P4.48

Therapeutic effect of rapamycine in a mouse model of autoimmune myositis

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Polymyositis (PM) and sporadic inclusion body myositis (sIBM) are inflammatory myopathies characterized by invaded muscle fibers by auto-reactive CD8 + T cells. Immunosuppressive drugs used in PM are not always efficacious and have no effect on sIBM. We have previously shown the positive effect of regulatory T cells (Treg) in a mouse model of experimental autoimmune myositis (EAM). We then tested in our. For EAM, mice were immunized once a week for 3 weeks with myosin emulsified in complete Freund's adjuvant. Muscle strength was evaluated by measuring the time (in s) the mouse was able to stay hanged. The histological severity of muscular inflammation was graded using the Kojima score (raging from 0: no inflammation to grade 4: diffuse, extensive infiltration). Mice received rapamacyne (3 mg/kg/j) during immunization protocol. Results were compared to a control group receiving placebo. In rapamycine treated mice, myositis was significantly less severe attested by the clinical (320 ± 152 s vs 56 ± 14 s, $p = 0.001$) and the histological scores (0.88 ± 0.75 vs 3.55 ± 0.7, $p = 0.001$). In draining lymph nodes of treated mice, we observed a significant decrease of percentage of T cells ($29 \pm 12\%$ vs $68 \pm 4.5\%$) and a significant increase of the Treg percentage ($16.4 \pm 1.2\%$ vs $9.8 \pm 1.8\%$ $p = 0.001$). Furthermore, auto-reactive activated T cells cannot leave the draining lymph nodes because of the increase of chemokines receptor CCR7 (1233 ± 343 vs 920 ± 128 MFI) and integrin CD62L (14271 ± 3574 vs 11280 ± 2180 MFI). These results show the efficacy of rapamycine in our model. The effect may be due to the increased percentage of immunosuppressive cells (Treg) associated with a decrease percentage of auto-reactive T cells blocked in the draining lymph node. These encouraging results have to be confirmed in human myositis.

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P4.49

Preventive therapy of severe neonatal myasthenia gravis during pregnancy

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We report the case of a 37 years woman with mild myasthenia gravis receiving immune therapy during her pregnancy in order to prevent a second case of severe neonatal MG with foetal involvement. Her previous child born in 2001, 8 months before the first maternal myasthenic symptoms, presented mild hydramnios, severe hypotonia and swallowing impairment, requiring at 24 h ICU survey for tube feeding during 7 weeks, recovering since the 4th month. At age 8 years, there was a persistent and disabling velo-pharyngeal impairment with anterior soft palate immobility. The mother was myasthenic since age 27 years, with initial and prominent ocular involvement, mild generalization signs, benign course; therapy included anticholinesterases, thymectomy and steroids. Basedow disease with mild ophtalmopathy was discovered at 33 years and treated by thyroidectomy. Anti-AChR ab titre was elevated between 150 and 100 nm, with a high foetal anti-AChR ratio (65%) quantified by $f \times$ TE671 assay. Despite the explanation of a high risk of severe NMG, the patient insisted for a third pregnancy beginning in February 2010 (37 years). All along pregnancy, myasthenic symptoms were minimal. In order to protect the foetus from maternal ab injury, steroids were increased from 5 to 20 mg/day, five IvIg infusions were administered from the 4th to the 9th month of pregnancy (each session, 2 days, 0.5 g per kg per day). Maternal Anti-AChR ab titre which was more than 100 nM 6 months before pregnancy decreased to 59 nM at 5 months and 49 nM at birth. The third baby was a girl born without any myasthenic symptoms. In conclusion, facing a high risk of severe NMG with foetal involvement, the proposition of a maternal protective therapy, aiming the decrease of amount of ab particularly pathogenic for the baby, is to be considered.

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P4.50

Mitochondrial therapy with Cyclosporine A in patients with Ullrich Congenital Muscular Dystrophy

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We are reporting the results of a prospective long-term open therapeutic trial to test the effect on muscle strength and respiratory function of CsA in six patients with UCMD. Three of these patients have been originally enrolled in the pilot trial(Merlini L et al. Proc Natl Acad Sci USA 2008;105:5225–9) with a biological endpoint in which treatment with CsA favorably affected mitochondrial function in ColVI myopathies in vivo, and dramatically decreased the frequency of muscle cell death in the patients. In the extended trial muscle strength improved significantly in 5 of the patients after a mean 22 months of treatment. Respiratory function slowly but continuously deteriorated in all the patients. Two patients required mechanical ventilation at night and one died after an acute respiratory infection. Long-term treatment with CsA has two different outcomes in UCMD patients: it improves muscle strength but does not impede the progressive deterioration of the respiratory function. The diaphragm is the most severely compromised muscle both in the otherwise mildly affected Col6a1−/− mice and in UCMD patients. Possible explanations for the contrasting results include at least the following: CsA may not be effective when the muscle is already too affected, different muscles respond differently to CsA, or both. The support of Telethon Fondazione Onlus – Grant GUP08006 – is gratefully acknowledged.

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P4.51

Correction of FKRP function via RNA *trans*-splicing

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Mutations in fukutin-related protein (FKRP), which are common in the Caucasian population, cause loss of sugar coating in dystroglycan and lead to several forms of muscular dystrophy, ranging from limb girdle muscular dystrophy to more severe congenital variants such as Walker Warburg Syndrome. No effective treatment exists for these conditions, characterised by deterioration of muscle and heart function and eventual early death of affected individuals. One approach to treatment is to restore

the function of the protein in muscle by correcting mutations in the FKRP gene. To this end we aim to employ a novel technology, RNA *trans*-splicing, which has shown promising results in preclinical models of diseases such as cystic fibrosis and Duchenne muscular dystrophy. In this form of gene therapy, a faulty exon within an endogenous transcript is targeted for *trans*-splicing-mediated replacement, which conveys the advantages of reduced transgene size and, importantly, retention of the endogenous regulation and expression pattern. We are currently testing a panel of pre-*trans*-splicing molecule (PTM) constructs in order to optimise the *trans*-splicing event with an exogenous FKRP minigene in a wild type cell line. Following selection of the most effective PTM and validation of the approach, we aim to apply the treatment to cell lines derived from patients and, subsequently, to FKRP-mutant mice to determine effectiveness *in vivo*. Ultimately, RNA *trans*-splicing could represent a novel form of therapy for patients with FKRP mutations and, appropriately modified, for patients with other mutations that are not easily corrected via conventional gene therapy.

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P4.52

Combining gene and stem cell therapy in the treatment of dysferlinophaties

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Mutations in gene encoding for dysferlin are involved in two main phenotypes of autosomal recessive muscular dystrophies: Miyoshi myopathy and Limb-Girdle Muscular Dystrophy type 2B. Both diseases are characterized by progressive weakness and wasting of skeletal muscles. No treatment is available for these diseases and development of effective therapies remains a big challenge. Dysferlin is expressed in skeletal and cardiac muscles where its function is membrane repair. Dysferlin contains calcium-dependent C2 lipid binding domains and an essential transmembrane domain. However, mildly affected patients in whom one or a large number of DYSF exons were missing have been described, suggesting that internally deleted dysferlin proteins can be functional. These evidences encourage to apply the exon skipping approach in dysferlinopathies. In a LGMD-2B patient carrying a deletion of the exon 32. Aim of this work was to extend the feasibility of exon skipping approach on the treatment of a patient carrying a deletion in exon 22 found at heterozygous composite state with a large deletion (ex 25–29) predicted to be in-frame. In this work we tested different antisense oligonucleotides (AONs) in order to induce the skipping of four DYSF exons and in the same time, we tested complete dysferlin protein delivery by lentivirus in CD133+ patient's blood cells and injected in scid/blAJ model. The LV-DYSF vector allowed dysferlin expression both in vitro, in mononuclear cells from our patients, and in vivo after intramuscular injection of transduced CD133+ stem cells in the scid/blAJ mouse model. We strongly believe that the combination of exon skipping strategy with lentivirus transduction of patient stem cell represents a useful tool for the development of new therapeutic approaches in dysferlinopathies. Moreover, lentivirus carrying complete dysferlin can be useful to bypass all unskippable mutations, as those located in transmembrane domains or other essential parts of the gene.

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P4.53

Effect of rituximab in two patients with dysferlin-deficient muscular dystrophy

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The administration of rituximab (RTX) in vivo results in B-cell depletion, evidence for multiple mechanisms of action have been reported. B cell depletion produced a response in patients with polymyositis, which is characterized as a T cell-mediated autoimmune disorder with biopsy findings similar to Miyoshi myopathy. In dysferlinopathies, there is evidence of immune system involvement including the presence of muscle inflammation and a down regulation of the complement inhibitory factor, CD55. Two patients were treated with four weekly infusions of RTX. To measure the improvement in muscle strength after treatment, the isometric hand grip maximal voluntary contraction (MVC) was measured by load cell four times during treatment, and again after one year. We determined the hand MVC analysis in 16 healthy subjects. We measured the number of B cells present in patients by flow cytometric analysis during the course of treatment. The analysis of B cell number during the course of treatment showed that CD20- and CD19-positive cells were depleted to 0–0.01%. The decrease in B cells was followed by an improvement in the mobility of the pelvic and shoulder girdles as shown by the MRC%. The MVC values of both patients began at values lower than normal whereas during treatment patients had improved percentage of muscle strength. The strength peak in both patients coincided with the minimum B cell values. We consider the increase in muscle strength observed in both treated patients to be a consequence of their treatment with RTX. To our knowledge, these are the first cases of increased muscle strength in patients with MM. The results of this study indicate that B cell depletion with RTX may be useful in the treatment of patients affected by MM, suggesting a possible role for B cells in the pathophysiology of this muscle disorder.

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P4.54

Increased oxidative stress and successful antioxidant treatment in a vertebrate model of RYR1 related myopathy

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The skeletal muscle ryanodine receptor is a critical mediator of excitation–contraction coupling and is essential for muscle calcium homeostasis. Mutations in RYR1 are associated with several childhood-onset myopathies (termed RYR1-related myopathies), including central core disease and minicore myopathy. Collectively, they are the most common non-dystrophic muscle diseases of childhood. No treatments currently exist for these disorders. While the major abnormality is hypothesized to be defective excitation–contraction coupling, other abnormalities related to altered RyR1 expression and function are likely to play a role in disease pathogenesis. To better understand the cellular changes found in RYR1 myopathies, we performed microarray analysis on a zebrafish model of RYR1