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immunology

Myasthenia Gravis: Immunological mechanisms of and novel immunomodulation approaches

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.

Regulatory T cells in myasthenia gravis: Ex vivo generated regulatory T cells modulate experimental autoimmune myasthenia gravis.

Naturally occurring CD4⁺CD25⁺ regulatory T (Treg) cells are key players in immune tolerance and have therefore been suggested as potential therapeutic tools for autoimmune diseases. In myasthenia gravis (MG), reduced numbers or functionally impaired Treg cells have been reported. We have observed that PBL from myasthenic rats contain decreased numbers of CD4⁺CD25^{high}FoxP3⁺ cells as compared with PBL from healthy controls and have tested whether Treg cells from healthy donors can suppress experimental autoimmune MG (EAMG) in rats. As the number of naturally occurring Treg cells is low we used an approach for large-scale ex vivo generation of functional Treg cells from CD4⁺ splenocytes of healthy donor rats. Treg cells were generated ex vivo from CD4⁺ cells by stimulation with anti-CD3 and anti-CD28 antibodies in the presence of TGF- β and IL-2. The obtained cells expressed high levels of CD25, CTLA-4 and FoxP3, and were capable of suppressing in vitro proliferation of T cells from myasthenic rats in response to acetylcholine receptor (AChR).

Administration of ex vivo generated Treg cells to myasthenic rats inhibited the progression of EAMG and led to down-regulation of humoral AChR-specific responses, and to decreased IL-18 and IL-10 expression. The number of CD4⁺CD25⁺ cells in the spleen of treated rats remained unchanged, but the subpopulation of CD4⁺CD25⁺ cells

expressing FoxP3 was significantly elevated. Our findings imply that Treg cells play a critical role in the control of myasthenia and could thus be considered as potential agents for the treatment of MG patients.

Suppression of experimental autoimmune myasthenia gravis by targeting the CXCR3/IP-10 signaling.

DNA microarray technology, supported by quantitative real time PCR, immunohistochemistry and flow cytometry, were previously used in our lab to identify new potential drug targets for MG and to delineate genes involved in the pathogenesis of the disease.

We have demonstrated that the chemokine IFN-gamma inducible protein 10 (IP-10) and its receptor CXCR3, are over-expressed in LNC and muscles of EAMG rats and in thymuses, muscles and CD4⁺ T cells of MG patients. Based on these results, we have recently initiated a study on the potential of inhibitors of IP-10/CXCR3 signaling to act as modulators of EAMG. We have shown that treatment by either a CXCR3 inhibitor or by IP-10-specific antibodies suppresses ongoing EAMG and lead to a reduction in humoral and cellular AChR-specific responses. These observations in EAMG suggest that inhibitors of IP-10/CXCR3 signaling should be considered as potential treatment modalities for MG.

Overexpression of phosphodiesterases in EAMG and EAE.

Phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cAMP and cGMP, and are therefore critical in determining the intracellular levels of these second messengers, which play a pivotal role in regulation of a wide range of cellular functions including cellular immune responses. A previous study in our lab employing 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 has been the first time that alterations in the expression levels of PDEs in an autoimmune disease have been demonstrated. Quantitative real time PCR analysis indicated that EAMG is characterized by an increase of PDE subtypes 1, 3, 4 and 7 in LNC, and of PDE subtypes 2, 3, 4 and 7 in muscles.

To find out whether alterations in PDE expression are specific for EAMG or are observed in other autoimmune diseases as well, we have initiated a study in collaboration with Prof. Ben-Nun from our department to examine PDE expression in experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis (MS). Real time PCR analysis revealed increased levels of PDE1, PDE2 and PDE4 in LNC of EAE rats compared to healthy controls at different stages of the disease. Changes in PDE levels in EAE suggest the involvement of PDE also in the pathogenesis of this disease. Thus, treatment of EAE and possibly MS as well might be considered as an optional therapeutic modality. Recent preliminary studies on MG and MS patients indicate an increase in the expression of several PDE subtypes in thymuses and PBL of myasthenic patients as well as in PBL of MS patients.

In view of the fact that PDE inhibitors are already used clinically with no serious side effects, the possibility of treating autoimmune diseases by such PDE inhibitors looks promising.

Suppression of experimental autoimmune myasthenia gravis by combination therapy: Pentoxifylline as a steroid-sparing agent.

Current treatment for MG and other autoimmune diseases involves in many cases the use of general immunosuppression, mainly corticosteroids. However, continuous treatment with steroids often results in severe adverse effects stressing the need for steroid sparing agents that would enable to lower the steroid dosage. We have previously shown that Pentoxifylline (PTX), a general phosphodiesterase (PDE) inhibitor effectively inhibits the progression of experimental autoimmune MG (EAMG) in rats. We have recently evaluated the therapeutic potential of a combination of suboptimal doses of methylprednisolone (Solumedrol) and PTX in rat EAMG. This combined treatment resulted in a pronounced suppressive effect on EAMG and was by far more effective than each of these drugs administered separately at these suboptimal doses. The suppressive effect on EAMG was accompanied by decreased humoral and cellular responses to the major autoantigen in MG - the acetylcholine receptor (AChR) as well as down regulation of Th1 cytokines and IL-10 in lymph node cells. The expression of PDE-4 and cathepsin-I, a marker for muscle wasting, decreased in the muscle. This study demonstrates the effectiveness of PTX when used as a steroid-sparing agent in combination with low dose steroids in the management of EAMG.

As both Solumedrol and PTX are already in clinical use, our results justify well-designed clinical trials to test the efficacy of their combination for the treatment of MG patients and possibly in other autoimmune diseases as well.

A disease-specific fraction isolated from IVIG is essential for the immunosuppressive effect of IVIG in experimental autoimmune myasthenia gravis

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 have demonstrated previously 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.

We have been employing this model system in an attempt to isolate from IVIG a disease-specific fraction involved in the therapeutic activity in myasthenia and to identify its properties and function. We demonstrated that chromatography of pooled human IVIG on immobilized immunoglobulin, isolated from either EAMG rats or from MG patients, results in a complete depletion of the suppressive activity of the IVIG preparation. Moreover, reconstitution of the activity-depleted IVIG with the eluted minute IVIG fractions that had been adsorbed onto the EAMG- or MG-specific columns, recovers the depleted immunosuppressive activity. This study demonstrates that a disease-specific anti-immunoglobulin fraction present in IVIG preparations is essential for the suppressive effect of IVIG and raises the possibility that such disease-specific fractions can be isolated from IVIG and considered as improved reagents for therapeutic purposes.

Selected publications

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