Editorial

Inclusion body myositis

Since the early description of cases of inflammatory myopathy with unusual filamentous inclusions in muscle fibres\(^1\)–\(^3\) inclusion body myositis has come to be recognised as a major form of idiopathic inflammatory myopathy with distinctive clinical and pathological features which accounts for up to a third of patients with inflammatory myopathy seen in a neuromuscular clinic. The condition, which predominates in males, is characterised by the insidious development of muscular weakness and atrophy which is curiously selective, involving particularly the quadriceps femoris muscles in the lower limbs and the forearm muscles, particularly the finger flexors, and especially the flexor digitorum profundus in the upper limbs.\(^4\)

Cutaneous and other systemic manifestations are usually absent although occasional patients have an associated connective tissue disorder, autoimmune disease, or immunodeficiency state.\(^5\)\(^6\) Asymmetry of muscle involvement is not uncommon and may be prominent in some cases, thereby compounding the diagnostic difficulty. With progression, which is usually protracted over a period of years, the weakness becomes more widespread and the selectivity of muscle involvement less apparent.\(^7\)

Dysphagia occurs in 20–30% of patients and is sometimes severe, requiring a cricothyroid myotomy or gastrostomy.\(^8\) Although most cases are sporadic, familial forms with a variable phenotype and either autosomal dominant or recessive inheritance also occur.

Affected people often present late in the course of the disease, when falls begin to occur because of quadriceps weakness and rarely respond to corticosteroid or immunosuppressive therapy.\(^9\)\(^10\) The diagnosis is often delayed because of lack of familiarity with the condition, an atypical pattern of muscle involvement, the fact that the serum creatine kinase concentration may be normal or only mildly increased, and the sometimes confusing finding of a mixed neuromyopathic pattern on electromyography. Some patients are initially misdiagnosed as having motor neuron disease. Confirmation of the diagnosis of inclusion body myositis requires a muscle biopsy, preferably an open biopsy from a muscle which is not too severely affected, and recognition of the characteristic rimmed vacuoles and hyaline eosinophilic inclusions in cryostat sections and intranuclear or cytoplasmic aggregates of 15–18 nm tubulofilamentous structures in muscle fibres by electron microscopy.\(^5\)\(^10\)\(^11\) T Cells, particularly those of the CD8+ phenotype, predominate in the inflammatory infiltrates and may invade non-necrotic muscle fibres together with macrophages.\(^12\) However, the extent of inflammatory infiltration is variable, sometimes being inconspicuous or even absent, especially in familial cases.

There have always been unresolved questions about this rather mysterious condition. Foremost among these is the significance of the muscle fibre inclusions and their relation to what seems to be an autoimmune T cell reaction occurring in the context of class I MHC expression by muscle fibres.\(^11\) Chou\(^1\) first suggested that the intranuclear and cytoplasmic tubofilamentous inclusions resembled myxovirus nucleocapsids and, on the basis of immunocytochemical findings, subsequently proposed that a defective mumps virus was the causative agent.\(^13\) This now seems unlikely, as further studies using in situ hybridisation\(^14\) and the sensitive polymerase chain reaction\(^15\) have failed to confirm the presence of myxovirus genomic material in muscle tissue. Of uncertain relevance also are the findings in some cases of inclusion body myositis of mitochondrial abnormalities and mtDNA deletions.\(^16\)

In 1991 Mendell \etal\(^17\) first reported the finding of amyloid deposits in association with the autophagic vacuoles in muscle fibres in inclusion body myositis. Recent immunohistochemical studies have shown the presence of β amyloid, ubiquitin, and an array of other proteins in the vacuoles.\(^18\)–\(^21\) These include the prion protein, apolipoprotein E, and hyperphosphorylated tau, which are also present in the cerebral plaques of Alzheimer’s disease. In addition, the nicotinic acetylcholine receptor and fibroblast growth factor and its receptor have been localised ultrastructurally to the tubofilaments which resemble the paired helical filaments that accumulate in nerve cells in Alzheimer’s disease. These findings have led to the intriguing suggestion that the degenerative process in the muscle fibres in inclusion body myositis has something in common with that in the brain in Alzheimer’s disease.

The exact relation between the mononuclear infiltrates and the inclusion bodies in inclusion body myositis remains unclear. Several possibilities exist.

(1) Autoimmunity to muscle components

Inclusion body myositis is usually considered to be an autoimmune muscle disease and the T cells which are present in the endomysial infiltrates are believed to be directed at antigens expressed by skeletal muscle cells.\(^5\) No target antigen has been identified but if a true autoimmune response is involved at least two scenarios are possible: (a) an autoimmune attack may cause muscle damage which in turn stimulates formation of inclusion bodies in...
response to this damage, or (b) the immune response may be directed at one or more of the protein components of the inclusions as a consequence of their excessive production and accumulation. Several histological and immunocytochemical features support the autoimmune hypothesis. These include the apparent invasion of non-necrotic muscle fibres by CD8+ T cells in biopsies,22 the expression of MHC class I13 and intercellular adhesion molecule-1 (ICAM-1)24 by non-necrotic muscle fibres which are invaded by the infiltrating T cells, the association of inclusion body myositis with other autoimmune diseases,25 and the strong HLA association with this disease (see below). Some information has also been gained regarding the properties of the infiltrating T cells. A proportion express the enzymes granzyme and perforin,26 which are thought to play a major part in cell mediated cytotoxic responses,27 and those associated with muscle fibre infiltration showed raised levels of expression of ICAM-1 and lymphocyte function associated antigen-1 (LFA-1) and a moderate increase in LFA-3 expression.24 These adhesion molecules play a part in T cell activation.28 Endomysial T cells were predominantly CD45RO+, indicative of memory rather than naive T cells.29 Raised expression of the adhesion molecules on lymphoid and muscle cells was, however, also demonstrable in patients with Duchenne muscular dystrophy.24

There is, however, very little direct in vitro evidence which shows an antigen specific myocytotoxic effect of T cells derived from patients with inclusion body myositis. The one published report, which investigated reactivity of T cell lines derived from patients with inclusion body myositis to autologous muscle cells, showed borderline cytotoxicity by cells from only one patient.30 Granzyme and perforin are likely to be expressed by cytotoxic cells which are activated non-specifically,27 as are ICAM-1, LFA-1, and LFA-3. The presence of CD8+ cells of the memory phenotype in the muscle lesions may simply reflect the fact that T cells with a memory phenotype are more likely to traffic through non-lymphoid tissues and can more readily cross the endothelium to enter inflamed or damaged tissues.31 Analysis of TCR V gene usage in muscle biopsies has not provided evidence to support an antigen specific immune response to muscle in inclusion body myositis. This has been examined by at least two groups using either polymerase chain reaction or immunocytochemical analysis.32,33 In each study Vβ3 positive T cells were commonly found but there was no evidence of monoclonality. It was suggested that the predominance of Vβ3 and Vα2 bearing T cells may have resulted from the influence of a local superantigen.32

Thus although some circumstantial evidence suggests that inclusion body myositis is an autoimmune disease this is far from proved.

(2) Inclusion body formation as a stimulus for muscle damage

The formation of intracellular inclusion bodies may be the primary event in inclusion body myositis. This may cause damage to muscle fibres and the inflammatory infiltrate may then occur as a consequence of this damage. Some evidence supports a distinction between the formation of the inclusions and the inflammatory response. In hereditary inclusion body myositis inclusion bodies containing the characteristic proteins can be shown but the inflammatory infiltrate is minimal.34 Primary muscle cultures prepared from patients with hereditary inclusion body myopathy have been shown to develop inclusions in culture in the absence of inflammatory cells and the formation of these inclusions is enhanced when these muscles are innervated in vitro.35 The overexpression of amyloid precursor protein (APP) in normal muscle cells after gene transduction in tissue culture has recently been shown to produce myotube vacuolization and degeneration along with the accumulation of Congo red positive material and the appearance of nuclear inclusions and tubulofilaments (Askanas et al, unpublished data). Furthermore, treatment of patients with inclusion body myositis with steroids has been shown to reduce the inflammatory component in muscle despite continued disease progression and formation of inclusions.36

The demonstration of increased levels of mRNA encoding APP, prion protein, and nicotinic acetylcholine receptor37-39 are consistent with the excessive endogenous production of inclusion body proteins. Synthesis of several of these proteins is also increased in regenerating muscle cells.40 Thus whether the overproduction of these proteins is a primary cause of muscle damage or is a response within damaged muscle is unclear. The overexpression of certain fragments of APP has been shown to be toxic to the expressing cells41 and accumulation of β44 has been reported to be neurotoxic, toxicity probably being dependent on the formation of β pleated sheets.42 Various mutant forms of APP which have been linked with Alzheimer's disease have been reported to confer overproduction of β44 by cells expressing these genes.43 It has been speculated that abnormal accumulation of one or a few of these proteins in inclusion body myositis results in accumulation of the others44 and the ultimate formation of inclusions. A high proportion of the proteins found in the inclusions is associated with the normal neuromuscular junction.44 An alternative hypothesis to explain their accumulation is the loss of control of expression of these genes via a "master-switch" gene product which may have particular relevance to proteins normally expressed at the neuromuscular junction.45 The net effect could be direct muscle fibre damage due to abnormal protein accumulation, an autoimmune response to one or more of these proteins, or both.

(3) Muscle fibre damage exacerbated by T cell infiltrates

T Cells have been shown to infiltrate skeletal muscle in situations in which muscle degeneration and regeneration is occurring without any autoimmune basis. Such situations include Duchenne muscular dystrophy22 and exercise induced muscle damage.39,40 Further, in vitro studies have shown that T lymphocytes can adhere to autologous and allogeneic myotubes and myoblasts in culture.41 This adherence is increased when myoblasts, in particular, are exposed to cytokines which upregulate the expression of adhesion molecules such as ICAM-1 and when the T cells are activated.42 This kind of environment presumably exists in damaged skeletal muscle; myoblasts are activated to proliferate, inflammatory cells may release a variety of cytokines, and muscle degeneration and regeneration are occurring. Human myoblasts can secrete IL-642 and perhaps other chemotactic factors which may contribute to the inflammatory process. In vitro studies also suggest that under certain conditions lymphocytes can form intimate membrane-membrane interactions with muscle cells by what is presumably an antigen/TCR-independent mechanism and as a result of this interaction certain enzymes can be delivered directly into muscle cells.43 The in vivo significance of this phenomenon is not known but it does indicate that lymphocytes can make intimate effective contact with muscle cells in situations other than an antigen-specific immune response.
The properties of T cells which accumulate in other pathological conditions such as tumours or atherosclerotic plaques may be relevant. Although having no apparent antigen specific function many of these T cells are capable of secreting basic fibroblast growth factor (BFGF) and heparin binding epidermal growth factor-like growth factor. Both of these molecules are potent mitogens for various cell types and BFGF, at least, has been shown to play an important part in muscle regeneration. Indeed, in regenerating murine muscle BFGF has been demonstrated in mononuclear cells, although a distinction between lymphocytes and macrophage/monocytes was not made. Taken together these data suggest that T cells can interact with muscle cells by means other than TCR antigen interaction and that the T cells are capable of delivering products which might cause muscle damage and influence the expression of the proteins found in the inclusion bodies. The molecular signals which attract the T cells to muscle cells in these situations are unknown, as are the molecules which facilitate the membrane-membrane interactions, although adhesion molecules such as ICAM-1 are likely to be involved. Recent observations regarding the interaction of T cells with muscle fibres in polymyositis may also have some relevance to inclusion body myositis. The points of membrane interaction of T cells with the invaded muscle fibres were shown to stain most intensely for APP (T cell) and NCAM-1 (muscle fibres) suggesting a possible interaction between these molecules during muscle fibre invasion.

Genetic factors in inclusion body myositis
An analysis of genetic associations with inclusion body myositis may provide clues to the aetiology of this disease. The most obvious candidate genes are those which have been associated with other autoimmune diseases and encoding the proteins which are found associated with the intramuscular inclusions. The most common markers which have been analysed in autoimmune diseases are those within the major histocompatibility complex (MHC) encoded on chromosome 6. This gene complex encodes the class I and class II HLA antigens which are responsible for the presentation of antigenic peptides to T lymphocytes. Also included in this complex are some of the genes encoding components of the complement system—namely, C4A, C4B, C2, and factor B. We have recently shown that over 90% (14/15) of our patients with inclusion body myositis possess the DR3 allele. Further, in most of these patients the DR3 is present on a haplotype which is marked by HLA B8 and has a deletion encompassing the C4A locus. In the remaining DR3 is present on a haplotype marked by HLA B18, BfF1, and a deletion of C4B. Both of these extended haplotypes have been associated with other autoimmune diseases such as myasthenia gravis, autoimmune thyroid disease, systemic lupus erythematosus (B8/DR3), and insulin dependent diabetes mellitus (B18/DR3). Recent reports that some patients with inclusion body myositis also present with common variable immunodeficiency are consistent with this finding as the B8/DR3 haplotype has also been associated with this condition. The very strong association with DR3 and its presence on two different extended haplotypes suggest a primary role for this allele in the predisposition to inclusion body myositis. Because most T cells seen in inclusion body myositis muscle are CD8+ any direct role for DR3 may be at the phase of CD4+ helper T cell sensitisation.

On the other hand the very high frequency of aberrations in the C4 region (over 90% of patients had at least one C4 gene deleted) raises the possibility that partial deficiency in complement activity may play some part in the pathogenesis of the disease. An increased frequency of C4 null alleles has been described in SLE and a role in the pathogenesis of that disease, by virtue of which defects on in the complex clearly cannot be ignored. A role in inclusion body myositis is less readily explained. Apart from the classic MHC and complement genes several other genes are encoded within the MHC. It has become clear that the DR3 bearing haplotypes associated with inclusion body myositis are extremely stable so that alleles of the genes which form part of these haplotypes (for instance, between B8 and DR3) are likely to be identical in unrelated people bearing them. Thus a role for a linked gene, the function of which has yet to be determined, cannot be excluded. One such gene which has recently been described and which is preferentially, although not exclusively, expressed in skeletal muscle may be worthy of investigation.

Genetic variants of the genes encoding the APP and prion protein, prominent components of the rimmed vacuoles of inclusion body myositis, have been associated with deposition of these proteins in diseases affecting the CNS. Mutations in or near the βA4 encoding region of the APP gene have been associated with Alzheimer’s disease and Dutch type hereditary cerebral haemorrhage and excessive production of the βA4 protein. We have sequenced this region of the APP gene in our patients with sporadic inclusion body myositis and found no evidence for an abnormal sequence in inclusion body myositis; nor have mutations been found in familial inclusion body myositis. Certain mutations in the prion gene have also been associated with the deposition of this protein in Gerstmann-Straussler-Scheinker syndrome and Creutzfeldt-Jakob disease, whereas homozgyosity for either of the two common polymorphic variants at codon 129 of this gene has been associated with Creutzfeldt-Jakob disease. Sequencing of the entire protein coding region of the prion gene in our group of patients with inclusion body myositis showed no mutations and a normal distribution of the codon 129 polymorphisms. A polymorphic variant of the APOE gene (e4) has been strongly associated with Alzheimer’s disease and in vitro experiments have shown that the e4 protein product has an increased binding affinity for βA4 but that its capacity to bind to full length APP has not been excluded. APOE has been found in several different forms and both e3 and e4 have been associated with with other alleles of APOE. It has been proposed that these properties influence both the deposition of βA4 and the rate of development of neurofibrillary tangles in Alzheimer’s disease. The parallels between the inclusions of inclusion body myositis and the amyloid plaques of Alzheimer’s disease—including the presence of APOE in the inclusions, as well as the association between Alzheimer’s disease and APO e4—led us, and others, to examine the allelic frequencies of the APO e4 gene in inclusion body myositis. The frequency of APO e4 was statistically increased in our group of patients with inclusion body myositis compared with normal controls and patients with other forms of inflammatory muscle disease whereas in a second study no statistically significant increase in the frequency of this allele was demonstrable. These studies comprised small numbers of patients and this point will require clarification. In normal skeletal muscle APOE has been shown to be concentrated at the neuromuscular junction and may be up-regulated as a consequence of injury. The APO e4 protein may influence the formation of the inclusions of inclusion body myositis by virtue of its increased binding to βA4 and its decreased affinity for the tau protein. Apart from its role in lipid redistribution between and within tissues APOE may also play a part in muscle regeneration.
and has been shown to be capable of modulating antigen specific and non-specific T cell activation. The allelic variants may differ in their capacity to perform these functions and these differences in function may also contribute to the development of inclusion body myositis. It is clear, however, that an association with APOE e4 will not provide an explanation for the development of inclusion body myositis in all patients. Development of this disease is likely to be multifactorial. Various combinations of genetic factors, probably together with an appropriate environmental stimulus, may interact to precipitate the condition seen as inclusion body myositis.

Summary
Concepts of inclusion body myositis have changed considerably over the past decade. Of particular importance has been the discovery that amyloid and some other proteins, which are also found in the brain in Alzheimer’s disease, are present in the vacuolar inclusions in inclusion body myositis and seem to accumulate as a result of their increased production in muscle fibres. The factors responsible for this increased production and accumulation remain to be determined. Although inclusion body myositis is usually considered to be an autoimmune disease of muscle, the evidence for this remains circumstantial and the relation between the T cell infiltrates and the abnormal protein accumulation in muscle fibres remains unclear. The variable extent of the inflammatory infiltrate in both sporadic and familial cases and the continued progression of the condition and of amyloid deposition to the contracture of muscle in the muscle-infiltrating lymphocytes. A mutagen in most cases suggest that the inflammatory process may be an epiphenomenon although it is possible that it may contribute to the muscle damage. Recent studies have shown that certain genetic factors may play a part in the pathogenesis, even in sporadic inclusion body myositis. These include certain HLA alleles (DR3) and complement haplotypes which could act by predisposing to the development of an immune response in muscle, and the APOE4 allotype which may favour the deposition of amyloid in muscle as has been postulated in Alzheimer’s disease. To date, no genetic variations have been found in the genes encoding the amyloid precursor protein or other proteins whose expression is downregulated in immunosuppressive trest. A mutation in a “master switch” gene which controls the production of all of these proteins remains a theoretical possibility.

Productive areas of investigation in sporadic inclusion body myositis are likely to include detailed analyses of topics such as the control of APP expression and processing in skeletal muscle cells, the molecular response of skeletal muscle cells to injury, and the interaction between healthy and damaged muscle cells and T lymphocytes at the cellular and molecular levels. Investigation of the genetic factors determining each of the above as well as the very strong association with DR3 and the rapidly increasing range of genetic factors which are being shown to be relevant in Alzheimer’s disease may provide important clues to the aetiology of this disease.

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NEUROLOGICAL STAMP

Valeriana officinalis (garden heliotrope)

Since ancient times people have believed garden heliotrope to be a cure for epilepsy and a host of other disorders, thus accounting for one of the plant's alternative names—all heal. In the 16th and 17th centuries, herbalists considered the herb a sedative for "nervous disorders" such as "hysterical complaints", an antispasmodic for convulsions, and a remedy for bad coughs and for constipation. It has been said that the herb drives cats into a frenzy.

It is pictured on a commemorative stamp for the International Congress of Pharmacology, issued by Czechoslovakia in 1971 (Stanley Gibbons 1982, Scott 1775).

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Editorial


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