

Inflammatory myopathies: disease mechanisms

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Purpose of review

Recent developments pertaining to disease mechanisms in the inflammatory myopathies are discussed, emphasizing those areas that are of particular interest to me.

Recent findings

The identification and further characterization of the type 1 interferon pathway in dermatomyositis is leading down a path of genomic medicine. Myonuclear structural abnormalities and the presence of nucleic acid-binding proteins, including the TAR DNA binding protein TDP-43, in sporadic inclusion body myositis (sIBM) sarcoplasm are important recent observations. This is an area likely to provide deep understanding of the mechanism of myofiber injury in sIBM. Proteomic characterization of proteins in sIBM muscle, muscle functioning as a lymphoid tissue, and the nature of belief systems, particularly one pertaining to beta-amyloid and sIBM, are other areas of interest.

Summary

Clarification of disease mechanisms is providing a basis for rational drug development for some patients with myositis.

Keywords

dermatomyositis, inclusion body myositis, inflammatory myopathies

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Introduction

Understanding the mechanisms by which myofibers are injured in myositis offers the best chance of developing well tolerated and effective therapies for these patients. The rapidly increasing number of US Food and Drug Administration (FDA)-approved and developing therapies that modulate the immune system or otherwise have potential for efficacy in myositis, in consideration with the resources and time required to test these agents, suggests that mechanistic understanding is crucial to successful drug development.

We know almost nothing about cause and effect in myositis because there are fundamental limitations for understanding disease mechanisms occurring in people's tissues. Even though one can categorize and quantitate molecules and cells in myositis tissue samples that are inappropriately present or absent, what these molecules are doing in these patient tissues can almost never be determined. Rather, models representing hypotheses of mechanisms can be constructed and can guide further mechanistic studies and rational therapeutic drug development.

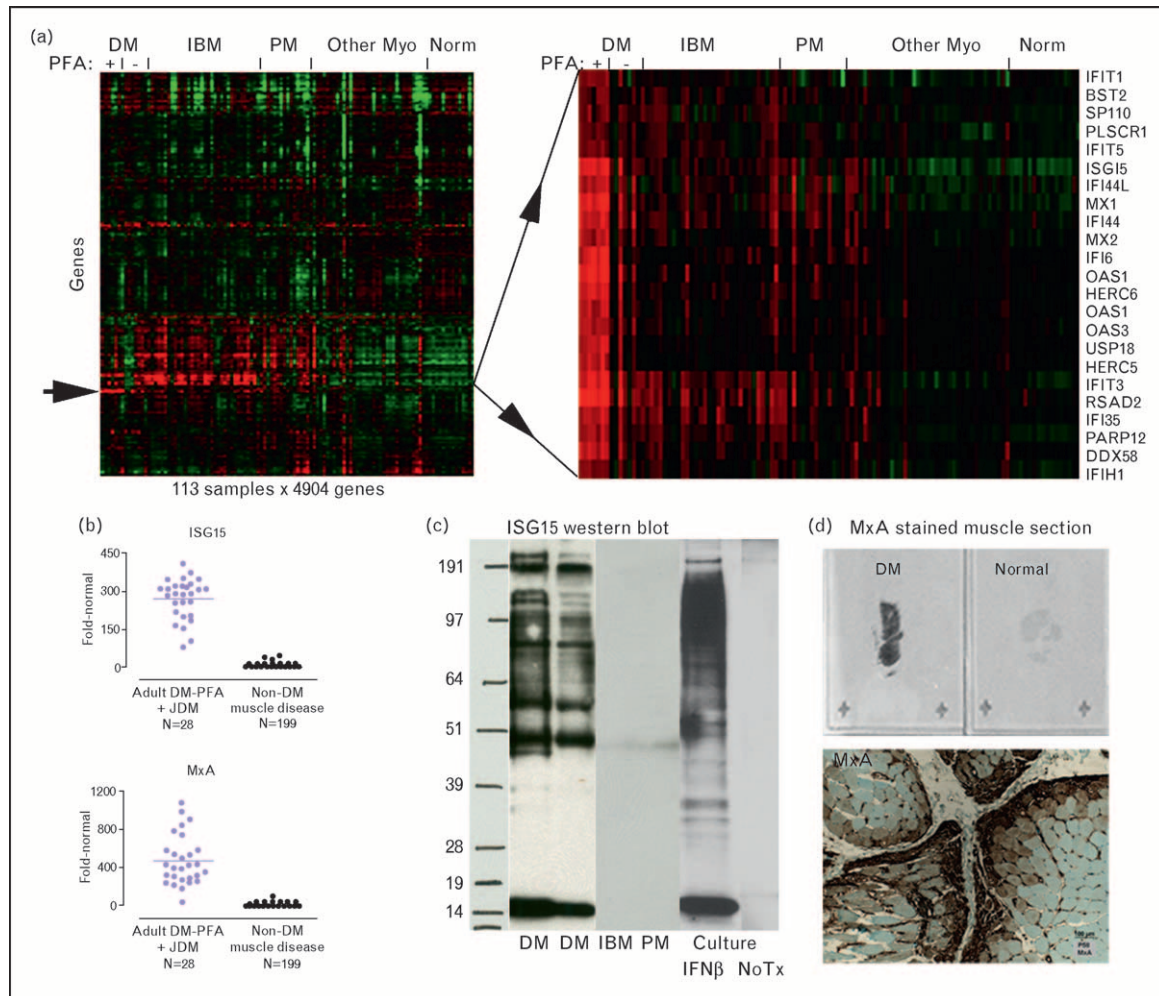
Approximately 895 PubMed indexed articles with keywords pertaining to myositis were published during the 18-month period between 1 October 2007 and 31 March 2009, of which approximately 80 address mechanisms (Supplementary Note 1). Here, I focus on disease mechanisms that seem most promising to me. Several areas are

only briefly mentioned including studies of molecules expressed by myofibers that may contribute to autoimmunity [1]; serum antibodies present in some patients with dermatomyositis [2], extending previous work on autoantibodies (recently reviewed in [3]); and the presence in myositis muscle of myofiber and endothelial precursor cells [4].

Dermatomyositis, type 1 interferons, and genomic medicine

The marked overproduction of type 1 interferon-inducible transcripts and proteins is a remarkably unique aspect of dermatomyositis muscle disease in comparison to all other muscle diseases that have been studied. The type 1 interferons are a class of molecules including interferon (IFN)- α and β . These cytokines can stimulate in target cells the transcription and translation of an entire class of genes (the type 1 interferon-inducible genes). Proteins produced from these genes' transcripts, such as Mx1, ISG15, OAS1, and approximately 50–100 others, remain inside of cells, functioning as defenses against viral infections through a variety of means such as inhibiting viral transcription, translation, or assembly of viral nucleocapsids.

Large-scale transcript measurement in muscle biopsy specimens by microarrays has provided remarkably strong and specific data. Within my lab's dataset of approximately 18 000 transcripts measured in each of

Figure 1 Genomic identification of type 1 interferon-inducible pathway activation in dermatomyositis muscle

(a) Analysis of 22 283 gene transcript probesets (4904 shown after filtering; one per row) in 113 muscle biopsy samples (one per column) disclosed a cluster of type 1 interferon-inducible genes specifically and highly up-regulated in dermatomyositis with perifascicular atrophy (PFA) (thin red stripe marked by an arrow). Enlargement of this arrowed region is shown on the right. Red and green indicate high and low expression. (b) High expression of transcripts for interferon-stimulated gene 15 (*ISG15*) and myxovirus resistance protein A (*MxA*) are extraordinarily specific to dermatomyositis. (c) Examples of *ISG15* western blots show free *ISG15* (the approximately 15-kDa band) and multiple *ISG15* conjugated proteins (the discrete bands and smear shown at higher molecular weights) in dermatomyositis but not other muscle biopsy samples. Cultured human skeletal muscle cell lines exposed to IFN- β develop the same pattern of free and conjugated *ISG15* as occurs in dermatomyositis. (d) *MxA* staining of dermatomyositis muscle is sometimes so impressive that it is evident on glass slides viewed without the aid of a microscope. *MxA* is preferentially located in perifascicular myofibers and in blood vessel walls. Adapted from Salajegheh M, *et al*. Interferon-stimulated gene 15 (*ISG15*) conjugates proteins in dermatomyositis muscle with perifascicular atrophy. *Ann Neurol* 2009 [Epub ahead of print].

113 muscle biopsy samples from patients with a wide range of myopathies, only dermatomyositis samples with perifascicular atrophy have marked elevation of type 1 interferon-inducible transcripts (Fig. 1a). In both my data and the publicly deposited microarray data collected by Drs. Lauren Pachman, Eric Hoffman, and colleagues (Gene Expression Omnibus dataset GDS1956), there is marked specificity of this pathway for dermatomyositis. For example, the transcript for the type 1 interferon-inducible gene *ISG15* is higher in every single adult patient with dermatomyositis and perifascicular atrophy ($n = 7$) and every single patient with juvenile dermatomyositis ($n = 21$) than in every one

of 199 non-dermatomyositis biopsy samples from a wide range of neuromuscular diseases that include other much more inflammatory myopathies ($n = 62$), muscular dystrophies, idiopathic myopathies, and neurogenic diseases (Fig. 1b). In these data, there is an *ISG15* transcript level above which there is 100% specificity for dermatomyositis.

Unlike transcript studies, the identification of type 1 interferon-inducible proteins in dermatomyositis samples is a much more laborious task, less amenable to high-throughput methods. The choice of which proteins to study first has been guided by microarray data; *MxA* and

ISG15 are of the greatest interest to me based on transcript profiling. These proteins are impressively and uniquely abundant in dermatomyositis myofibers with perifascicular atrophy and in dermatomyositis capillaries [5]. ISG15, a ubiquitin-like modifier, is furthermore attached to many other proteins in dermatomyositis muscle, the identities of which have not been determined (Fig. 1c). Exposure of human skeletal muscle cell cultures to IFN- α or IFN- β produces a similar picture of ISG15 conjugation present in human dermatomyositis samples (Fig. 1d).

Genomic technologies are not only being applied to the mechanistic study of myositis, but may play a role in patient management. A class of blood biomarkers that reflect diagnosis and disease activity in myositis have been identified and their potential utility for patient management is being defined [6,7]. The often publicized concept of ‘personalized medicine’ based on genomic markers may become a future reality in myositis.

What causes tubuloreticular inclusions and perifascicular atrophy in dermatomyositis?

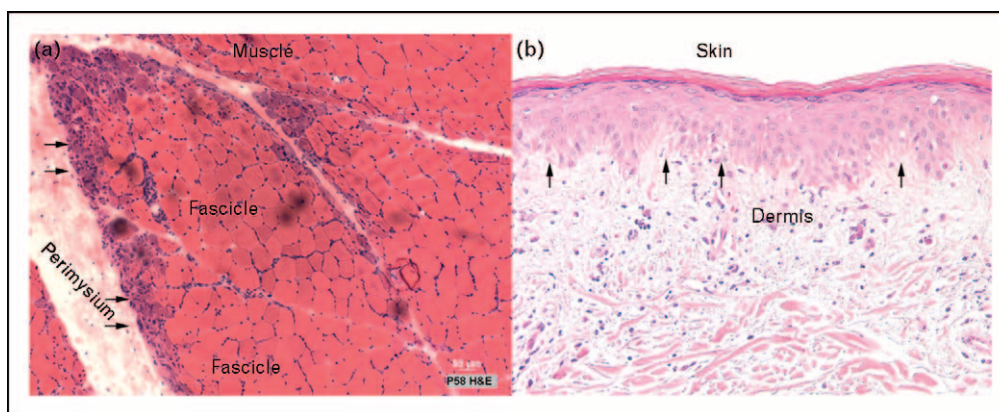
Two pathological findings in dermatomyositis muscle biopsies are the footprints of its disease mechanism, unique to it among all known muscle diseases. These are tubuloreticular inclusions and perifascicular atrophy. Following these footprints will lead to deep understanding of dermatomyositis mechanism.

The association of tubuloreticular inclusions within intramuscular endothelial cells was noted as a distinguishing feature of adult and juvenile dermatomyositis at least as early as 1970 [8] and further defined in the juvenile form in 1975 [9], when tubuloreticular inclusions were found in

endothelial cells in 76–98% of all intramuscular blood vessels. Their unique association with dermatomyositis has been emphasized by many investigators [9–11]. Systemically administered recombinant IFN- α has been known since the early 1980s to induce tubuloreticular inclusions in various patient tissues [12–14]. Cultured endothelial [15] and other cells [16–18] exposed to type 1 interferons, but not the type 2 gamma-interferon [17], were shown to develop tubuloreticular inclusions as early as 1981. For uncertain reasons, however, for more than 20 years these data remained unconnected to dermatomyositis in the literature; no publication commented on the clear mechanistic relationship between type 1 interferons and these nearly pathognomonic lesions in dermatomyositis muscle until 2004 [19]. A recent review of tubuloreticular inclusions in myositis has also not connected these published data to dermatomyositis [20].

Perifascicular atrophy is more aptly called perimysial perifascicular myofiber injury, emphasizing the observation that injury does not occur equally to all of the myofibers at the periphery of a fascicle but rather those myofibers that border on the connective tissue of the perimysial spaces (Fig. 2a). Perifascicular myofibers bordering neighboring perifascicular myofibers of another fascicle tend to be less affected. This ‘border’ effect may also be seen in dermatomyositis skin [21], constituting an ‘interface dermatitis’ in which the basal layer of epidermal keratinocytes bordering the connective tissue of the dermis is preferentially affected (Fig. 2b). The nature of the pathology of these myofibers is a fascinating question that goes right to the core of one of dermatomyositis’s most impairing clinical manifestations, muscular weakness. The possibility that the production of one or more type 1 interferon-inducible transcripts or proteins by and within these myofibers leads to their injury has been considered [5].

Figure 2 Perifascicular atrophy and interface dermatitis in dermatomyositis skin and muscle



(a) Myofiber injury preferentially occurs in myofibers bordering perimysial connective tissue (arrows), not myofibers bordering other fascicles. (b) Keratinocyte injury similarly preferentially involves the basal layer of keratinocytes (arrows) bordering dermal connective tissue. Reproduced with permission from [21].

Sporadic inclusion body myositis, myonuclei, and TDP-43

The first published reports delineating distinct pathological features of sporadic inclusion body myositis (sIBM) from polymyositis were written by Chou in 1967 [22] and 1968 [23]. These emphasized substantial myonuclear abnormalities, further detailed by Carpenter and colleagues in 1978 [24], and between 1993 and 1996 [25–28]. These investigators formulated a hypothesis that rimmed vacuoles, a feature that distinguishes sIBM from polymyositis on hematoxylin and eosin (H&E) 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 (see Supplementary Note 2). The isolation of this knowledge regarding sIBM myonuclear abnormalities through the lack of citation to it has been a sad chapter in the history of sIBM research.

Though the published expert opinion of the sIBM research community reflected through this citation bias has been that sIBM myonuclear abnormalities are not a valuable area of study, it is my opinion that research in this area will lead to fundamental understanding of sIBM disease mechanisms, for several reasons. First, rimmed vacuoles are likely derived from myonuclei. Their linings are blue on H&E and red on trichrome-stained sections, as are myonuclei. In quantitative studies of paired adjacent sections, 73% of H&E-identified rimmed vacuoles were found lined with the nuclear membrane proteins lamin A/C and emerin (Fig. 3a) [29]. Nonquantitative studies have also reported emerin and a nuclear histone protein lining these vacuoles [30], and two other proteins visible in normal muscle only in myonuclei, valosin-containing protein (VCP) [31] and TAR DNA binding protein (TDP)-43 [32••]. Second, investigators using electron microscopy to study sIBM have emphasized the distinct myonuclear morphological changes present [23,24,29].

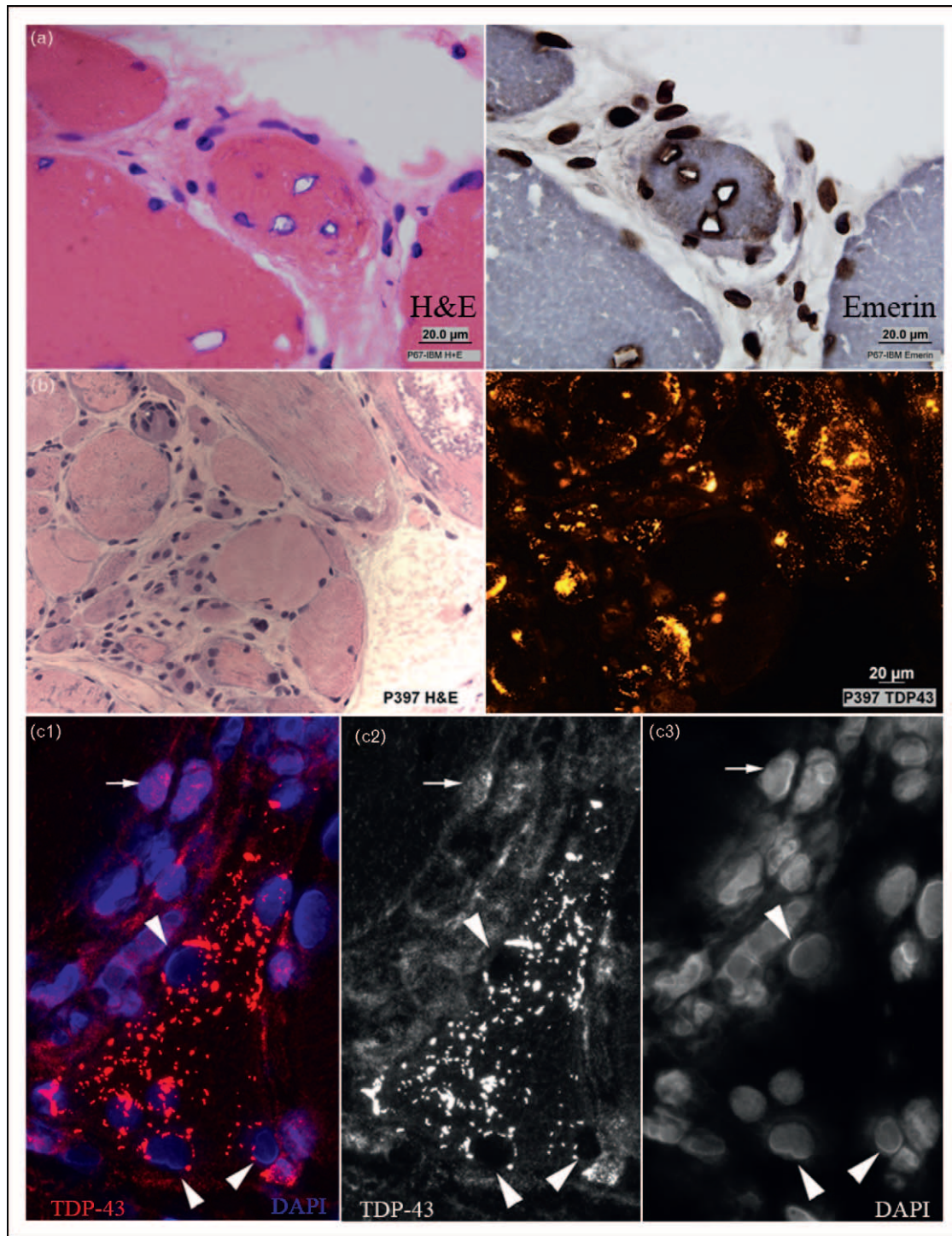
Third, 15 years ago, experiments attempting (and failing) to confirm claims of specific beta-amyloid precursor protein (β APP) transcript abundance in sIBM muscle instead found an unidentified nucleic acid-binding protein lining vacuoles of some sIBM myofibers [26]. Recently, the nucleic acid-binding protein TDP-43 was identified by Dr Conrad Weihl and colleagues in non-nuclear sarcoplasm in sIBM and hereditary inclusion body myopathy (hIBM) due to VCP mutations [32••]. This important observation (Fig. 3b) has been made in two other studies as of 4/1/09, and extended to patients with myofibrillar myopathy, with myotilin and desmin mutations [33•] and in one patient not genetically characterized [34••]. One study found ‘several’

affected muscle fibers in each of five sIBM patients [33•]; since typical biopsy sections contain 1000 or more myofibers, these investigators appeared to find very little TDP-43 abnormalities (if ‘several’ is interpreted as ‘3’, this would perhaps imply less than 0.3% of myofibers affected). In a separate quantitative study, however [34••], sarcoplasmic accumulations of TDP-43 were found in a mean of 23% of sIBM myofibers across 23 patients. The presence of more than 1% of such affected myofibers in a muscle biopsy specimen was 91% sensitive and 100% specific for sIBM among 50 patients with inflammatory myopathies. sIBM sarcoplasmic TDP-43 was accompanied by myonuclear depletion, present in 12% of myonuclei of such fibers compared to 99% of myonuclei in fibers lacking sarcoplasmic accumulation (Fig. 3c) [34••]. This is similar to the redistribution of neuronal TDP-43 from nuclei to cytoplasm that was previously identified in frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) and in amyotrophic lateral sclerosis (ALS) [35,36].

The mechanisms and consequences of TDP-43 redistribution from myonuclei to sarcoplasm in a high percentage of sIBM myofibers are uncertain. Two related observations are noteworthy. First, like in sIBM, rimmed vacuoles and nuclear tubulofilaments develop in oculopharyngeal muscular dystrophy (OPMD), a disease associated with mutations in a predominantly nuclear (like TDP-43) nucleic acid binding protein (PABP2). PABP2 mutations have been sought in sIBM [37] (and not found) because of some of its clinical similarities with OPMD. Second, siRNA-mediated depletion of TDP-43 from nuclei in several nonmuscle cell lines leads to abnormalities in the integrity of the inner nuclear membrane evident through visible gaps in lamin A/C and emerin staining [38•]. The likely relevance of this observation to inclusion body myopathies was noticed by Dr J. Paul Taylor (St Jude’s Hospital, Memphis, TN, USA). It is possible that the nuclear localization of TDP-43 may be necessary for maintenance of intact nuclear envelope. Abnormal accumulation of extranuclear TDP-43 may additionally lead to deleterious interaction with mRNAs or other RNA-binding proteins and affect the translation of specific proteins in sIBM.

Inclusion body myositis: what’s missing?

Pronounced atrophy of muscle, particularly forearm flexors and quadriceps, is a long recognized feature of sIBM. The simple question of what specific proteins are missing from these atrophied sIBM muscles is only recently beginning to be addressed. One approach is through the analysis of proteins from muscle biopsy samples separated in two-dimensional gels [39]. This technique likely has poor reproducibility [40•]. Another approach is through mass spectrometry-based shotgun proteomics

Figure 3 Myonuclear abnormalities in sporadic inclusion body myositis

(a) Lining of hematoxylin and eosin (H&E)-rimmed vacuoles with nuclear membrane protein emerlin. (b) Adjacent H&E and TDP-43-stained sections show multiple sarcoplasmic TDP-43 foci. (c) Myonuclei (arrowheads) are depleted of TDP-43 in myofibers containing sarcoplasmic TDP-43. Normal myonuclear localization of TDP-43 (arrow) is evident in myofibers without such sarcoplasmic accumulation. Reproduced with permission: (a) from [29], (b) and (c) from [34**].

[41*]. Technically demanding for methods development specific to the study of human muscle biopsy samples, this approach has the potential to quantitate the relative abundance of 1000s of specific proteins within each

sample. These studies have suggested substantial depletion in fast-twitch sarcomeric and glycolytic enzyme proteins in sIBM samples compared to other inflammatory myopathy and normal muscle. By

comparing microarray-measured transcript abundance with protein abundance from the same samples, it appears that this protein loss may be due to failure of translation not transcription. Whether this hypothesis is correct and whether these findings have mechanistic value to sIBM are uncertain.

Muscle has taken up certain lymphoid tissue functions

In many patients with inflammatory myopathies, muscle has taken up functions ordinarily performed by the secondary lymphoid tissues, lymph node and spleen. Such function was suspected in some patients with myositis and overlap syndromes whose biopsies contained nodular collections of inflammatory cells at least as early as 1996 [42]. Antigen-mediated clonal expansion of B cells into antibody-producing plasma cells producing antibodies in muscle is definitely present in many patients with dermatomyositis, polymyositis, and sIBM [43–45]; it is especially strong in sIBM, a disease which until recently was widely believed to have sparse B cells present in muscle. This process occurs even in the absence of typical B cell dense zones such as are present in lymphoid germinal centers. A permissive environment that might allow such B cell development, but does not necessarily indicate its occurrence, is present in juvenile dermatomyositis muscle [46•].

Myeloid dendritic cells, an immune system cell specialized at the task of antigen presentation to and activation of T cells, are abundant, invade myofibers in polymyositis [47] and sIBM [47,48•], and appear in stellate morphologies in close contact with T cells, suggesting but not proving that the activation of T helper cells through antigen presentation and costimulation by these professional antigen-presenting cells occurs directly in muscle in polymyositis and sIBM.

Skeletal muscle may not be well adapted to serving both as a contractile force-generating organ and a lymphoid organ. Although the mechanism of immune-mediated myofiber injury in polymyositis and sIBM has been modeled exclusively as one in which cytotoxic T cells invade myofibers, this aspect of disease is relatively less than the much larger numbers of myofibers that are simply surrounded and often displaced by T cells and other immune system cells, but otherwise not invaded. One speculation is that molecules secreted by these many noninvading immune cells injure myofibers.

The significance and implications for patients of lymphoid activity within muscle are uncertain, but the presence of these mechanisms within muscle and the possibility of their contribution to muscle injury provide rationale for drug development. A large number of biological therapies either in development or FDA-approved

for other indications target these processes [5,49•] and may be attractive candidates for clinical trials in myositis.

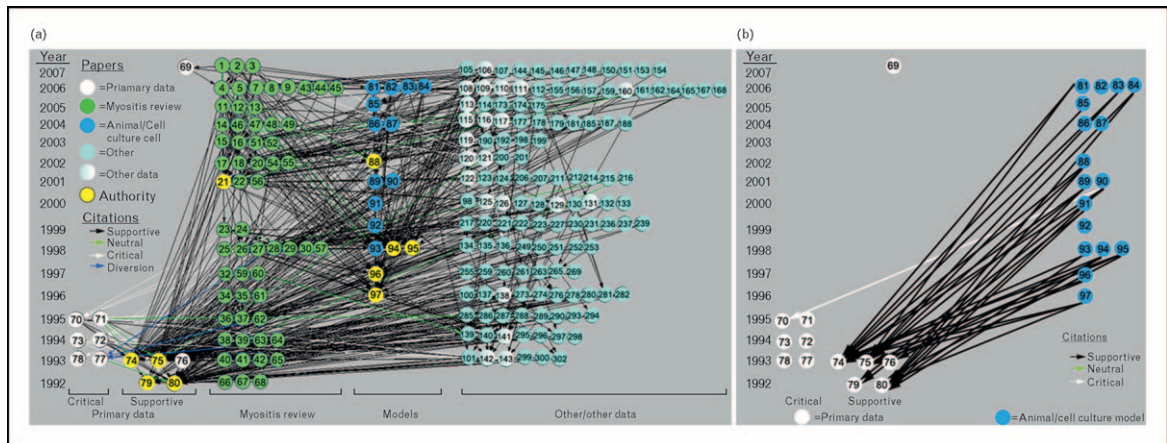
The nature of belief

Whether scientific claims are valid depends on the soundness of data supporting them; whether they are believed depends on different factors. The path from data to belief is established within an individual paper (methods are clearly described and results are required to be convincing), but how data lead to belief shared by a community across all papers within a field has been little studied, in part because of major technical barriers. I have recently developed methods that address these barriers and applied these methods to the study of claims pertaining to the role of beta-amyloid (A β) in inclusion body myositis [50••].

Citation is a scholarly method of connecting ideas to the broader medical literature. In addition to this scholarly use, however, citation also has social uses ('social citation') – for self-serving purposes and as a tool for persuasion. In this study [50••], social network theory was applied to the entire citation network representing claims regarding the presence and roles of β APP and A β in sIBM, a network that as of 10/26/2007 contained 242 papers and 220 609 citation paths (chains of citations among these papers). All statements of such claims in PubMed-indexed English language papers were collected and the patterns of citations supporting these claims studied. Through computational approaches, I detected certain distortions in the pattern of citations that would not be present had only a scholarly use of citation been used (Fig. 4a). These distortions turned out to be a result of extensive use of citation as a persuasive tool. The paper addresses the nature of the persuasive citation methods (citation bias, citation diversion, amplification, and invention) and the consequences of their use on belief in the claim, all documented exactly and quantitatively. The use of such methods to obtain National Institutes of Health (NIH) research funding and justify the validity of animal models (Fig. 4b) is also discussed, as well as the incentives that may propel the continued evolution of unfounded belief systems. These concerns are not strictly academic, as the lives and well being of patients with sIBM are critically dependent on what lines of research are pursued and what resources support them.

A previous attempt to point out the crucial technical limitations, contradictions, and biases supporting claims that A β is a 'hallmark of IBM' [51•] was met with a response [52•] that such data-based views were incorrect because they conflicted with widespread belief in these claims. It is my hope that my more recent study [50••] will allow readers to more easily understand not just what the limitations of these claims are but why such widespread belief in them exists.

Figure 4 Citation bias used to support belief regarding beta-amyloid significance in sporadic inclusion body myositis



(a) The claim-specific citation network of papers (nodes) and citations (directed arrows) containing statements of beta-amyloid significance in sporadic inclusion body myositis (sIBM). Primary data addressing the claim is present in 12 papers published between 1992 and 1995, and one in 2007. Six of these (70–73, 77, 78) report data that conflicts with the claims; these have rarely been cited by subsequent papers. Instead, five papers have been repeatedly cited and have achieved authority status in this belief network. (b) Citations from animal and cell culture model papers to Primary Data to support rationale for overproduction of β APP mRNA as a valid model of sIBM. Only 1 of 32 citations flows to papers that present data that conflict with the validity of these models. Adapted with permission from [50**].

Conclusion

Substantial advances are being made in understanding the disease mechanisms of inflammatory myopathies. These provide rationales for clinical trials of existing FDA-approved and developing products, especially biological agents, several of which are in myositis clinical trials at the time of this writing.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 559).

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