

tain antibodies play a role by recruiting macrophages to the muscle in an ADCC process, remains unclear.

In uncontrolled studies, PM and DM respond to prednisone to some degree and for some period of time; adding an immunosuppressive drug (Azathioprine, Cyclosporine, Mycophenolate, Methotrexate) may have a steroid-sparing effect but their benefit is uncertain. In contrast, IBM is resistant to most of these therapies, most times. Controlled studies have shown that IVIg is effective and safe for the treatment of DM. The clinical benefit, which can be impressive in patients with early disease, is associated with improvement in the muscle cytoarchitecture and resolution of the aberrant immunopathological parameters, including interception of complement activation and downregulation of ICAM-I, VCAM, TGF- β , MHC-I and various immunoregulatory and structural genes. IVIg seems to be also effective in patients with PM but offers transient help to a small number of patients with IBM.

New agents currently on the market may be promising new therapies for the treatment of inflammatory myopathies. Among them include the monoclonal antibodies or fusion proteins against: a) molecules associated with T-cell-signaling pathways, such as the anti-CD52 (CAMPATH), anti-LFA/ICAM (Leukocyte Functional Antigen/ Intracellular Adhesion Molecule), and anti-IL2 receptor (IL2-receptor antagonist). Further, two cytophillin-binding drugs, Tacrolimus and Rapamycin, that prevent the IL2-induced T cell proliferation or transcription, may be candidate agents for certain conditions; b) B cells using the monoclonal antibody directed against CD20, expressed on B cells (Rituximab) or the humanized version Ocrelizumab; agents against B-cell growth factors, such as BAFF and APRIL are in the offing; c) Complement C5 (Eculizumab), which might be appropriate for some complement-mediated disorders like DM or NAM; d) cytokines, especially agents against TNF- α , IL1 or IL1 β ; and e) Cellular Adhesion and T cell transmigration molecules. Such agents include Natalizumab directed against α 4 β 1 integrin (VLA4) on lymphocytes, a drug approved for multiple sclerosis, and Fingolimod, an anti-T-cell-migration agent that traps lymphocytes in the lymphoid organs.

I-8 Muscle maturation and early pathogenic findings in spinal muscular atrophy: any clues for therapy?

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder that affects motor neurons. It is caused by mutations in the survival motor neuron gene 1 (SMN1). The SMN2 gene, which is the highly homologous SMN1 copy that is present in all patients, is unable to prevent the disease. SMA patients can be classified into four groups based on age at onset and acquired milestones (type I or severe acute disease, with onset before 6 months; type II, before 18 months; type III, after 18 months and type IV, in adult life). The explanation for the neuromuscular phenotype in SMA is to assume that insufficient SMN protein causes motor neuron dysfunction and death, and that muscle at-

rophy is a consequence of denervation. However, investigation is ongoing to ascertain whether muscle, neuromuscular junctions, or motor neurons alone are the critical target tissue in SMA. The neuropathologic description of SMA comes largely from postnatal necropsy samples, which describe the end-stage of the disease. The human developmental period appears to play an essential role in SMA pathogenesis. With the exception of severe congenital SMA (type 0), varying age at onset in the four SMA types provides evidence of a latency period without clear manifestations in most SMA patients. Given that studies of patients' preclinical status are lacking, the main objective of our work is to study SMA during development to gain insight into the mechanism of disease in the prenatal and presymptomatic stage. Prenatal SMN tests performed at around 11-13 weeks allowed us to identify fetuses predicted to develop SMA in families with a previous patient affected by type I disease. SMA fetuses were collected from therapeutic abortions after confirmation of a homozygous deletion of exon 7 and 8 of the SMN gene by chorionic villi DNA analysis. In these samples we systematically studied histology, cell death and gene and protein expression in spinal cord and muscle, the key tissues involved in the disease. The study of terminal peripheral nerves and neuromuscular junctions identify possible links between the two tissues in the pathogenesis of the disease. By confocal and electron microscopy we observed a variable degree of changes in the acetylcholine receptors clustering, presynaptic retention of vesicles and terminal nerve degeneration in the motor endplates of fetuses with severe SMA. Furthermore, ultrasound fetal movements were investigated at these stages. At the gestational age examined, we did not observe a qualitative early limitation of movements in fetuses with SMA. Our results support the view of SMA as a developmental disorder and the hypothesis that motor neurons, terminal peripheral nerves, neuromuscular junctions and skeletal muscle may play all together a role in the pathology of SMA. These studies may help to define therapeutic targets and delineate a possible early intervention in SMA.

Supported by FIS 08-0729 and GENOME PROJECT.

I-9 Opportunities and challenges of pharmaceutical research and development today

L. Middleton

Not arrived

I-10 Pathogenesis of muscle degeneration in periodic paralyses and DMD

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Mutations in Cav1.1 and Nav1.4 channels cause hypokalemic periodic paralysis, a dominantly inherited muscle disorder characterized by episodic weakness and chronic progressive weakness. In-vivo ²³Na magnetic resonance imaging (MRI), fat-suppressed 1H-MRI (STIR), and force assessment were performed to determine intramuscular Na⁺ load, edema, and muscle strength in patients under different conditions. Membrane

potentials and twitch force were measured in muscle strips obtained from patients and controls. Of the 36 patients, 25 presented with chronic muscle weakness of varying degrees, up to wheelchair-dependence. The weakness was associated with intracellular Na⁺ overload and edema. Older patients revealed a vacuolar myopathy or a progressive muscular dystrophy. Weakness, intracellular Na⁺ overload and edema were increased and further raised by cooling and glucose/insulin, and almost completely normalized by 4 weeks of treatment with the carbonic anhydrase inhibitor acetazolamide (Jurkat-Rott et al., 2009). In vitro, the chronic weakness correlated to membrane depolarization, and acetazolamide repolarized the membrane and restored force. We conclude that membrane depolarization associated with intracellular Na⁺ overload and edema causes both episodic and permanent muscle weakness. The chronic weakness is reversible in muscles which show mild or only moderate fatty degeneration. Acetazolamide has direct and beneficial effects on weak muscle and can markedly improve both forms of weakness.

In addition, we tested whether the edema in Duchenne muscular dystrophy (DMD) is caused by an osmotic effect due to increased myoplasmic Na⁺ content or by inflammation. The muscle edema was quantified on STIR images using background noise as reference. Na⁺ was quantified by a muscular tissue sodium concentration (TSC) sequence. A novel inversion-recovery (IR) sequence allowed us to determine mainly the myoplasmic Na⁺ by suppression of the extracellular ²³Na signal, e.g. from vasogenic edema. Both intracellular TSC and water content were markedly increased in DMD compared to volunteers ($p < 0.001$). We conclude that the elevated myoplasmic Na⁺ concentration in DMD is osmotically relevant and causes a mainly intracellular muscle edema that contributes to the pathogenesis of DMD.

We hypothesize that antiedematous treatment can reverse the edema and prevent the edema-induced muscle degeneration.

I-11

News in non dystrophic myotonias

F. Deymeer

Not arrived

I-12

Timothy Syndrome and Cardiomyopathy

R. Bloise

Not arrived

I-13

Muscle ryanodine receptor in congenital myopathies and channelopathies

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Central core disease (CCD) and malignant hyperthermia (MH) have been linked to point mutations in the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel (ryanodine receptor), which is localized on human chromosome 19 (RYR11). Central core disease is a relatively mild, slowly progressive autosomal dominant myopathy, characterized histologically by the presence of centrally located cores running the length of the muscle fibres. MH is a pharmacogenetic induced hypermetabolic disease. CCD linked RyR1 mutations are associated with depletion of thapsigargin-sensitive stores and to an increase of the resting calcium level. Influx of Ca²⁺ from the extracellular environment is a major factor influencing the level of the resting intracellular [Ca²⁺]. Our working hypothesis is that decrease of sarco(endo)plasmic reticulum Ca²⁺ load via leaky ryanodine receptor channels and/or alteration of calcium influx via store operated channels or excitation-coupled Ca²⁺ entry (ECCE), may account for, at least in part, the phenotype of patients with CCD, including muscle weakness and abnormal secretion of inflammatory cytokines from muscle cells. We set out to test the validity of our hypothesis by directly investigating the mechanisms activating calcium influx in myotubes from normal individuals and from patients with CCD and MH by TIRF microscopy. Our data shows that some mutations in RYR1 affect ECCE in human myotubes from CCD and MH patients; this enhanced Ca²⁺ entry is accompanied by the generation of reactive nitrogen species and enhanced nuclear localization of NFATc1, which in turn may be responsible for the increased IL-6 released by myotubes from patients with central core disease.

I-14

Molecular pathomechanism of DM2/PROMM: similarities and differences between DM1 and DM2

G. Meola

Not arrived