NEW ADVANCES IN MYOPATHIES: OVERVIEW FROM THE IX INTERNATIONAL CONGRESS ON NEUROMUSCULAR DISEASES - ADELAIDE, AUSTRALIA, 1998

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INTRODUCTION

The IX International Congress on Neuromuscular Diseases was held in Adelaide, Australia, between 30 August and 4 September 1998. The Congress covered both basic and clinical science across the field of neuromuscular disorders with 30 lectures, 10 symposia and over 80 workshops, covering many aspects of anterior horn cell disease, peripheral neuropathy, neuromuscular junction disorders and myopathies. The neuromuscular field covers many hereditary disorders and the most exciting advances in this field in the last decade have been in the area of neurogenetics.

At the last International Neuromuscular Congress, which was held in Kyoto, Japan four years previously, the excitement in the neurogenetic field was enormous, with the mutations that underlie a range of neuromuscular disorders being reported at that Congress. By contrast, at the Adelaide Congress the overall impression was that there had been a phase of consolidation over the previous four years, the range of mutations associated with well-established genetic disorders was further defined, and new work looking at disease mechanisms in light of genetic discoveries was presented. The main themes of this Congress were advances in neurogenetics (molecular neurology) and in nerve and muscle regeneration. Some of the advances in muscle disease are briefly presented here.

ADVANCES IN MUSCULAR DYSTROPHY

There have been considerable advances in understanding the pathoaetiology of common forms of muscular dystrophy associated with abnormalities of the complex which anchors the extracellular matrix to the sarcolemma and to the contractile apparatus. The identification of deficiency of the large cytoskeletal protein, dystrophin, by Louis Kunkel and his group some years ago provided the first insight into this area. Professor Kunkel's demonstration of large deletions in the dystrophin gene provided a molecular explanation for the commonest form of muscular dystrophy, Duchenne dystrophy; he gave an update in this area.

Although the muscular dystrophies are a heterogenous group of genetic disorders, the most common are caused by mutations in the **dystrophin gene**. Additional forms of muscular dystrophy are caused by mutations in dystrophin-associated proteins. These include the **sarcoglycans**, which are a complex of four transmembrane dystrophin-associated proteins, mutations of which lead to various forms of limb girdle muscular dystrophy. Analysis of sarcoglycan mutations in limb girdle muscular dystrophy patients is ongoing and correlation of these mutations made with problems with complex assembly at the muscle membrane. Mutations in one sarcoglycan gene tend to lead to depression of levels of the other major sarcoglycans, although the extent of this effect is variable. The sarcoglycans play a critical role in the pathogenesis of most forms of limb girdle muscle dystrophy.

Professor Beckmann (France) presented the experience of his group with limb girdle muscular dystrophy syndromes. Both autosomal recessive and autosomal dominant forms are now recognised. Mutations in any of four sarcoclycans can lead to this syndrome. By contrast, limb girdle LGMD2A is caused by mutations in a gene in coding for a muscle specific calpain 3, a member of the calcium-activated neutral protease family. Over 70 distinct mutations in this gene have been identified. This observation is of interest in that mutations in structural proteins underlie most forms of muscular dystrophy and the identification of a protease mutation suggests that another pathophysiological pathway operates in some cases. He suggested the term 'structuropathy' to encompass cases with structural protein abnormality as opposed to enzymopathy, as exemplified by the calpain 3 mutation cases.

Professor Ozawa (Japan) also overviewed new advances in the field of **sarcoglycanopathies**. His group first proposed the name sarcoglycan complex in 1994 and were responsible for the identification of absence of all three sarcoglycan proteins from the muscle biopsy from a child with severe childhood autosomal recessive muscular dystrophy. If one of the sarcoglycan genes in a patient with muscular dystrophy has a mutation which is either homozygous or if a compound heterozygous mutation is present, the protein product of the mutated gene is lost, and this leads to difficulty in assembly of the sarcoglycan complex with loss of expression of the other sarcoglycan subunits in the patient's muscle. It appears that integrity of the whole sarcoglycan complex is necessary before binding to dystrophin can occur.

RARER MUSCULAR DYSTROPHIES

Bethlem myopathy

This autosomal dominant myopathy was described in 1976 and is characterised by progressive limb girdle and joint contractures. This myopathy has been linked by a Dutch group to chromosome 21q22.3, an area in which are found several of the subunits that code for the extracellular matrix protein collagen components. Shireen Lamande presented a study carried out in Australia in which a new Bethlem myopathy mutation had been identified which resulted in skipping of COL6A1 exon 14 during pre-mRNA splicing with the deletion of 18 amino acids from the triple helical domain of the collagen VI chain. This data provided the first evidence of the biosynthetic consequence of a structural collagen VI mutation and suggested that a protein haploinsufficiency may be a common mechanism in Bethlem myopathy. This demonstration of the extracellular component, collagen VI, present in half the normal amount as a cause of a mild myopathic disturbance, adds yet another mechanism to the causes of muscular dystrophy.

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Emery-Dreifuss muscular dystrophy

This has been an area of considerable genetic excitement in recent times. Professor Arahata (Japan) presented an overview of new findings in this disease which is characterised by slowly progressive muscle wasting and weakness with a humeroperoneal distribution and by early contractures of elbows, Achilles tendons and neck muscles, as well as a cardiomyopathy with severe atrio-ventricular block. It is an X-linked condition but is distinct from the dystrophinopathies. Bione's group recently identified the STA gene as being responsible for the X-linked form of Emery-Dreifuss muscular dystrophy and subsequent immunochemical analysis of the gene product, emerin, has provided new insights into this disorder. Emerin is a 254 amino acid protein which is normally found at the intercalated discs in control muscle and may have a role as a cellular adhesion molecule.

Myoshi myopathy

Another rare type of muscular dystrophy which is characterised by distal weakness confined to the legs and marked calf involvement with very high CK levels. Professor Brown and Dr Bushby showed that Myoshi myopathy mapped to chromosome 2p13 in the same area as limb girdle muscular dystrophy 2B. They have identified a candidate gene which may be important in calcium binding, and further studies are under way to determine whether mutations in this gene underlie both Myoshi myopathy and a form of limb girdle muscular dystrophy.

Congenital muscular dystrophies

These represent another rare type of muscular dystrophy in which there is a problem with binding of the extracellular matrix to the sarcolemma in many cases: a workshop organised by Professor Fernando Tomé overviewed this area. The congenital muscular dystrophies have been known for many years and several forms are described. There is now a major subdivision into cases which lack merosin (laminin A2 chain deficient CMD) and cases where merosin is preserved. There are now at least five forms of congenital muscular dystrophy, one of which is related to merosin deficiency. Cerebral involvement may be associated with its severity ranging from profoundly retarded children who are bedridden to children who become transiently ambulant and achieve a limited knowledge of reading and writing.

Fascioscapulohumeral muscular dystrophy

Reviewed by Professor Padberg, this is an autosomal dominant myopathy of extremely variable expressivity with age of onset varying from 10 until 50 years of age and a large proportion of gene carriers remaining asymptomatic for decades. As well as the characteristic facial and pectoral girdle involvement, some involvement of foot extensors and abdominal muscles is characteristic. High-tone hearing loss is found in 75% of cases and a few patients have a retinal vasculopathy. The gene for FSHD was located to chromosome 4q35 by Padberg's group in 1990: in this area there are between 10 and 100 almost identical tandemly-arranged repeating units each of 3.3 kb, and in patients a number of these repeats are deleted. No gene has been identified within the area of this deletion, suggesting that these deletions execute a position effect on a more centromeric located gene.

MYOTONIC MUSCULAR DYSTROPHY

In myotonic muscular dystrophy somatic instability of an expanded CTG repeat is evidenced by heterogeneity of the repeat alleles within and between tissues. One of the characteristic clinical features of myotonic dystrophy is anticipation, where the clinical phenotype becomes more severe in affected individuals in sequential generations, and it has been known for some time that this relates to an increase in the size of the CTG expansion in more severely affected individuals. Ashizawa suggested that this instability may involve two mechanisms with frequent mutations involving gain or loss of a small number of repeat units, and infrequent large repeat size changes with a bias towards deletion. The triplet repeat in myotonic dystrophy is effectively located between two genes, one of which encodes a protein kinase. The expression pattern of this protein kinase does not correlate well with selective involvement of muscle groups and results were presented by Dr Winchester (Glasgow), looking at the expression pattern of the second gene, called myotonic dystrophy associated homeodomain protein gene. This gene was found to be expressed in many tissues during embryogenesis and had an emphasis for tissues preferentially affected in myotonic dystrophy patients, including the lens. Further study of this protein in myotonic dystrophy is clearly important.

A transgenic mouse has been developed carrying the human DM region, study of which may provide further insights into disease mechanisms. Dr Groenen and colleagues presented their studies of mouse models for myotonic dystrophy, indicating that models with a simple gain or loss of function of the associated protein kinase gene does not lead to an animal model which closely mimics myotonic dystrophy, although second generation mouse models are currently being studied.

Moxley reviewed therapies in myotonic dystrophy, suggesting that certain anabolic steroids such as IGF-1, DHEA and an insulin-enhancing drug, troglitazone, may be potential therapies.

THERAPEUTIC POSSIBILITIES IN THE DYSTROPHIES

John McHowell reviewed gene therapy in the dystrophies, indicating that this has great potential as a method of treatment and that considerable advances have been made but much fundamental work remains to be done. It is noted that the Muscular Dystrophy Association of the United States has applied for permission to undertake a clinical trial of gene therapy in boys with Duchenne muscular dystrophy, and that this should start in the near future. For gene therapy to be practical in human dystrophies, gene expression must be obtained and sustained in appropriate tissues. Studies in the Golden Retriever muscular dystrophy model may be very useful, either before, or ancillary to, human trials. Problems that remain to be addressed include the limited spread of expression from the site of injection into the surrounding muscle, the immune response of the host to the vector and to newly-expressed dystrophin, and the need for systemic spread which would lead to significant expression in many muscles.

Improved cell-based therapies for muscle diseases were discussed by Professor Terry Partridge. Myogenic cells have mechanisms which enable them to rebuild and repair muscle fibres damaged by genetic disease, and during repair muscle fibres can be replaced or augmented by addition of

myogenic cells. There are different types of myogenic cells in mature skeletal muscle, some of an early progenitor type and some of a more typical adult satellite cell type which behave differently during muscle regeneration and on transplantation. Although trials of cell-based therapy have had some problems to date, further optimisation of myoblast transplantation technique has the potential to lead to improved outcomes. It is possible that the behaviour of myogenic cells can be altered by transgenesis in a way that may improve their powers of dispersion and their ability to restore muscle structure and function.

Professor Tremblay noted that clinical trials of myoblast transfer had produced limited results in the main due to three problems, namely an immune response which rapidly killed many of the transplanted cells, limited migration of transplanted myoblasts and an inflammatory reaction. With major immunosuppression in a mouse model the results were improved with studies in an mdx mouse (dystrophin deficient) indicating that up to 95% of muscle fibres express dystrophin.

Issues that need to be addressed in ongoing cell therapy trials include the nature of the immunosuppressive regimen recommended and methods to improve myoblast migration when injected directly into muscle, such as by using a metalloproteinase gene. Although initial studies with myoblast transplant have been disappointing there is clearly the potential for further studies in this area based on a better understanding of the scientific problems that have to be overcome.

Looking ahead it can be reasonably anticipated that by the next neuromuscular congress, which will be held in Vancouver in four years time, the remaining questions about the link between genetic abnormalities and disease pathogenesis in the dystrophy area will have been largely solved and there will have been further solid steps towards both successful gene and cell therapy.

INFLAMMATORY MYOPATHIES

Professor Frank Mastaglia (Western Australia) discussed clinical and MRI findings in response to treatment and outcome in the major forms of idiopathic inflammatory myopathy. The three major forms of inflammatory myopathy are polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). Professor Mastaglia's group followed 72 patients for periods of up to seven years: 24 DM, 9 PM, 21 with an overlap syndrome and 18 with IBM. In IBM, MRI scan proved a useful investigation, confirming selective and asymmetric involvement of the *vasti* and *medial gastrocnemius* in the lower limbs, and of the *flexor digitorum profundus* in the upper limbs. The majority of patients with PM or DM responded favourably to treatment but some residual muscle weakness was found in most patients who were considered to be in remission when strength was compared to gender and age-matched control data from 200 normal subjects.

Considerable advances have been made in recent years in characterisation of cellular, humoral and molecular aspects of muscle inflammation in inflammatory myopathies. Mantegazza and colleagues (Milan) looked at the nature of the inflammatory infiltrate in different cases: the infiltrating cell phenotype was mainly CD8+ T cells in polymyositis and inclusion body myositis. A restricted disease specific V_b T cell receptor family repertoire was found in both PM and IBM. The nature of the antigens which provoke this are still unclear. Immunohistochemical and molecular studies indicate that muscle fibre injury is mediated by cellular release of cytotoxic enzymes whereas no apoptotic signals were observed. Immune-mediated mechanisms play a primary role in the pathogenesis of PM and DM, but the primary target antigen is unknown and applied immunotherapies non-specific. Many patients with PM and DM respond to steroids to some degree and for some period of time. Professor Dalakas suggested that although azathioprine, methotrexate, cyclosporine and cyclophosphamide are used sometimes with mild-to-moderate benefit, response may be disappointing. Combination immunotherapies are gaining ground. Plasmapheresis has been largely disappointing. High-dose intravenous immunoglobulin may be a helpful modality, especially in dermatomyositis. Pulse intravenous methylprednisolone has been reported as beneficial also, mainly in dermatomyositis. Diltiazem may reduce muscle calcification.

Inclusion body myositis was discussed in two workshops. Professor Serratrice examined the clinical features necessary to make this diagnosis. Onset is typically in the sixth to eighth decade with a male preponderance. Asymmetrical muscle weakness and atrophy, both distal (foot dorsiflexors, wrist and finger flexors or extensors) and proximal, and ankle areflexia is typical. Some muscle involvements are hallmarks, including sterno-mastoid involvement, quadriceps, peroneal, wrist and finger extensors and flexors. Typical cases may present with multifocal involvement and asymmetrical pseudoneuropathies. Pseudopolymyositic forms may be seen with dysphagia, myalgia and proximal weakness.

Questions poised by Professor Serratrice as currently unresolved include: Is refractory polymyositis a misdiagnosed IBM? Is IBM an inflammatory or degenerative disease? How do we treat this untreatable disease? Hereditary inclusion body myopathy was also discussed in a workshop. Professor Argov discussed the clinical features indicating that the spectrum of HIBM based on clinical features is wide but current linkage analysis data may redefine this. He discussed a recessive quadriceps sparing myopathy (QSM) which is a form of HIBM and has been described in at least 100 patients from more than 40 families. This may be associated with facial weakness. CNS white matter changes have been reported in one French-Canadian family, and another characteristic hereditary IBM syndrome is Japanese distal myopathy. In most forms onset is in early life with slow progression and maintenance of ambulation into the fourth to the sixth decade. Genetic studies in HIBM were presented by Eisenberg and colleagues who concluded that the HIBM gene resides in a 1 megabase interval on chromosome 9. A similar chromosomal location has been reported by a Japanese group for autosomal recessive distal myopathy with rimmed vacuoles. An autosomal dominant form of inclusion body myositis is also recognised and remains to be more clearly defined at a genetic level. There are some similarities in the pathological appearance in skeletal muscle to changes seen in the brain in Alzheimer dementia but it has not been possible to link hereditary inclusion body myositis to any of the recognised gene loci in hereditary Alzheimer's disease. Inclusion body myositis, both sporadic and hereditary, is an area of major ongoing study at present, with major doubts as to whether it is truly an inflammatory myopathy.

METABOLIC AND MITOCHONDRIAL MYOPATHIES

A major lecture from Dr John Morgan-Hughes and a symposium and several workshops were devoted to the area of mitochondrial myoencephalopathy. Much work in recent years has been devoted to defining the nature of mutations in mitochondrial DNA, which underlie various myoencephalopathies. A range of tRNA mutations which tend to be closely associated with a particular myoencephalopathic syndrome is well defined, although some overlap is recognised. Large-scale rearrangements tend to be associated with CPEO syndromes. Point mutations in structural subunits in complex I tend to lead to Leber's Hereditary Optic Neuropathy. Although the aetiology of these mitochondrial DNA disorders has been defined in large measure, much remains to be understood about pathogenetic mechanisms, in particular selective patterns of tissue involvement unique to particular mutations. Dr Poulton discussed the mitochondrial bottleneck as a mechanism which may allow switching to a new mitochondrial variant within a single generation.

A workshop was devoted to mitochondrial failure in senescence and in neurodegenerative disease, a controversial area where the significance of the mitochondrial abnormalities reported is not always clear. A significant fall in respiratory chain activity occurs in a range of human tissues with ageing. Immunocytochemical studies have demonstrated patchy deficiency of cytochrome oxidase in cerebral neurones in elderly individuals which may be more marked in AD dementia. Abnormalities of mitochondrial function have also been identified in Parkinson's disease and Huntington's disease: the significance of the mitochondrial changes in these areas remains to be clarified. By contrast, it is clear that mitochondrial dysfunction has a central role in Friedreich's ataxia, where Pandolfo presented a paper overviewing the role of frataxin, a mitochondrial protein associated with the inner membrane which is involved in iron transport and in which mutations lead to Friedreich's ataxia.

OVERVIEW

In this short overview of advances in myology presented at the IX International Congress on Neuromuscular Diseases only a small number of the many symposia and workshops in the Congress devoted to this area have been addressed. A full listing of the topics with abstracts can be found in the Conference Handbook[1] (lectures and symposia), and in the issue of *Muscle & Nerve* [2] devoted to the Congress (workshops and free communications).

ACKNOWLEDGEMENTS

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REFERENCES

1 Byrne E. *IX International Congress on Neuromuscular Diseases. Movement Along the Final Common Pathway. Conference Handbook* Adelaide, Australia, 1998. (Copies available from SAPMEA Conventions).

2 *Muscle & Nerve*. Supplement 7, 1998.