

Theories of the Pathogenesis of Inclusion Body Myositis

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Abstract Inclusion body myositis is a progressive disease of the skeletal muscle. Here, specific theories of its pathogenesis are reviewed and general considerations pertaining to modeling of this disease discussed. Understanding of inclusion body myositis disease mechanism remains extremely poor. Current published animal models do not represent the disease. Future studies need to consider the critical role of biomarkers and methodologic issues in their discovery.

Keyword Inclusion body myositis

Introduction

Inclusion body myositis (IBM) is a poorly understood disease affecting skeletal muscle. Its distinctive clinical features (onset at age 40 years or older, preference for asymmetric finger and wrist flexors and quadriceps muscles) and pathology, characterized by impressive myofiber abnormalities (termed *degeneration*), and immune system cell infiltration have yielded many ideas regarding its disease mechanisms. Enigmatic of the confusion in this field, even the name IBM derives from a case report of a patient who did not have the disease (a 26-year-old with quadriceps sparing and a severe limb-girdle pattern of weakness since age 18 years) [1]. Several recent reviews have addressed its potential disease mechanisms [2, 3–6, 7].

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Specific Theories

Myofiber Injury by β -Amyloid

The belief that the protein β -amyloid ($A\beta$) is produced by IBM myofibers and injures them is widely believed to be fact, having been stated as such in at least 200 PubMed-indexed papers as of October 2007. According to this theory, unknown factors result in the overproduction by myofibers of the mRNA of the $A\beta$ precursor protein (β APP), followed by translation of this transcript to the β APP protein itself, and the cleavage of this protein into $A\beta$ peptide. The overabundance of $A\beta$ inside of myofibers is then theorized to cause myofiber degeneration, atrophy, and death through a variety of mechanisms of “toxicity” [8]. Congo red staining material (“amyloid”) in IBM myofibers is an entirely distinct issue, and this material, whose nature is likely diverse but certainly unknown, should not be confused with the protein $A\beta$ simply because both substances share a common word in their names.

Several concerns about the scientific foundation for the $A\beta$ toxicity IBM theory and the nature of the widespread published belief in it have been reported [4, 9•]. For example, no Western blot study demonstrating the claimed presence of $A\beta$ in IBM muscle (let alone blots comparing IBM with other muscle diseases) has ever been published, a remarkable fact given the claim's wide acceptance. Claims regarding $A\beta$ presence in IBM muscle continue to be made using immunohistochemical reagents (eg, the 6E10 antibody) that react with β APP [10] despite knowledge of this technical pitfall [9•, 11], a critical issue because the protein β APP has been reported previously as being nonspecifically present in regenerating myofibers in all muscle diseases [12, 13]. However, it is examination of the central claim of this theory, that β APP transcript is overproduced

by IBM myofibers, that most succinctly outlines its critical problems.

The central problem with the claim that β APP transcript is specifically overproduced by IBM myofibers is that it has no supporting evidence and was contradicted by even the most vigorous current proponents of this theory. The same laboratory reporting that β APP transcript was overproduced in IBM myofibers in a 1993 publication [14] reported in a 1994 publication [13] that it was similarly overproduced in regenerating muscle fibers in all muscle diseases studied, including polymyositis, dermatomyositis, amyotrophic lateral sclerosis, and Duchenne muscular dystrophy (“the pattern and intensity of β APP-mRNA expression in regenerating muscle fibers did not differ in relation to the diagnosis or age of a patient”) [13]. However, as of October 2007, the 1993 publication had received 54 citations from other papers supporting claims of this transcript’s overproduction in IBM, whereas the 1994 publication had been cited only four times [9••].

The only independent attempt to confirm the 1993 publication reporting IBM myofiber β APP transcript overproduction using the same methodology (in situ histochemistry) found the method technically unreliable in IBM because of the presence of one or more sarcoplasmic nucleic acid-binding proteins (“in contrast to the findings of Sarkozi et al., we were unable to show an increase in mRNA for beta-amyloid precursor protein in IBM fibers by using antisense RNA probes”) [15]. This article, whose authors included the late George Karpati, is the most important IBM mechanistic paper published in the 1990s and early-2000s (see the subsequent “Nuclear Abnormalities” discussion), yet it has rarely been cited by the neuromuscular community despite thousands of citations made to other IBM mechanistic papers [9••].

No quantitative studies of β APP transcript abundance in IBM and appropriate control samples were reported until almost a decade later; these microarray studies showed no difference in β APP transcript abundance in IBM compared with polymyositis [16]. Not until 2008 were the first polymerase chain reaction-based studies of β APP transcript in IBM muscle reported [17], and these showed no difference between the transcript’s abundance in IBM and polymyositis, with even greater amounts of β APP transcript in dermatomyositis than IBM. By this time, nine publications had already reported β APP transcript-overproducing animals as models of IBM [18–26]. How this theory could have progressed to a point in which transgenic animals overproducing β APP transcript could be used as models of IBM without the most basic polymerase chain reaction studies examining whether β APP transcript was even specifically overproduced in IBM muscle is a remarkable story in its own right, a story understood through a study of the citation network around this theory [9••].

Myofiber Injury by Tau

At least 41 publications have stated that accumulation of phosphorylated forms of the microtubule-associated protein tau occurs in and contributes to the pathogenesis of IBM [27•]. IBM has been called a “tauopathy” [28], and the use of lithium in clinical trials for patients with IBM has been recommended based on reductions in tau pathology in β APP transgenic mice treated with lithium [25].

Evidence that tau is present in IBM myofibers is entirely limited to its immunohistochemical detection by antibodies (“tau” immunoreactivity), with the most commonly used one being SMI-31. What is remarkable about this literature is that immunoreactivity with this antibody has repeatedly been used to claim tau presence even though this antibody’s primary target (to which it was developed) is a different protein, phosphorylated neurofilament H. Recent studies have provided evidence that “tau” immunoreactivity is present in normal muscle myonuclei and that some or all of this immunoreactivity is due to the presence of neurofilament H [27•]. All literature claiming that SMI-31 immunoreactivity demonstrates phosphorylated tau in IBM myofibers is incorrect. There is no evidence that tau plays any role in the mechanism of myofiber injury in IBM.

Myofiber Injury and Other Accumulated Molecules

Many other molecules have been reported to accumulate in IBM myofibers and have been suggested to play a role in IBM myofiber injury. These include, but are not limited to, the following (references not included here because of space limitations): prion protein, ubiquitin (and mutant ubiquitin), cathepsins B and D, α 1-antichymotrypsin, α B crystallin, desmin, cdk5, semicarbazide-sensitive amine oxidase, presenilin-1, α -synuclein, ApoE, redox factor-1, BACE1, BACE2, low-density lipoprotein receptor, very low-density lipoprotein receptor, liver-regulating protein, rapsyn, nicotinic acetylcholine receptor, transforming growth factor- β 1, fibroblast growth factor, fibroblast growth factor receptor, cystatin C, neuronal nitric oxide synthase, inducible nitric oxide synthase, nitrotyrosine, insulin-like growth factor-1, Hsp27, Hsp40, Hsp65, Hsp70, superoxide dismutase-1, interleukin-1 α , interleukin-1 β , interleukin-6, calnexin, calreticulin, glucose-regulated protein-78, glucose-regulated protein-94, endoplasmic reticulum protein-72, extracellular signal-regulated kinase, Vaccinia virus complement control protein, clusterin, γ -tubulin, synemin, Bcl-2, Bax, LMP2, LMP7, MECL1, myostatin, glycogen synthase kinase-3 β , RNA polymerase II, survival motor neuron protein, NOGO-B, neprilysin, nuclear factor- κ B, transglutaminase 1 and 2, γ -tubulin; the 19S, 20S, PSMB8, PSMB9 proteasome subunits, HERP, parkin, RNF5, casein kinase-1a, and sequestosome-1.

The validity and meaning to IBM pathogenesis for many of these biomarkers is uncertain. Many of these studies are incomplete in defining the significance of these biomarkers, lacking disease control studies or quantitation. For example, RNF5 overexpression was interpreted from immunohistochemical studies and formed the basis for a transgenic RNF5 transcript-overproducing murine model claimed to represent IBM [29]. However, RNF5 transcript levels were not studied in IBM, and no studies were conducted in any other inflammatory myopathy samples, such as polymyositis or dermatomyositis. What if RNF5 was overproduced in all inflammatory myopathies, or many other muscle diseases? Many reports, including disease control studies, have stated ambiguously that various accumulated molecules were not present in “immunoreactive inclusions that were characteristic of IBM” [9••], leaving open the possibility that these molecules were present in disease control myofibers in other visible patterns; the statement of the control results is a tautology because the control diseases do not have “inclusions.”

Myofiber Injury by the Immune System

IBM muscle usually contains an impressive adaptive immune system response [30]. Whether the immune system contributes substantially to myofiber injury is uncertain. In considering this area, it is important to recognize implications of cellular constituents of the immune system (generally what is called *inflammation* based on the microscopic visualization of cells) and soluble secreted molecules of the immune system present, but not visible by microscopy, in IBM muscle.

T Cells

The invasion of apparently viable IBM myofibers by cytotoxic T cells has been emphasized since the mid-1980s [31]. A great deal of evidence indicates that these T cells have been stimulated by antigen and developed through successive generations highly specific antigen-directed T-cell receptors [reviewed in 30]. Although the presence of cytotoxic T-cell myofiber invasion has been widely emphasized, this occurs in a relatively small number of myofibers based on cross-section examination, and many IBM biopsies show far greater numbers of CD4⁺ T cells (not cytotoxic) surrounding and pushing apart, but not invading, myofibers. Many morphologically abnormal myofibers typically have no nearby T cells visible on cross-sections. Whether these T cells are injuring muscle (eg, through secretion of soluble molecules) or contributing to other immune cell myofiber injury is unknown.

B Cells

Although B cells as defined by the surface markers CD19 and CD20 were long thought to be sparse or absent from IBM muscle, recent studies have shown that differentiated B cells (CD138⁺ antibody-secreting plasma cells) are not only abundant in IBM muscle but are transcriptionally active, producing and secreting immunoglobulins within muscle, and that these immunoglobulins are from clonally expanded, highly refined antigen-directed plasma cells [32, 33•].

Although the consequences of such antibody production are unknown, the key insight gained from these discoveries is that they open the door to possibly identifying antigens against which both T and B cells may be directed because of the principle of linked recognition (B-cell-aided maturation of T cell requires that both B-cell immunoglobulin and T-cell receptors recognize the same molecular complex). The use of patient-derived antibodies for antigen identification is a technically easier strategy than T-cell approaches. This strategy has been used successfully, identifying an immune response against α B crystallin in several patients with IBM [34]. α B Crystallin previously had been identified as a molecule of interest in IBM because of its distinctive immunohistochemical appearance in IBM compared with other inflammatory myopathies [35].

Soluble Immune Molecules

IBM muscle is likely an environment rich in soluble immune cell-secreted proteins. Certainly the RNA transcripts of such immune molecules are greatly amplified in IBM muscle [16]. Studies of their proteins are hampered by technical challenges: most of these are likely washed away during the preparation of immunohistochemical sections. The accurate measurement of cytokine proteins in IBM muscle by other methods is fraught with difficulties. The mechanistic consequences of this likely cytokine-rich environment, containing particularly abundant interferon- γ and possibly tumor necrosis factor- α based on transcript studies and the abundance of T cells and macrophages present, are unknown.

Nuclear Abnormalities

Nuclear abnormalities and their implications in IBM recently have been reviewed [7•]. The first published reports delineating distinct pathological features of IBM from polymyositis were written by Chou [36, 37] in 1967 and 1968. These emphasized substantial myonuclear abnormalities that were further detailed by Carpenter and colleagues in 1978 [38] and between 1993 and 1996 [15, 39, 40]. These investigators formulated a hypothesis that rimmed vacuoles, a feature that distinguishes IBM from

polymyositis on hematoxylin- and eosin- and trichrome-stained muscle sections, derived from the breakdown of myonuclei. Between 1996 and 2007, few published papers mentioned these data. No review papers, typically the most influential type of publication in shaping opinion, including at least 31 written during this period, mentioned the existence of these data or their implications.

Most rimmed vacuoles are lined with nuclear membrane proteins, suggesting they frequently derive from myonuclear breakdown [41]. Further evidence for this hypothesis is reviewed elsewhere [7•]. Fifteen years ago, experiments attempting (and failing) to confirm claims of specific A β precursor protein transcript abundance instead found a nucleic acid-binding protein lining vacuoles of some IBM myofibers [15]. The recent discovery of the nucleic acid-binding protein TDP-43 in IBM non-nuclear sarcoplasm is a major advance in this long dormant theory [42•, 43••, 44, 45]. Abnormalities in the distribution of TDP-43 in IBM myofibers with fluorescent microscopy are the most impressive of all microscopic IBM biomarkers I have seen, present in a mean of 23% of IBM myofibers, most of which appear morphologically normal or only minimally abnormal on parallel hematoxylin and eosin sections [43••]. In these fibers, TDP-43 has redistributed from its normally nuclear location to the sarcoplasm.

The mechanisms and consequences of TDP-43 redistribution from myonuclei to sarcoplasm in a high percentage of IBM myofibers are uncertain. Abnormal accumulation of extranuclear TDP-43 may lead to deleterious interaction with or sequestration of certain particular mRNA or microRNAs, and affect the translation of specific proteins in IBM.

Future Study of IBM Pathogenesis

There are lessons to be learned from several decades of research into IBM disease mechanism that has not disclosed fundamental insights benefiting people. Some of these are outlined here.

Animal Models in IBM: Progress or Circularity?

Thirteen publications have reported animal experiments as actual or potential models of IBM [18–26, 29, 46–48]. It is my opinion that physicians have inordinate reverence for such animal models and need to consider more critically their validities and potential utilities for delivering translational results to patients. Reasonable animal models based on unequivocal temporally and causally defined events (such as DNA mutations, infectious organisms, or mechanical trauma) can potentially be useful in the study of disease mechanism. Because in IBM, no such temporally and causally defined

events have been defined from the study of human tissue samples, two approaches have been used to justify animal models: circularity and invention of temporality.

Circularity involves the production of an animal model and then the claim that “it looks like” the human disease to persuade others that the animal model includes the “key” disease-causing molecular events. For example, animals are genetically engineered to overproduce β APP, their tissues examined to show β APP overproduced, and they are then confirmed as IBM models because IBM also is reported as having β APP in muscle. In another example, histochemical studies from animals fed high-cholesterol diets containing abundant artifact have been interpreted as resembling the degenerative changes of IBM myofibers [47].

Invention of temporality has been carefully documented for the claim that A β accumulation occurs early and precedes other abnormalities in IBM muscle [9••]. Initially stated as hypothesis in 1992 and 1993, 27 papers as of October 2007 have made this temporal claim, which spontaneously transformed from hypothesis to fact through citation alone, a process called *citation transmutation*. Authors have repeatedly stated it as fact (eg, “the appearance of A β -positive nonconophilic deposits precedes vacuolization in IBM muscle fibers”), supporting these statements to citations to papers in which it had only been proposed as hypothesis (“may represent early changes of IBM”). This invented temporal claim has been used to justify National Institutes of Health funding for animal experimentation [9••].

Until such time as IBM temporally and causally defined events are defined through further study of human disease biomarkers, animal models are highly unlikely to be productive endeavors for individuals with IBM. However, paradoxically, animal models, with little dependence on their validity, are very productive for biomedical researchers, aiding the ability to publish papers and obtain research funding. Because they allow for hypothesis-driven research (even on the wrong hypotheses), they allow for the use of key ingredients for successful grant proposals: carefully controlled scientific experiments with exciting novel technologies.

The Crucial Role of Biomarkers

Biomarkers are biological observations associated with disease. For example, dystrophin DNA sequence variants in blood cells are biomarkers of Duchenne muscular dystrophy, and serum creatine kinase is a biomarker in some disorders of cardiac and skeletal muscle.

All theories of IBM pathogenesis have relied critically on muscle biomarkers. Biomarker specificity is the key issue for the future. Finding molecular biomarkers that distinguish IBM muscle from both other inflammatory myopathy muscle (polymyositis and dermatomyositis) and from a range of

other myopathies containing rimmed vacuoles would advance mechanistic understanding. Great care needs to be taken in establishing the validity of biomarkers, and three issues need particular attention: immunohistochemical methodology, the use of controls, and quantitation.

Immunohistochemical Methodology

Immunohistochemical approaches have dominated the field. Assuming that immunoreactive signal indeed represents target can be very problematic, as just because an antibody was raised against a specific protein does not ensure that its binding in muscle indicates the presence of that protein. Interpreting antibodies known to react to multiple proteins as indicative of the presence of a specific protein is an invalid practice that has pervaded the field (eg, 6E10 indicative of A β , though it reacts also to β APP; SMI-31 indicative of tau, though it reacts to neurofilament H and is stated as such on its manufacturer's datasheet).

The interpretation of artifact as immunohistochemical signal is a major problem in the field. Many published images in the IBM literature appear to me to show artifact, not immunohistochemical signal. The problem of autofluorescing material visible in both red and green channels without the presence of primary antibodies is particularly concerning (Fig. 1), as many published images have been interpreted as demonstrating this material as immunoreactive and have been used to conclude that certain proteins aggregated in IBM myofibers. It is not sufficient to simply state in methods that autofluorescence was excluded; claims of fluorescent immunoreactive material need to be sup-

ported by single fluorochrome staining accompanied by images using both red and green fluorescent filter sets (Fig. 1).

Disease Controls

Appropriate controls in all studies are essential. Demonstrating that a given biomarker is increased in IBM compared with normal muscle is of limited value. What needs to be demonstrated is that the biomarker differs in IBM from an appropriate range of other diseases. How else can we know whether the biomarker is key to IBM? The consequences of not performing disease control studies are substantial for IBM research. Why are not transgenic muscle-overproducing β APP animals reported as models of amyotrophic lateral sclerosis rather than IBM, as β APP was easily demonstrated as fourfold increased in amyotrophic lateral sclerosis muscle Western blots [49]? Why are RNF5 transgenic animals interpreted as models of IBM based on immunohistochemical studies of RNF5 in IBM alone, when RNF5 transcript elevation has not been reported in IBM, and RNF5 transcript and protein not even studied in other inflammatory myopathies [29]?

Quantitation

Is it sufficient to report that a molecule is "present" in immunohistochemical studies of IBM myofibers and interpret it as mechanistically important, but not to report that it was seen in only 3 of 1,000 myofibers examined? Frequently ignored issues of quantitation are crucial to

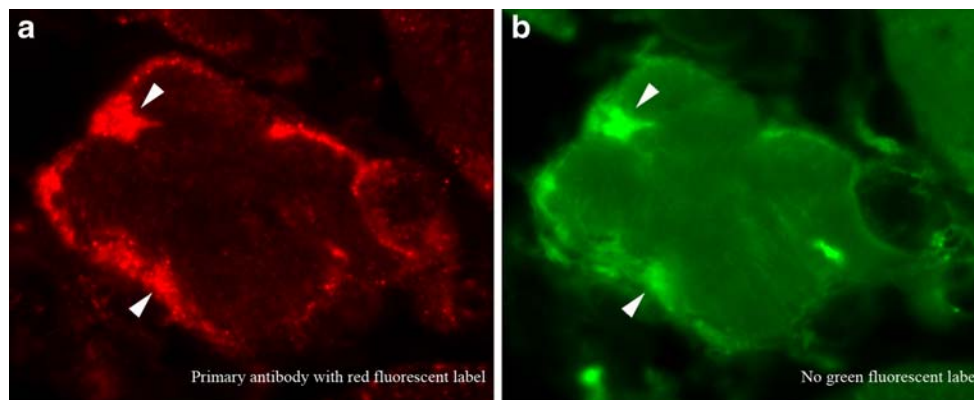


Fig. 1 The potential for misinterpretation of artifact as protein aggregates in inclusion body myositis (IBM) myofibers. Immunofluorescence microscopy using a primary antibody (not disclosed here) with a red fluorochrome (**a**). The IBM literature is filled with similar images interpreted as demonstrating the accumulation of a specific protein. The same image viewed through a green fluorescent filter set (**b**), however, confirms that the material producing most, if not all, red

fluorescence seen in (**a**) also emits green fluorescence. Because no green fluorescent molecules were used in this experiment, this material is autofluorescent. No protein aggregates can be concluded to be present. Had these studies been performed with a second green fluorescent-labeled antibody, they may have been interpreted incorrectly as showing colocalization of two different proteins in IBM aggregates. *Arrowheads* highlight some of the autofluorescent regions

understanding the value of a biomarker to disease mechanism. For example, reporting on studies of muscle from IBM patients that A β was present in “almost 100% of their vacuolated fibers” is difficult to interpret when unaccompanied by a statement as to how many vacuolated fibers were seen in those same samples. As some of the same laboratory’s samples contain “only two or three vacuolated muscle fibers” [9••] (see discussion at <http://www.bmj.com/cgi/data/bmj.b2680/DC1/1>, page 10), and typical biopsy sections contain 1,000 or more myofiber cross-sections, the percentage of A β -containing myofibers in such patients may be actually less than 0.3%. Does this degree of presence justify the status of A β as a disease-causing biomarker? Does it support claims of “the universal presence of A β in IBM” and that it is a “hallmark” of the disease [11]? Can studies reporting no visible β APP or A β in three of five IBM patient muscle biopsy samples and its presence in “only a few fibres” in the other two samples be validly cited as showing “ β APP in IBM fibers has been confirmed by others” [9••]? Quantitation matters for biomarker significance.

Trends: Good for Researchers, Bad for Patients

Two roads lie before researchers contemplating the pursuit of relevant disease mechanisms in IBM: pursue unbiasedly generated or fortuitously found clues, or join a trend. The former is more likely to produce scientific advances helpful to patients, the latter safer and more likely to produce grant funding and successful publication helpful to the researcher.

Trends are very powerful mechanisms for successful publication and funding. For example, at the time of this writing, there were 100,802 PubMed-indexed papers containing the search term *nitric oxide*, with 42,735 papers having nitric oxide in their title. Such widespread interest leaves opportunity for exploration of this molecule’s presence in IBM [50]. The more diseases in which a biomarker is found to be abnormal, the lower its specificity for any particular disease and the less likely it is to be critically involved in that disease’s pathogenesis. Widespread interest in a molecule should make it less valuable an area of study for IBM, yet paradoxically, the greater the interest in the molecule, the more likely peers are to approve grant funding and manuscript acceptance. Every new trend allows opportunity for scientific studies in many unrelated diseases. Although no one can know whether the pursuit of any given trend will turn out to be productive for people with IBM, in my opinion, the odds are strongly against the benefit of adapting ideas from other fields into models of IBM disease mechanism unless they are driven by independent justification. Trends that IBM research has seen include concepts of “stress” (“endoplasmic reticulum stress,” “oxidative stress”), myofiber breakdown (“autoph-

agy,” “proteasome dysfunction,” “unfolded protein response”), and “molecular toxicity” (A β , tau, prion protein).

Conclusions

After two to three decades of intense interest in IBM, there is almost no understanding as to the cause of this disease and the mechanisms by which myofibers are injured. Large gaps exist between the identification of disease biomarkers and their causal relationships to muscle injury. Currently advocated animal models do not represent this disease and should not be relied upon for therapeutic development. Careful attention to biomarker specificity, quantitation, and methodology is crucial to progress for patients with IBM.

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