulation in the cytoplasm of vacuolated and even normal appearing muscle fibres, as well as in small regenerated fibres.

Plectin is a highly conserved and ubiquitously expressed intermediate filament – associated protein concentrated at sites of mechanical stress, such as the hemidesmosomes in skin, the Z disk of skeletal muscle fibers and the intercalated disks in cardiac muscle cells. Plectin is also normally associated with the sarcolemma, the postsynaptic membrane, the nuclear membrane, and the intermyofibrillar network of skeletal muscle. Plectin is known to associate with vimentin, integrin, desmin, lamin B, myosin II, vimentin and actin. Plectin is also associated with mitochondria and is important in the localization of intracytoplasmic organelles. We analysed the expression of plectin in three MM cases and we found in all of them increased immunostain in abnormal fibres. All the patients with s-IBM had conspicuous plectin deposits in inclusion bodies of vacuolated muscle fibres, as well as in normal appearing fibres.

Vimentin, a member of the intermediate filament family, is absent in healthy mature muscle fibres, being expressed only in developing muscle, colocalized with desmin, and in regenerating muscle fibres. We found increased expression of vimentin in five of the six MM cases in the sarcoplasmic inclusions as well as in regenerated fibres. Nine of ten patients with IBM also showed increased expression of vimentin in small and degenerated fibres, but also in vacuolated fibres.

EXPRESSION OF CHAPERONE PROTEINS

αB-crystallin is a member of the small heat shock protein family, which exerts a role as molecular chaperones by binding unfolded or denatured proteins, suppressing irreversible protein aggregation and consecutive cell damage, their essential role in neuromuscular disorders being corroborated by the observation that a mutation of the human αB-crystallin gene causes an autosomal dominant myofibrillar myopathy morphologically characterized by αB-crystallin and desmin accumulation and granulofilamentous material by electron microscopy. We found strong expression of αB-crystallin in sarcoplasmic inclusions in all MM cases, colocalized with desmin, as well as in all IBM cases, in the vacuolated muscle fibres at the border of vacuoles and in inclusion bodies, but also in normal appearing fibres, as previously suggested.

The selective degradation of many short-lived proteins in eukaryotic cells is carried out by the ubiquitin system. Abnormalities in ubiquitin-mediated processes have been shown to cause pathological conditions. In our study we observed accumulation of ubiquitin in all cases with MM, two of them showing strong aggregation in the cytoplasmic bodies, in the other the increase was only mild. Muscle biopsy specimens from IBM patients also revealed enhanced expression of ubiquitin in vacuolated fibres and, focally, also in nonvacuolated ones. These findings indicate that muscle fibres contained undesirable protein material targeted for non-lysosomal degradation, but the mild ubiquitin positivity in some of the cases suggest operation of an alternative pathway of protein degradation.

Heat shock proteins play an important role in protein-protein interactions, including folding and assisting in establishing proper protein conformation, and prevention of inappropriate protein aggregation. Heat shock proteins are synthesized under different stress conditions and act as molecular chaperones for protein molecules. We found increased expression of HSP 72/73 in all our MM cases as well as in all IBM muscle biopsy specimens. In the first group, HSP 72/73 was mainly found at
subsarcolemmal and central sites in muscle fibres; in the latter group, HSP 72/73 was enhanced in degenerated and regenerated muscle fibres and in inflammatory infiltrates invading muscle fibres, as well as in vacuolated fibres, more conspicuous around vacuoles.

DISCUSSION

Our study shows, as previously expected, some similarities and partial overlaps in protein expression between MM and IBM, but also some dissimilarities.

The mechanisms responsible for formation of the multiprotein inclusions in IBM muscle are not understood, although the histopathology of s-IBM has been well described, but it seems that unfolding and misfolding of proteins probably play a role, as well as the cellular aging that promotes accumulation of abnormal proteins and slows degradation of normal and abnormal proteins.

Only a minority of MM cases were, up to now, shown to be caused by mutations in desmin, alpha B-crystallin, myotilin and selenoprotein N1 genes, suggesting that the majority of them are due to yet unidentified gene defects or are non-genetic at all, also requiring further mutational analyses of other genes such as for paranemin, synemin and syncoilin. On the contrary, IBM is a sporadic disease. Both disorders share accumulation of different types of proteins, indicating a partially common pathogenesis.

Accumulation of extrasarcomeric cytoskeletal proteins was a common feature in both diseases. We found increased expression of desmin, alpha B-crystallin and plectin in all the muscle biopsies, showing a marked disturbance of filamentous inter-myofibrillar cytoskeleton, with its important role in structural and functional maintenance of striated muscle fibres in response to stress. Thus, we amplified the findings of other investigators [9] who described desmin and alpha B-crystallin accumulation in both MM/DRM and IBM. We found accretion of plectin, with its essential role in the proper spacing, stabilization and subcellular attachment of intermediate filaments. We also confirmed the occurrence of increased immunomarcation of alpha B-crystallin in abnormal vacuolated muscle fibres, as well as in the normal appearing fibres in IBM, as described by Banwell et al., [9]. Heat shock protein 72/73, a “stress marker”, was also found overexpressed in both disorders. We observed a similar expression of sarcomeric proteins in both diseases, as actin and alpha actinin aggregates. Concerning the expression of myotilin, a recently discovered protein that, when mutated, causes MM, we found in all the cases increased accumulation in the cytoplasm of vacuolated fibres in IBM patients, as well as in the sarcoplasmic inclusions in our DRM/MM specimens, thus supporting the suggestion that myotilin is the most reliable immunocytochemical marker for abnormal regions in the muscle fibres in MM [4]. Our study shows additional abnormal myotilin immunomarcation in the vacuolated muscle fibres in IBM.

Only normal immunolabelling of nuclear membrane proteins was encountered in both diseases, but there are data that these proteins may be found aggregated in sarcoplasmic bodies in MM. Further studies may eventually correlate these accretions with a particular genetic profile.

The pattern of transsarcolemmal protein immunolabelling showed, in our studied cases, consistent dissimilarities. We found prominent dystrophin colocalisation with desmin aggregates in MM cases, our IBM cases always showing a normal immunolabelling at the sarcolemmal level. The same pattern was found for alpha and beta dystroglycans. The sarcoglycans alpha, beta and gamma were always normally expressed in the muscle fibres of IBM patients, but occasionally aggregated in the cytoplasmic bodies in MM. Delta sarcoglycan was markedly increased in MM specimens, and occasionally also in IBM specimens. Merosin showed normal expression in IBM, but was frequently found coaggregated with desmin in MM cases, as were beta and gamma laminin. Dysferlin and caveolin were overexpressed in both MM and IBM cases. These observations point to a more severe impairment of the sarcolemmal architecture in MM than in IBM.

CONCLUSIONS

1. There is considerable aggregation of proteins in both IBM and DRM.
2. A large number of same proteins accrue in both groups of conditions.
3. In each of the two groups, however, there are proteins aggregated only in one group, not in the other.
4. In both groups of disorders, impairment in extralysosomal protein degradation is an important pathogenetic principle, high-
lighted by the common involvement of the chaperone protein α-B crystallin and by the involvement in both groups of disorders of proteins engaged in the ubiquitin proteasomal degradative pathway of proteins. While DRM consist of both desminopathies, i.e. desmin-accumulating myopathies, and other forms related to mutations in other genes such as selenoprotein N1, and myotilin, suggesting that mutant proteins form part of the protein aggregates, no such mutant proteins have been identified in h-IBM.

While s-IBM is considered a sporadic and acquired condition, among patients with DRM, a non-hereditary form has also been suggested but, perhaps, will be more difficult to prove as a fair number of genes in DRM may indicate that there are still unidentified genes involved as well and, thereby, recognizing true acquired DRM most exclusively by exclusion of any hereditary form.

Acknowledgment: A.B. was supported by a fellowship of the European Neurological Society (ENS).

Corresponding author: Alexandra Bastian
“Colentina” Clinical Hospital, 19, Şos. Ștefan cel Mare, Bucharest
E-mail: aleca.bastian@yahoo.com

REFERENCES


Received August 8, 2010
Fig. 1. – Immunocytochemical aspects in inclusion body myositis.
Fig. 2. – Immunocytochemical aspects in mm.