Myasthenia Gravis: New genes, new targets and novel immunomodulation approaches

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Myasthenia gravis (MG) and its animal model, experimental autoimmune MG (EAMG), are autoimmune disorders in which the acetylcholine receptor (AChR) is the major autoantigen. Our lab focuses on the elucidation of molecular, cellular and immunological mechanisms underlying the elicitation and progression of these diseases and on attempts to develop novel gene targets and new immunomodulation approaches for EAMG.

IP-10 and its receptor CXCR3 are overexpressed in myasthenia gravis. DNA microarray technology, supported by quantitative real time PCR, immunohistochemistry and flow cytometry, were used to identify new potential drug targets for MG and to delineate genes involved in the pathogenesis of the disease.

The chemokine IFN-γ-inducible protein 10 (IP-10, CXCL10), and its receptor CXCR3 were found to be overexpressed in lymph node cells (LNC) of EAMG rats. Real time PCR confirmed these findings and also revealed upregulated mRNA levels of Mig, another chemokine that activates CXCR3. TNF-α and IL-1β were also upregulated. These upregulations were observed in both immune response effector cells, namely LNC, and in the target organ of the autoimmune attack, the muscle, and were reduced following antigen-specific suppression of EAMG. The relevance of IP-10/CXCR3 signaling in myasthenia was validated by similar observations in MG patients. A significant increase in IP-10 and CXCR3 mRNA levels in both, thymus and muscle was observed in MG patients compared to age-matched controls. Our results demonstrate the involvement of IP-10/CXCR3 signaling in the pathogenesis of myasthenia and suggest that CXCR3 and IP-10 may be suitable new drug targets for treatment of MG and other autoimmune diseases.

Overexpression of phosphodiesterases in EAMG and Suppression of disease by a phosphodiesterase inhibitor. DNA microarray analysis followed by quantitative real time PCR analysis revealed increased levels of several phosphodiesterase (PDE) subtypes in LNC and muscles of EAMG rats, compared to healthy controls. This is the first time that alterations in the expression levels of PDEs in an autoimmune disease have been demonstrated.

Pentoxifylline (PTX), a general PDE inhibitor, inhibited the progression of EAMG when treatment started at either the acute or chronic stages of disease. This suppression was associated with downregulation of humoral and cellular AChR-specific responses as well as downregulation of PDE4, TNF-α, IL-18, IL-12 and IL-10 in LNC and of PDEs 1, 4, 7 and TNF-α in muscles. The expression of Foxp3, a transcription factor essential for CD4+CD25+ regulatory T cell function, is increased in splenocytes although the number of these cells remains unchanged. Interestingly, PTX also reduces the expression of the endopeptidase cathepsin-l, a marker of muscle damage, in EAMG muscles, suggesting a possible direct effect of PTX on muscles.

Data mining and comparison of the muscle transcriptome in MG and EAMG. The transcriptomic approach has been employed to analyze the expression of genes in muscles of EAMG rats and in muscle biopsies from MG patients. Rat muscle genes were analyzed by Affymetrix chips and human muscle genes by Agilent cDNA chips. The lists of deregulated genes in MG and EAMG were compared. Statistical analyses of the muscle transcriptomes revealed two major groups of deregulated genes common to both MG and EAMG: 1) Genes linked to muscle biology, including muscle proteins regulating contraction such as myosin polypeptides and myosin binding proteins; 2) Genes belonging to the chaperone protein category including several heat shock proteins. Besides these major categories, there was evidence for deregulation of genes involved in transcription and translation in human MG patients and for overexpression...
of genes involved in metabolism and cell death in EAMG rats. There were no inflammation-associated deregulated genes in MG or EAMG. As muscle genes are clearly deregulated in myasthenia, modulating their protein products could represent a new therapeutic approach.

**Combined therapies for MG: Synergistic effect in EAMG by combined treatment with corticosteroids and pentoxifylline (PTX).** Current treatment for MG and other autoimmune diseases involves in many cases the use of general immunosuppression, mainly corticosteroids that have undesirable side effects. The ultimate therapy for MG and other autoimmune diseases may combine more than one treatment modality, possibly operating through different mechanisms. Moreover, any treatment that will allow to reduce the dose of general immunosuppressive drugs would be extremely beneficial.

We attempt to employ combined therapy for MG that will include corticosteroid administration at sub-optimal doses together with other antigen-specific or antigen-nonspecific treatment modalities. In view of our study on the therapeutic effect of PTX treatment in EAMG (see above), we have attempted combined therapy with corticosteroids (Solumedrol) and PTX. We demonstrate that treatment of EAMG with a combination of sub-optimal doses of both Solumedrol and PTX gives excellent therapeutic results, by far better than any of these drugs separately. The dose of steroids used in the combined treatment modality could be ten times lower than the doses used in steroid treatment alone.

**Immunosuppression of EAMG by pooled immunoglobulins is mediated by an antigen-specific anti-idiotypic activity.** Intravenous immunoglobulin (IVIG) administration has been used in recent years for the treatment of a variety of autoimmune diseases including MG. The mechanism of action of IVIG treatment and the fraction responsible for its therapeutic effect are still not identified. By studying the effects of IVIG administration in rat EAMG we showed that IVIG treatment can successfully prevent the induction of EAMG and immunosuppress an ongoing disease. The mechanism by which IVIG modulates EAMG involves suppression of Th1 cells and B cell proliferation but probably does not act via regulatory T cells.

In collaboration with Prof. O. Laub and Dr. R. Meidler from Omrix we have employed this model system to test the possible involvement of antigen-specific anti-idiotypic activity in the therapeutic effect of IVIG. Chromatography of human IVIG on immobilized rat anti-AChR IgG results in a complete depletion of the suppressive activity of the IVIG. Moreover, the eluted immunoglobulin fraction that had been adsorbed onto the anti-AChR antibodies and comprises as most only 1/100000 of the IVIG preparation, retains the immunosuppressive activity of IVIG. This supports the notion that the therapeutic effect of IVIG is mediated by an antigen-specific anti-immunoglobulin activity (anti-idiotypes) present in pooled human IgG and raises the possibility that disease-specific anti-idiotypic activity can be fractionated from IVIG and become a general improved reagent for disease treatment.

**Selected publications**