Inclusion Body Myositis Pathogenesis: Steady Progress

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] and 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 1975[10]) 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, largely for 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 studies[19–21] 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 aggregation[35] were important steps forward for IBM research. Advances in understanding muscle protein processing machinery (the immunoproteasome[36]), endoplasmic reticulum (ER) stress,[37] and altered autophagy (with impaired p62 binding to LC3[38]) in IBM[39] have led to diagnostic muscle biomarkers[40,41] such as p62,[42] TDP-43,[43] and LC3[44] 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 in IBM lies within the major histocompatibility gene [MHC] loci[47–49]). In addition, there is diffuse upregulation of MHC-1, antigen-directed (T cells and plasma cells[19]) and innate (myeloid dendritic cells[50] and macrophages) immune cells, and their secreted products.[51] Functionally, there is evidence that such an environment can result in degenerative changes: (1) upregulation of the immunoproteasome (present in IBM muscle[36,52]) causes abnormal protein processing[36]; (2) inflammation is highly correlated with mitochondrial pathology[53]; (3) cytokines (interleukin 1b and interferon-gamma together) can produce abnormal protein aggregates in cultured myofibers[54]; and (4) 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 >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 (eg, prionlike material capable of inducing protein misfolding).

A variety of other data provided in this article address previously published issues pertaining to anti-cN1A antibodies, 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 Sjögren 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%) patients with SLE.[30] Conversely, other previous studies have reported anti-cN1A antibody positivity in 23 to 36% of SS and 14 to 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 2 studies.[26,29]

What is 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 parallel attempts to identify the functionally active molecules in the anti-cN1A–positive patient IgG fractions should be undertaken. An initial "hypothesis-driven" approach, according to which anti-cN1A antibodies themselves are responsible, can readily be 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 antiganglioside antibodies as a biomarker.[61] However, 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

S.A.G. is an inventor of intellectual property pertaining to diagnostics and therapeutics owned and managed by Brigham and Women's Hospital, performs sponsored research for Pfizer, and is a founder of the Inclusion Body Myositis Foundation and Abcuro.

Steven A. Greenberg, MD

Department of Neurology, Brigham and Women's Hospital

Children's Hospital Informatics Program

and Harvard Medical School

Boston, MA

References

1. Chou SM. Myxovirus-like structures in a case of human chronic polymyositis. Science 1967;158:1453–1455.
2. Badrising UA, Maat-Schieman ML, van Houwelingen JC, et al. Inclusion body myositis. Clinical features and clinical course of the disease in 64 patients. J Neurol 2005;252:1448–1454.
3. Cox FM, Titulaer MJ, Sont JK, et al. A 12-year follow-up in sporadic inclusion body myositis: an end stage with major disabilities. Brain 2011;134(pt 11):3167–3175.
4. Lloyd TE, Mammen AL, Amato AA, et al. Evaluation and construction of diagnostic criteria for inclusion body myositis. Neurology 2014;83:426–433.
5. Hilton-Jones D, Brady S. Diagnostic criteria for inclusion body myositis. J Intern Med 2016;280:52–62.

6. Benveniste O, Guiguet M, Freebody J, et al. Long-term observational study of sporadic inclusion body myositis. Brain 2011; 134(pt 11):3176–3184.
7. Price MA, Barghout V, Benveniste O, et al. Mortality and causes of death in patients with sporadic inclusion body myositis: survey study based on the clinical experience of specialists in Australia, Europe and the USA. J Neuromuscul Dis 2016;3:67–75.
8. Hogrel JY, Allenbach Y, Canal A, et al. Four-year longitudinal study of clinical and functional endpoints in sporadic inclusion body myositis: implications for therapeutic trials. Neuromuscul Disord 2014;24:604–610.
9. Cortese A, Machado P, Morrow J, et al. Longitudinal observational study of sporadic inclusion body myositis: implications for clinical trials. Neuromuscul Disord 2013;23:404–412.
10. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–497.
11. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I: Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. Ann Neurol 1984;16: 193–208.
12. Engel AG, Arahata K. Monoclonal antibody analysis of mononuclear cells in myopathies. II: Phenotypes of autoinvasive cells in polymyositis and inclusion body myositis. Ann Neurol 1984;16: 209–215.
13. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. III: Immunoelectron microscopy aspects of cell-mediated muscle fiber injury. Ann Neurol 1986;19:112–125.
14. Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. IV: Cell-mediated cytotoxicity and muscle fiber necrosis. Ann Neurol 1988;23:168–173.
15. Amemiya K, Granger RP, Dalakas MC. Clonal restriction of T-cell receptor expression by infiltrating lymphocytes in inclusion body myositis persists over time. Studies in repeated muscle biopsies. Brain 2000;123(pt 10):2030–2039.
16. Salajegheh M, Rakocevic G, Raju R, et al. T cell receptor profiling in muscle and blood lymphocytes in sporadic inclusion body myositis. Neurology 2007;69:1672–1679.
17. Greenberg SA, Pinkus JL, Amato AA, et al. Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. Brain 2016;139(pt 5):1348–1360.
18. Greenberg SA, Sanoudou D, Haslett JN, et al. Molecular profiles of inflammatory myopathies. Neurology 2002;59:1170–1182.
19. Greenberg SA, Bradshaw EM, Pinkus JL, et al. Plasma cells in muscle in inclusion body myositis and polymyositis. Neurology 2005;65:1782–1787.
20. Bradshaw EM, Orihuela A, McArdel SL, et al. A local antigen-driven humoral response is present in the inflammatory myopathies. J Immunol 2007;178:547–556.
21. Salajegheh M, Pinkus JL, Amato AA, et al. Permissive environment for B-cell maturation in myositis muscle in the absence of B-cell follicles. Muscle Nerve 2010;42:576–583.
22. Salajegheh M, Lam T, Greenberg SA. Autoantibodies against a 43 KDa muscle protein in inclusion body myositis. PLoS One 2011;6: e20266.
23. Pluk H, van Hoeve BJ, van Dooren SH, et al. Autoantibodies to cytosolic 5′-nucleotidase IA in inclusion body myositis. Ann Neurol 2013;73:397–407.
24. Larman HB, Salajegheh M, Nazareno R, et al. Cytosolic 5′-nucleotidase 1A autoimmunity in sporadic inclusion body myositis. Ann Neurol 2013;73:408–418.
25. Greenberg SA. Cytoplasmic 5′-nucleotidase autoantibodies in inclusion body myositis: isotypes and diagnostic utility. Muscle Nerve 2014;50:488–492.
26. Goyal NA, Cash TM, Alam U, et al. Seropositivity for NT5c1A antibody in sporadic inclusion body myositis predicts more severe motor, bulbar and respiratory involvement. J Neurol Neurosurg Psychiatry 2016;87:373–378.
27. Lloyd TE, Christopher-Stine L, Pinal-Fernandez I, et al. Cytosolic 5′-nucleotidase 1A as a target of circulating autoantibodies in autoimmune diseases. Arthritis Care Res (Hoboken) 2016;68:66–71.
28. Herbert MK, Stammen-Vogelzangs J, Verbeek MM, et al. Disease specificity of autoantibodies to cytosolic 5′-nucleotidase 1A in sporadic inclusion body myositis versus known autoimmune diseases. Ann Rheum Dis 2016;75:696–701.
29. Lilleker JB, Rietveld A, Pye SR, et al. Cytosolic 5′-nucleotidase 1A autoantibody profile and clinical characteristics in inclusion body myositis. Ann Rheum Dis 2017. doi: 10.1136/annrheumdis-2016-210282. [Epub ahead of print].
30. Kramp SL, Karayev D, Shen G, et al. Development and evaluation of a standardized ELISA for the determination of autoantibodies against cN-1A (Mup44, NT5C1A) in sporadic inclusion body myositis. Auto Immun Highlights 2016;7:16.
31. Chou SM. Myxovirus-like structures and accompanying nuclear changes in chronic polymyositis. Arch Pathol 1968;86:649–658.
32. Carpenter S, Karpati G, Heller I, Eisen A. Inclusion body myositis: a distinct variety of idiopathic inflammatory myopathy. Neurology 1978;28:8–17.
33. Oldfors A, Larsson NG, Lindberg C, Holme E. Mitochondrial DNA deletions in inclusion body myositis. Brain 1993;116(pt 2):325–336.
34. Rifai Z, Welle S, Kamp C, Thornton CA. Ragged red fibers in normal aging and inflammatory myopathy. Ann Neurol 1995;37:24–29.
35. Mendell JR, Sahenk Z, Gales T, Paul L. Amyloid filaments in inclusion body myositis. Novel findings provide insight into nature of filaments. Arch Neurol 1991;48:1229–1234.
36. Bhattarai S, Ghannam K, Krause S, et al. The immunoproteasomes are key to regulate myokines and MHC class I expression in idiopathic inflammatory myopathies. J Autoimmun 2016;75:118–129.
37. Vattemi G, Engel WK, McFerrin J, Askanas V. Endoplasmic reticulum stress and unfolded protein response in inclusion body myositis muscle. Am J Pathol 2004;164:1–7.
38. Nakano S, Oki M, Kusaka H. The role of p62/SQSTM1 in sporadic inclusion body myositis. Neuromuscul Disord 2017;27(pt 4):363–369.
39. Güttsches AK, Brady S, Krause K, et al. Proteomics of rimmed vacuoles define new risk allele in inclusion body myositis. Ann Neurol 2017;81:227–239.
40. Dubourg O, Wanschitz J, Maisonobe T, et al. Diagnostic value of markers of muscle degeneration in sporadic inclusion body myositis. Acta Myol 2011;30:103–108.
41. Hiniker A, Daniels BH, Lee HS, Margeta M. Comparative utility of LC3, p62 and TDP-43 immunohistochemistry in differentiation of inclusion body myositis from polymyositis and related inflammatory myopathies. Acta Neuropathol Commun 2013;1:29.
42. Nogalska A, Terracciano C, D'Agostino C, et al. p62/SQSTM1 is overexpressed and prominently accumulated in inclusions of sporadic inclusion-body myositis muscle fibers, and can help differentiating it from polymyositis and dermatomyositis. Acta Neuropathol 2009;118:407–413.
43. Weihl CC, Temiz P, Miller SE, et al. TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. J Neurol Neurosurg Psychiatry 2008;79:1186–1189.
44. Lunemann JD, Schmidt J, Schmid D, et al. Beta-amyloid is a substrate of autophagy in sporadic inclusion body myositis. Ann Neurol 2007;61:476–483.
45. Benveniste O, Stenzel W, Hilton-Jones D, et al. Amyloid deposits and inflammatory infiltrates in sporadic inclusion body myositis:

the inflammatory egg comes before the degenerative chicken. Acta Neuropathol 2015;129:611–624.

46. Weihl CC, Mammen AL. Sporadic inclusion body myositis—a myodegenerative disease or an inflammatory myopathy. Neuropathol Appl Neurobiol 2017;43:82–91.
47. Garlepp MJ, Laing B, Zilko PJ, et al. HLA associations with inclusion body myositis. Clin Exp Immunol 1994;98:40–45.
48. Badrising UA, Schreuder GM, Giphart MJ, et al. Associations with autoimmune disorders and HLA class I and II antigens in inclusion body myositis. Neurology 2004;63:2396–2398.
49. Rothwell S, Cooper RG, Lundberg IE, et al. Immune-array analysis in sporadic inclusion body myositis reveals HLA-DRB1 amino acid heterogeneity across the myositis spectrum. Arthritis Rheumatol 2017. doi: 10.1002/art.40045. [Epub ahead of print].
50. Greenberg SA, Pinkus GS, Amato AA, Pinkus JL. Myeloid dendritic cells in inclusion-body myositis and polymyositis. Muscle Nerve 2007;35:17–23.
51. Greenberg SA. Pathogenesis and therapy of inclusion body myositis. Curr Opin Neurol 2012;25:630–639.
52. Raju R, Dalakas MC. Gene expression profile in the muscles of patients with inflammatory myopathies: effect of therapy with IVIg and biological validation of clinically relevant genes. Brain 2005; 128(pt 8):1887–1896.
53. Rygiel KA, Miller J, Grady JP, et al. Mitochondrial and inflammatory changes in sporadic inclusion body myositis. Neuropathol Appl Neurobiol 2015;41:288–303.
54. Schmidt J, Barthel K, Wrede A, et al. Interrelation of inflammation and APP in sIBM: IL-1 beta induces accumulation of beta-amyloid in skeletal muscle. Brain 2008;131(pt 5):1228–1240.
55. Freret M, Drouot L, Obry A, et al. Overexpression of MHC class I in muscle of lymphocyte-deficient mice causes a severe myopathy with induction of the unfolded protein response. Am J Pathol 2013;183:893–904.
56. Tawara N, Yamashita S, Zhang X, et al. Pathomechanisms of anti-cN1A autoantibodies in sporadic inclusion body myositis. Ann Neurol 2017 doi: 10.1002/ana.24919. (in press).
57. Toyka KV, Brachman DB, Pestronk A, Kao I. Myasthenia gravis: passive transfer from man to mouse. Science 1975;190:397–399.
58. Cappellano G, Orilieri E, Woldetsadik AD, et al. Anti-cytokine autoantibodies in autoimmune diseases. Am J Clin Exp Immunol 2012;1:136–146.
59. Limaye VS, Lester S, Blumbergs P, Greenberg SA. Anti-C N1A antibodies in South Australian patients with inclusion body myositis. Muscle Nerve 2016;53:654–655.
60. Field EJ, Hughes D. Toxicity of motor neurone disease serum for myelin in tissue culture. Br Med J 1965;2:1399–1401.
61. Kollewe K, Wurster U, Sinzenich T, et al. Anti-ganglioside antibodies in amyotrophic lateral sclerosis revisited. PLoS One 2015; 10:e0125339.

DOI: 10.1002/ana.24920