

Inclusion Body Myositis Pathogenesis: Steady Progress

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Inclusion body myositis (IBM) is a slowly progressive skeletal muscle disease for which no effective pharmacological therapy is available. Since its initial pathological description 50 years ago,¹ substantial progress has been made in our clinical understanding of IBM. We now have extensive understanding of its demographics, pattern of muscle involvement,^{2,3} diagnostic criteria,^{4,5} and large amounts of cross-sectional,^{6,7} and more limited longitudinal,^{3,8,9} data regarding disease duration and clinical severity.

Progress in understanding the pathogenesis of IBM, crucial to identifying rational therapeutic strategies, has come more gradually. A series of pivotal publications from 1984-1988 reported the use of a novel technology, monoclonal antibodies (invented in 1975¹⁰) as histochemical reagents, to study the types of immune cells present in IBM and other disease muscle.¹¹⁻¹⁴ These studies identified cytotoxic T cell injury to IBM myofibers and launched a highly productive line of still-continuing investigation of the properties of these T cells.¹⁵⁻¹⁷ These studies have suggested that in IBM muscle there are specific molecules against which the adaptive immune system has concentrated its attack (an IBM autoantigen). Unfortunately, for largely technical reasons, studies of these T cells have failed to identify any of these molecules.

In contrast, these pivotal studies found little involvement in IBM of B cells, another key arm of the adaptive immune system.¹¹ No studies of IBM pertaining to B cells, the plasma cells they differentiate into, or the antibodies they produce were published for at least 15 years. The use of another novel technology in 2002 (gene expression profiling by microarrays, a technique developed in the early 1990s), however, identified robust B cell and plasma cell activation in IBM muscle.¹⁸ This new method launched a series of studies¹⁹⁻²¹ that led in 2011 to the identification in approximately 50% of IBM patients of a serum autoantibody present against an incompletely characterized muscle protein.²² This protein was identified in 2013 as cytoplasmic 5' nucleotidase (NT5C1A; cN1A).^{23,24} Anti-cN1A antibody detection is now used as a diagnostic test with approximate sensitivity of 35% and specificity of 95%.²⁵⁻³⁰

In parallel to these observations pertaining to autoimmunity, various muscle pathological abnormalities have been observed and collectively called "degeneration". The recognition of myonuclear degeneration,^{31,32} mitochondrial pathology,^{33,34} and abnormal protein aggregation³⁵ were important steps forward for IBM research. Advances in understanding muscle protein processing machinery (the immunoproteasome³⁶), endoplasmic reticulum (ER) stress,³⁷ and altered autophagy (with impaired p62 binding to LC3³⁸) in IBM³⁹ has led to diagnostic muscle biomarkers^{40,41} such as p62,⁴² TDP-43,⁴³ and LC3⁴⁴ aggregates.

The relationship between autoimmunity and degeneration in IBM has been a source of speculation and debate.^{45,46} Notable in this regard is IBM's striking autoimmune genetic background (the only robust association of genetic variation of IBM lies within the major histocompatibility gene (MHC) loci⁴⁷⁻⁴⁹). In addition, there is diffuse upregulation of MHC-1, antigen-directed (T cells and plasma cells¹⁹) and innate (myeloid dendritic cells⁵⁰ and macrophages) immune cells and their secreted products.⁵¹ Functionally, there is evidence that such an environment can result in degenerative changes: (i.) Upregulation of the

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immunoproteasome (present in IBM muscle^{36,52}) causes abnormal protein processing³⁶; (ii.) inflammation is highly correlated with mitochondrial pathology⁵³; (iii.) cytokines (interleukin 1b and interferon-gamma together) can produce abnormal protein aggregates in cultured myofibers⁵⁴; and (iv.) MHC-1 expression is sufficient in a murine model to result in ER stress and a severe myopathy with rimmed vacuoles.⁵⁵ These observations, however, are indirect.

In the current issue of the Annals of Neurology, Tawara et al.⁵⁶ provide more direct evidence addressing this debate. The passive transfer of patient sera to *in vitro* or *in vivo* models has been a productive investigational method for the study of certain human diseases, for example myasthenia gravis over 40 years ago.⁵⁷ Tawara et al. now report for the first time the use of this functional approach for IBM. Specifically, the authors exposed a cell culture model (*in vitro*) to and injected into mice (*in vivo*) purified blood immunoglobulin G (IgG) fractions from 3 anti-cN1A positive IBM patients and 3 anti-cN1A negative IBM patients. The sera IgG from the anti-cN1A positive IBM patients, but not the anti-cN1A negative patients, resulted in a muscle “degenerative” histological change (p62 protein aggregation) similar to that present in IBM muscle. Muscle from these mice additionally showed atrophic fibers and macrophage infiltration. Thus, although caution is warranted in generalizing because of the small number of patients studied, Tawara et al. have demonstrated that circulating molecules in a subgroup of anti-cN1A positive IBM patients can induce in model systems one of the prominent muscle molecular degenerative changes present in people with IBM. This is potentially a large step forward for the field.

It is important to note that Tawara et al. have not demonstrated that anti-cN1A antibodies themselves can induce degenerative changes in model systems. Rather their data suggest that blood anti-cN1A antibody positivity marks IBM patients who have circulating molecules in the IgG fraction that are capable of inducing muscle degenerative changes, specifically p62 aggregation. What are likely candidates for these degenerative-pathology inducing molecules? The purified IgG antibodies could potentially interact with a vast array of molecules. Additionally, any soluble molecules bound to these IgGs or caught in the same serum fraction as a contaminant may have been present. For example, antibodies bound to cytokines are present in many autoimmune diseases,⁵⁸ and may have been present. In addition, purified IgG preparations may contain contaminating serum proteins that may not even be of immune origin (e.g., prion-like material capable of inducing protein misfolding).

A variety of other data provided in this paper address previously published issues pertaining to anti-cN1A antibodies, sometimes agreeing or differing with previous observations. The diagnostic performance of anti-cN1A antibodies among patients was 36% sensitivity and 92% specificity, similar to most previous studies.^{22-30,59} None of 15 patients with either Sjogren’s syndrome (SS) or systemic lupus erythematosus (SLE) had detectable anti-cN1A antibodies, in agreement with a previous report finding anti-cN1A antibodies in none of 20 (0%) patients with SS and 2 of 33 (6.1%) of patients with SLE.³⁰ On the other hand, other previous studies have reported anti-cN1A antibody positivity in 23-36% of SS and 14-20% of SLE.^{27,28} Immunohistochemically detected cN1A aggregates were present in the perinuclear region in IBM muscle, as previously seen.²⁴ There was no correlation seen between anti-cN1A antibody status and age of onset, similar to findings from other studies,^{24,29} and no differences in disease severity, in contrast to findings in two studies.^{26,29}

What’s next for the pursuit of this publication’s key finding? A reasonable approach would be confirmation of the experiments in a larger number of patients to first understand whether anti-cN1A antibody positivity is truly a robust marker of the presence of IgG fractions that can functionally induce p62 aggregation *in vitro* and/or *in vivo*. Much of this initial work can proceed efficiently and productively in human cell culture models. Additional proteins known to aggregate in IBM can also be studied, such as TDP-43. Subsequent, or in parallel,

attempts to identify the functionally active molecules in the anti-cN1A positive patient IgG fractions should be undertaken. An initial “hypothesis-driven” approach, that anti-cN1A antibodies themselves are responsible, can be readily tested by purifying these molecules with recombinant cN1A, and testing both the purified anti-cN1A IgG and the remaining IgG fraction (minus the anti-cN1A antibodies) for functional activity. Other experiments may include using high salt concentrations in protein G columns to first elute any molecules bound to IgG Fab regions, and then separately eluting IgGs.

Lastly, as important as the Tawara et al. publication is as a first study demonstrating a degenerative-pathology inducing soluble (and likely immune) factor in a very small number of anti-cN1A antibody positive IBM patients, an historical reminder of caution is warranted. As an example, the toxicity of serum from patients with amyotrophic lateral sclerosis (ALS) attributed to immune molecules was noted at least as early as 1965.⁶⁰ The ALS field has included theories of humoral autoimmunity and degeneration for decades, with recent lack of validation of anti-ganglioside antibodies as a biomarker.⁶¹ Yet, there is still little perceived likelihood of therapeutic progress based on this line of research. Nevertheless, given a preponderance of molecular evidence of marked autoimmunity in IBM and the robust findings reported by Tawara et al., the pursuit of this line of investigation is likely to further our understanding of the pathogenesis of IBM.

Potential Conflicts of Interest

SAG is an inventor on intellectual property pertaining to diagnostics and therapeutics owned and managed by Brigham and Women’s Hospital; receives sponsored research from Pfizer; and is a founder of the Inclusion Body Myositis Foundation, Inc. and Abcuro.

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