Molecular Immunology and Genetics of Inflammatory Muscle Diseases

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Polymyositis, dermatomyositis, and inclusion body myositis, although immunopathologically distinct, share 3 dominant histological features: inflammation, fibrosis, and loss of muscle fibers. Progress in molecular immunology and immunogenetics has enhanced our understanding of these cellular processes. Based on the T-cell receptor gene rearrangement, the autoinvasive CD8+ T cells in polymyositis and inclusion body myositis, but not dermatomyositis, are specifically selected and clonally expanded in situ by heretofore unknown muscle-specific autoantigens. The messenger RNA of cytokines is variably expressed, except for a persistent up-regulation of interleukin 1β in inclusion body myositis and transforming growth factor β in dermatomyositis. In inclusion body myositis, the interleukin 1, secreted by the chronically activated endomysial inflammatory cells, may participate in the formation of amyloid because it up-regulates β-amyloid precursor protein (β-APP) gene expression and β-APP promoter and colocalizes with β-APP within the vacuolated muscle fibers. In dermatomyositis, transforming growth factor β is overexpressed in the perimysial connective tissue but is down-regulated after successful immunotherapy and reduction of inflammation and fibrosis. The degenerating muscle fibers express several antiapoptotic molecules, such as Bcl-2, and resist apoptosis-mediated cell death. In myositis, several of the identified molecules and adhesion receptors play a role in the process of inflammation, fibrosis, and muscle fiber loss, and could be targets for the design of semi-specific therapeutic interventions.
recognizes an antigen is the CD3 region, which is encoded by genes in the V-J and V-D-J segments of the TCR gene. If the endomysial T cells are selectively recruited by a muscle-specific autoantigen, the use of the V and J genes of the TCR should be restricted, and the amino acid sequence in their CD3 region should be conserved. Three independent laboratories (Department of Neurology, Stanford University School of Medicine, Stanford, Calif; along with the Istituto Nazionale Neurologico “Carlo Besta,” Milan, Italy; National Institutes of Health [NIH], Bethesda, Md; and Neuroimmunology Laboratory, Max Planck Institute, Martinsried, Munich, Germany) have examined the TCR gene families of the infiltrating or the autoinvasive CD8+ T cells with polymerase chain reaction and immunocytochemistry and showed that in patients with PM, but not in those with DM, only certain T cells of specific TCRα and TCRβ families are recruited to the muscle from the circulation.3,5 Cloning and sequencing of the amplified endomysial or autoinvasive TCR gene families demonstrated a restricted use of the Jβ gene with conserved amino acid sequence in the CD3 region. These findings indicate that in PM, the CD8+ cells are specifically selected and clonally expanded in situ by muscle-specific autoantigens, the nature of which remains unknown. Such antigens, presented to the autoinvasive CD8+ T cells by the class I major histocompatibility complex antigen on the sarcolemma, are expected to be either endogenous muscle peptides or viruses. The failure, however, by several laboratories to amplify known viral RNA from muscles affected by PM points to endogenous muscle proteins, rather than viruses, as the likely candidate autoantigens.1

In patients with s-IBM, similar studies have also shown an oligoclonal pattern of the TCRγ gene expression. In contrast to the case in PM, however, in s-IBM the use of the Jβ gene by the endomysial T cells was random and the sequences in the CD3 region inconsistent, suggesting a non–antigen-specific T-cell recruitment.6 New observations combining immunocytochemistry with polymerase chain reaction and sequencing of the most prominent TCR families have shown that the autoinvasive, but not the perivascular, CD8+ cells are also clonally expanded in patients with s-IBM.7 This finding is enhanced by our observations, based on sequential muscle biopsy specimens performed in a 2-year period in 3 patients with IBM that showed persisting clonal restriction of the same VB families among autoinvasive CD8+ T cells.8 Despite the apparent resistance of s-IBM to immunotherapies, it appears that autoantigenic muscle peptides may also drive the T-cell activation in patients with s-IBM in a pattern similar to that in patients with PM. These observations complement the strong immunogenetic association of IBM with HLA-DR3 alleles,9 its frequent occurrence with other autoimmune diseases,10 and the immunopathologic features of T-cell–mediated and class I major histocompatibility complex–restricted cytotoxicity.11,12 The nature of the muscle antigen and whether it is different or the same in PM and IBM remain to be determined.

ROLE OF CYTOKINES AND ADHESION RECEPTORS IN INFLAMMATION, FIBROSIS, AND AMYLOID

After binding to their receptors on the cell surface, cytokines induce dimerization and phosphorylation of the Janus Kinase (JAK) family of kinases, followed by phosphorylation of the signal transduction and activation of transducers family of proteins that translocate to the nucleus for gene transcription and cell proliferation. In the muscle biopsy specimens of patients with PM, DM, and IBM, there is an overexpression of signal transduction and activation of transducers type 1, indicative of cytokine up-regulation. This has been confirmed by polymerase chain reaction studies that have shown a varying degree of amplification of messenger RNA of interleukin (IL) 1, IL-2, tumor necrosis factor α, interferon gamma, transforming growth factor β (TGF-β), granulocyte-macrophage colony-stimulating factor, IL-6, and IL-10 (Figure 1). The expression of each cytokine, however, has varied among the studied specimens of PM, DM, and IBM muscles and has not always related to the degree of endomysial inflammation. The exception has been for 2 pleiotropic cytokines that have been consistently up-regulated in the muscle tissues, IL-1β in IBM and TGF-β in DM (Figure 1), prompting us to examine further their pathogenetic role for each disease.

TRANSFORMING GROWTH FACTOR β AND DM

Transforming growth factor may be beneficial by suppressing the local inflammatory response or detrimental, when in excess, by stimulating the extracellular matrix

Figure 1. Cytokine messenger RNA expression in 3 patients with inflammatory myopathy shows a variable degree of cytokine amplification. Interleukin (IL) 1β and transforming growth factor β1 (TGF-β1) are the strongest and most consistently amplified cytokines. M indicates molecular weight; GAPDH, glyceraldehyde-phosphate dehydrogenase; IFN-γ, interferon gamma; TNF-α, tumour necrosis factor α; and GM-CSF, granulocyte-macrophage colony-stimulating factor.
proteins, resulting in fibrosis and chronic inflammation.\textsuperscript{11} Mice double knockout for the \textit{Tgfb} gene (\textit{Tgfb}^{−/−}) lack TGF-\textbeta and develop prominent endomysial inflammation similar to that seen in human PM (M.C.D., S. Wahl, PhD, unpublished observations, 1997). Because the adhesion of \textit{T} cells is mediated by the up-regulated integrins on their lymphocytic surface and cellular fibronectin is a ligand for \textbeta\textsubscript{i} integrin, treatment of the \textit{Tgfb}^{−/−} mice with fibronectin peptides has suppressed the endomysial inflammation (M.C.D., S. Wahl, PhD, unpublished observations, 1997). These findings suggest that fibronectin or other small peptides that interfere with the binding of integrins to their respective ligands on the endothelial cell wall may provide new, promising therapeutic approaches for the treatment of patients with PM and DM.

The deleterious effect of TGF-\textbeta in chronic inflammation and fibrosis is best evident in the muscles of patients with DM, where fibrosis is prominent and TGF-\textbeta and TGF-\textbeta messenger RNA are up-regulated.\textsuperscript{12} In the repeated muscle biopsy specimens of patients with DM who improved after successful immuno-therapy, we have observed not only the suppression of class I major histocompatibility complex antigen, vascular cell adhesion molecule, intracellular adhesion molecule-1, endomysial inflammation, and fibrosis,\textsuperscript{13} but also substantial down-regulation of TGF-\textbeta and the TGF-\textbeta messenger RNA. More direct anti–TGF-\textbeta strategies, therefore, may be reasonable future therapeutic strategies in suppressing the deleterious inflammatory fibrosis in patients with DM.

**IL-1\textbeta AND s-IBM**

In s-IBM, the excess of IL-1\textbeta is derived by activated endomysial macrophages and \textit{T} cells and probably by endomysial \textbeta-amyloid precursor protein (APP), which is a known enhancer of IL-1\textbeta production.\textsuperscript{14} There appears to be a closed loop between IL-1\textbeta and \textbeta-APP because IL-1\textbeta up-regulates the \textbeta-APP gene expression and \textbeta-APP promoter through protein kinase C.\textsuperscript{14} Consistent with these data is the observation that IL-1\textbeta co-localizes with \textbeta-APP not only in the amyloid plaques of patients with Alzheimer disease\textsuperscript{15} but also within the vacuolated muscle fibers of patients with s-IBM (\textbf{Figure 2}).\textsuperscript{16} It can be proposed that the microglia in the brain and the macrophages in the muscle, both cells of the same lineage, promote through the production of IL-1 the evolution and continuous formation of amyloid in both the brain of patients with Alzheimer disease and muscle of patients with IBM, as depicted in \textbf{Figure 3}.

The endomysial excess of IL-1\textbeta in patients with s-IBM may also be connected with the development of abnormal mitochondria and the ragged-red fibers that are commonly seen in this disease. In human myotubes, treatment with IL-1 causes cellular destruction and abnormal mitochondria that immunoreact with anti–IL-1 (C. Mora, MD, M.C.D., unpublished observations, 1996-1998). Furthermore, antiseraum to IL-1 immunostains the subsarcolemmal mitochondrial accumulations of the ragged-red fibers in these patients’ muscle biopsy specimens, as shown in \textbf{Figure 2}. Because ragged-red fibers are seen only in s-IBM where inflammation is prominent, but not in the hereditary form of IBM where inflammation is absent, a connection of pathogenic significance may be proposed between chronic inflammation, IL-1\textbeta production, and mitochondrial toxicity.

**APOPTOTIC OR ANTIAPOPTOTIC MOLECULES IN PM AND IBM**

Cytotoxic \textit{T} cells induce cell death either through the perforin pathway or the \textit{Fas-FasL}–dependent process. In PM and IBM, the autoinvasive activated \textit{T} cells contain perforin granules that are reoriented toward the surface of the muscle fibers and, when released, induce pores on the plasma membrane, causing osmotic cell lysis.\textsuperscript{18} Whether the \textit{Fas-dependent} pathway is also involved in myocytic cell death is unclear. Many of the regenerating and degenerating muscle fibers in patients with PM or IBM express the Fas antigen,\textsuperscript{19} and the autoinvasive CD8+
cells express the Fas-L. Despite these Fas-Fas-L interactions, however, no signs of apoptosis have been detected in the muscle fibers of patients with inflammatory myopathies. This is the case even in the muscles of patients with myositis associated with human immunodeficiency virus infection where apoptosis of CD8+ cells takes place in the circulation.20 In the muscle, the expression of Fas does not seem to imply susceptibility to apoptosis, probably because the Fas-positive muscle fibers coexpress neutral cell adhesion molecule or Bcl-2, a 26-kd antiapoptotic protooncogene protein, both molecules associated with regeneration or the prevention of apoptosis.21 Bcl-2 is also expressed on satellite cells, which renders the multinucleated muscle fibers even more resistant to apoptotic death. The balance of the interacting proapoptotic or antiapoptotic molecules and the signals responsible for the transduction of myocytic cell death in the inflammatory myopathies need further study.

GENETICS IN HEREDITARY IBM

Among the autosomal recessive or dominantly inherited adult-onset, nondystrophic myopathies, there exists a heterogeneous group of vacuolar myopathies, collectively called hereditary IBM, owing to the presence of endomyosial vacuoles and tubulofilamentous inclusions identical to those seen in s-IBM.21 Patients with hereditary IBM, with or without IBM, have various clinical phenotypes, some of which are prevalent in certain ethnic groups.21 One subset of hereditary IBM, initially described in Iranian Jews as a quadriceps-sparing, noninflammatory vacuolar myopathy, has now been seen in other ethnic groups in several countries—United States, Mexico, India, and Morocco—and has been linked to chromosome 9p.21 A clinicohistologically similar type of hereditary IBM described in Japan has also been linked to chromosome 9. When this area is narrowed down within a sequencing size, the gene responsible for hereditary vacuolar myopathies may be identified.

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REFERENCES


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