List of Abstracts

Speaker Abstract

Ocular MG Prednisone Treatment Trial
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Ocular Myasthenia Gravis (OMG) causes visual disability and dysfunction of daily living activities, but Generalized Myasthenia Gravis (GMG) is a potentially life-threatening illness particularly when the muscles of respiration and swallowing become weak. Preventing the progression of OMG to GMG with the proposed low-cost therapy would decrease the risk of severe neurological illness, with few major adverse effects. Since the risk of developing GMG decreases markedly beyond one year after the onset of OMG, treatment during the first year may be highly effective in preventing GMG.

We will perform a prospective double masked randomized control trial on 402 subjects with newly diagnosed OMG (≤ 12 wks) at 40 sites in the US/Canada to test a Primary outcome hypothesis: Treatment of OMG, with doses of corticosteroids that do not cause significant systemic complications, will reduce the one-year incidence of GMG (by 26%; power 80%, 2-sided α = 0.05), as determined by a masked, standardized, reproducible clinical evaluation. Secondary outcome hypotheses include: A chronic, low dose corticosteroid regimen will improve functional binocular vision more often than placebo and pyridostigmine; improved binocular vision will reduce disability and improve the quality of life; major adverse events due to corticosteroid therapy can be prevented by close monitoring and early co-interventions. At entry subjects will be age ≥18 years, have no GMG and no contraindications to prednisone. Treatment assignment is to either prednisone tablets: 50 mg tapered over 36 weeks (continued for ocular symptoms at < 10 mg daily) and pyridostigmine or placebo tablets for prednisone and pyridostigmine. The primary outcome measure, the 1-year incidence of GMG as assessed by survival analysis methods, is determined by signs and symptoms of weakness in non-ocular muscles on GMG Specific Questions and the MG Manual Muscle Test without other explanation. Secondary outcome measures include Incidence of GMG between months 12 and 24, severity of OMG, general and GMG-related quality of life, incidence of side effects of prednisone, osteoporosis prevention.

Poster Abstracts

1. Polygenetic Disease Associations in Thymoma MG
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Background: Relevant genetic markers for myasthenia gravis (MG) include TNFA, TNFB, FCGR2A and IL-10. The corresponding gene products are thought to be involved in the MG pathogenesis.

Objective: To investigate whether MG susceptibility correlates with specific combinations of genetic markers, and to compare the contribution of each marker.

Patients and Methods: 47 MG patients and 92 healthy blood donors were examined for TNFA, TNFB, FCGR2A and IL-10 genotypes, and autoantibodies (Ab) against nicotinic acetylcholine receptor (AChR), titin, and ryanodine receptor (RyR).

Results: MG susceptibility increased with an increasing number of genetic markers characteristic for both thymoma MG and MG with titin antibodies. There was no increase in disease susceptibility with increased number of genetic markers for early onset MG. For thymoma MG patients, FCGR2A polymorphisms seem to be the most important determinant of disease.

Conclusion: Specific combinations of polymorphisms, individually associated with MG, potentiate the risk for thymoma MG and MG with titin-Ab.
2. Downregulation of Foxp3 Transcript in PBL from Myasthenic Subjects

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Regulatory T cells (Tregs) are crucial players in the induction of peripheral tolerance to self antigens and hence in preventing/controlling autoimmunity. In Myasthenia gravis (MG) thymic abnormalities might cause a functional defect in the generation and/or export of Tregs in the periphery. By real-time PCR we evaluated relative levels of Foxp3 (marker for Tregs), IFN-γ (Th1), IL10 (Th2) transcripts in peripheral blood lymphocytes (PBLs) from 10 MG patients and 9 healthy donors (HD). PBLs were challenged in vitro with ConA and anti-CD3/CD28 mAbs (aspecific stimulators), and with a pool of 5 αAChR Tcell epitopes (α58–77, α97–116, α111–130, α132–151, α144–163), peptide ε180–200, and Torpedo AChR (TACChR), for 4h. No differences in Foxp3 transcript were found in unstimulated PBLs. On the contrary, Foxp3 mRNA levels were overall down-regulated in MG patients compared with HD following in vitro stimulation. Foxp3 relative expression ($2^{-\Delta\Delta C_{T}} \pm$ SE) were: 4.63 ± 0.83 vs 7.07 ± 0.98 (ConA), 2.32 ± 0.63 vs. 4.36 ± 0.48 (p = 0.027, anti-CD3/CD28); 85 ± 0.8 vs 1.44 ± 0.20 (p = 0.016, peptides pool); 0.98 ± 0.11 vs. 3.00 ± 1.65 (ε180–200); 1.36 ± 0.21 vs. 2.14 ± 0.35 (TACChR). Moreover, increased levels of IL10 mRNA were found after ConA, anti-CD3/CD28, and TACChR stimulation in MG, while a significant down-regulation was found for IFN-γ mRNA following ConA and anti CD3/CD28 stimulation. Molecular data for IFN-γ and IL10 were confirmed by ELISPOT. These results show an altered induction of Foxp3 in MG PBLs following aspecific stimuli, with an associated IL10 up-regulation and IFN-γ down-regulation. Extended longitudinal studies of these molecules will provide functional data to be correlated to disease activity/progression in MG patients.

3. European MG Network: Development of a European-Wide Clinical and Biological Database

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EuroMyasthenia Network aims to set-up a European-wide Myasthenia Gravis (MG) network to improve information and knowledge for MG, promote a better classification, optimize therapeutic strategies and support actions to reduce inequalities in MG care. Its objectives are: standardize biological, clinical and histological criteria all over the EU countries; promote surveys among the organisations of MG patients to determine the influence of psychological and socio-economical determinants in the onset and/or aggravation of the disease; develop a database at a European scale to serve as a basis for epidemiological studies; promote the establishment of a specific European Card for MG patients; disseminate data via the EuroMyasthenia.eu site. In this context, an European database is being established. In a first step, it will be organized in independent tables specific for: patient’s information and identification code (master table); clinician’s details; MG diagnostic criteria; availability of biological samples; clinical follow-up. This structure will be easily modified to better address specific questions raised by partners of the Network, as they arise. Data entry will be allowed by secure client-server connection; the database will be accessible/viewable on internet via the EuroMyasthenia.eu web site. The database collects clinical data from European MG patients and allows the sharing of MG biological samples among EU scientists/researchers. Specific criteria to guarantee anonymity of the patients (and of healthy subjects) are also granted, by the definition of an informed consent on the final uses of the database (i.e. sharing of clinical and bioimmunological data, sharing of biological samples etc. among the European clinical and research community, and establishing epidemiological studies). The project is partially supported by EU grant DG-SanCo Contract No. 2005105.

4. Regulatory Properties of Anergic CD4+CD25+ T Cells Derived from Spleens of Naive and EAMG Lewis Rats

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Recruitment of naturally occurring CD4+CD25+ regulatory T (Treg) cells is a promising approach to modulate autoimmune reactions. In the present study we analyzed splenic Treg cells derived from EAMG and healthy rats. FACS analysis revealed no differences in CD4+CD25+ subset (11.0% ± 3.8 vs 10.7% ± 1.1) as well as in the CD25high population (2.0% ± 1.1 vs. 1.9% ± 0.2) between EAMG and healthy rats. Splenic Treg cells were purified for CD4 and subsequently for CD25 by MACS MicroBeads; IFN-γ, IL-6 and IL-10 mRNA transcripts were then studied by Real-time PCR. Mean IFN-γ mRNA levels were
higher in EAMG Treg (7.0 ± 3.4) compared with control Treg cells used as calibrator (1.2 ± 0.4), while no difference was recorded in IL-6 and IL-10 mRNA levels. Treg from EAMG and healthy rats were tested in vitro and resulted anergic to stimulation with the immunodominant rat α97-116 peptide and ConA. Their suppressive activity was then evaluated by modulating the proliferative responses of a rat T-cell line raised to α97-116. Treg from healthy rats co-cultured at 1:1 cell ratio with p97-116 specific T cell line showed a marked suppressive capacity (21188 ± 2248 cpm ± SE vs 81131 ± 14623 cpm ± SE) compared with EAMG Treg (48817 ± 5426 cpm ± SE). We then assayed the effect on primary proliferative TACChR response of lymph node cells from rats i.p. treated with 1.5 x 10⁶ Treg purified from healthy rats, 24 hours after TACChR-immunization. Ex vivo TACChR responses were significantly reduced (2309 ± 667 cpm ± SE) compared with vehicle treated rats (36872 ± 2897 cpm ± SE), while ConA specific responses were unaffected (114387 ± 7641 vs 100306 ± 5160). Our data indicate that CD4+CD25+ from normal and EAMG rats, despite antigen unresponsiveness, show different suppressive activity in vitro; CD4+CD25+ cells from normal rats could be used to explore cellular and molecular mechanisms leading to tolerance induction to AChR.

5. Increased TLR4 Expression in Thymic Epithelial Cells of MG Patients by LPS: Role of Innate Immunity in MG
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Some lines of evidence indicate that thymic epithelial cells (TECs) are involved in the primary autosensitization step in myasthenia gravis (MG), even if the initial events that lead to the AChR-specific autoantibody production are still unknown. By considering the role of innate immune system in autoimmunity, a link between innate immunity signaling pathways and the intrathymic events involving TECs might be hypothesized. We explored this hypothesis by analyzing the expression of TLR4, a member of Toll-like receptor family, in TECs isolated from MG patients with hyperplasia, thymoma or thymic involution, and stimulated with LPS for 3, 6, 24 and 48h. By RT-PCR, we found that LPS was able to induce TLR4 gene expression in each cell lines analyzed. TLR4 transcriptional levels increased more rapidly in TECs purified from hyperplastic and involuted thymus (24h) than in TECs from thymoma (48h). Double immunofluorescence stainings confirmed the increased levels of TLR4 in TECs after LPS stimulation with the maximal effect at 48 h. We also observed that LPS up-regulated IL-6, with a peak of expression at 3h of stimulation, suggesting that IL-6 synthesis might be an early response to LPS preceding TLR4 induction, and biglycan, a component of extracellular matrix known to activate macrophages through TLR4 to secrete pro-inflammatory cytokines and chemokines. Our findings provide the first evidence that intracellular TLR-4-mediated pathways can be elicited by “danger” signals in MG TECs, suggesting their possible role in the pathological events causing MG.

6. Regulation of Chemokine Production by Estrogens: Implication for MG
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In myasthenia gravis (MG), there is a clear relationship between thymic pathology and gender, with thymic hyperplasia affecting essentially young female patients (9:1). We have demonstrated that the expression of α and β estrogen receptors (ER) is significantly elevated in thymocyte populations of hyperplastic MG thymuses as compared to control thymuses, an observation which may be related to upregulation of ER expression by proinflammatory cytokines. To further investigate the contribution of estrogens in the formation of thymic GC, we analyzed their effect on the expression of molecules involved in the anti-acetylcholine receptor (AChR) response (MHC class II, AChR) and of chemokines implicated in germlinal center (GC) development (CXCL13, CCL21, and CCL19), Stimulation of thymic epithelial cells (TEC), which express AChR and MHC proteins, in the presence of β-17 estradiol resulted in profound decrease in HLA-DR at both mRNA and protein levels; decreased expression of AChR subunits was also observed, albeit not to the same extent. Real-time PCR analysis of CXCL13, CCL21, and CCL19 showed that β-17 estradiol down-regulates the expression of these three chemokines in TEC; however, little or no difference was observed at the protein level. Interestingly, the expression of certain cytokines, upregulated in a proinflammatory environment, was downregulated in the presence of β-17 estradiol. Our results suggest that the balance between estrogens and proinflammatory cytokines could
be of importance in thymus homeostasis and influence the progression of the autoimmune response in MG patients.

7. Mutations of the Extracellular Domain of Human AChR γ-Subunit Exhibit Improved Characteristics
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Muscle acetylcholine receptor (AChR) is the major autoantigen in the autoimmune disease Myasthenia Gravis (MG), in which pathogenic autoantibodies bind to, and inactivate, the AChR. Our aim was to obtain satisfactory amounts of soluble protein, through the expression of recombinant extracellular domains (ECD) of the AChR subunits and to subsequently use them as starting material for structural and therapeutic studies. In order to optimize the characteristics of the previously expressed γ1-218 ECD, we constructed, expressed and purified a set of mutant forms of this protein. Substitution of either Cys-61 to Ser in the γECD (first mutant) or the Cys-106 and Cys-115 (second mutant) to Ser has not significantly ameliorated the characteristics of the protein. The third mutant form, in which the hydrophobic Cys-loop region (amino acids 128-142) has been replaced by the hydrophilic Cys-loop from the acetylcholine binding protein (AChBP), is water-soluble, expressed at higher levels and apparently forms dimers. A fourth mutant form carrying both the Cys-loop of the AChBP and the two Ser in place of Cys 106 and 115, is also water-soluble, has a greater expression yield and probably forms monomers. Immunoabsorption tests with MG sera have showed that all four mutants are recognized by human MG anti-γ antibodies, and also that the two mutants which contain the AChBP loop, appear to be more efficient immunoabsorbents than the wild type γECD. These results suggest that the mutants retain the characteristics of the γECD, and those which contain the AChBP loop are improved with respect to the expression yield, solubility and antigenicity. Therefore the latter mutants are more suitable for our purposes, which are to use these recombinant polypeptides as tools for the study and treatment of MG.

8. Branched Peptides for Sensitive Diagnosis and Possible Removal of Antibody Sub-Populations in MG
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Myasthenia Gravis (MG) is probably the best characterized autoimmune disease in terms of molecular mechanisms of pathogenesis and can be seen as a model for productive connection between biological and molecular studies and clinical applications. MG is an antibody-mediated disease of the neuromuscular junction. More than 80% of MG patients have circulating antibodies (Ab) against the muscle nicotinic receptor (nAChR) that can be measured through a radio-precipitation assay. The pathogenetic role of anti-nAChR Ab have been experimentally demonstrated and is also testified by the short-term beneficial effect of plasmapheresis for MG patients. Nonetheless, a direct correlation between antibody titer and disease severity has never been demonstrated. This can be in part ascribed to the diversity of Ab populations among MG patients. The classical radio-assay does not measure anti-AChR Ab directed to the acetylcholine-binding site. The relevance for MG pathogenesis of this Ab population, which might directly interfere with receptor conductance, is still an open matter. We synthesized different peptide mimotopes of the nAChR binding site, obtained by combinatorial chemical libraries, in a tetrabranchined form. We demonstrated a much higher efficiency of dendrimers when used as antigen on EIA plates for binding α-bungarotoxin or MG sera with respect to correspondent monomeric linear or cyclic peptides. Binding efficiency of mimotopes was also evaluated by BIACORE. A selected branched peptide was immobilized on a Sepharose column and used to test removal of anti-binding site Ab from MG sera. Antibodies were measured by radio assay and by a competition assay with 125I-α-bungarotoxin, before and after passage through the peptide column. Anti-binding site Ab were completely removed from serum and could be recovered by elution from the column.

9. Immune Complexes Affect EAMG Severity Via FCGAMMA Receptor III Mediated Complement Production
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Immune complexes and classical complement pathway (CCP) have essential pathogenic roles in experimental autoimmune myasthenia gravis (EAMG) induction. A well-characterized pathogenic mechanism is activation of CCP by acetylcholine receptor (AChR)-anti-AChR antibody (Ab) immune complexes located at the neuromuscular junction (NMJ).
To characterize additional mechanisms by which immune complexes induce EAMG, we immunized Fcgamma receptor III (FcyRIII) knockout (KO) and wild-type mice with AChR. FcyRIII KO mice were highly resistant to EAMG and had reduced NMJ IgG and complement deposits, lymph node cell (LNC) IL-6 and IFN-γ production and serum C1q and C3 levels. These results suggest that circulating immune complexes affect EAMG severity by stimulating the production of certain cytokines and complement factors via FcyR interaction. Mice deficient for CCP factor C4 have been previously shown to be resistant to EAMG induction. To test if EAMG resistance of FcyRIII KO mice was primarily associated with the reduced production of another CCP factor, C1q, C57BL/6 and RIIIS/J mice were treated with anti-C1q or isotype Ab before or after EAMG induction. Anti-C1q Ab effectively prevented and treated EAMG in both mouse strains by reducing NMJ deposits and LNC IL-6 production as observed in FcyRIII KO mice.

Our results show that immune complex and FcyR interactions are involved in EAMG induction. CCP inhibitors applied with or without FcyR blockers might be beneficial in the future treatment of myasthenia gravis.

10. PTPN22 and CTLA4 Genotypes Synergize in Predisposing to Thymoma-Associated MG

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Myasthenia gravis (MG) in thymoma patients depends critically on intratumorous generation and export of mature autoreactive CD4(+) T cells. Our previous study implicated the CTLA4 high genotype +49A/A in predisposing to paraneoplastic MG in thymoma patients, even though it protects against several autoimmune diseases. Here we studied a single nucleotide polymorphism of the protein tyrosine phosphatase PTPN22 in subjects of known CTLA4 genotype. Interestingly, the PTPN22 +1858T(+) genotypes, which are predisposing to several autoimmune diseases, also exert a predisposing effect to both early-onset MG (EOMG) and thymoma-associated MG. In addition, there is a synergic predisposing effect of CTLA4 +49A/A and PTPN22 +1858T(+) to thymoma-associated MG but not EOMG. Recently, the autoimmunity-prone PTPN22 +1858T(+) genotypes have been found to confer a more potent inhibition of T-cell activation, which is against the theory of decreased inhibition of T-cell activation in the peripheral immune system. Furthermore, PTPN22 has been known to exhibit conserved, strong expression in murine and human thymocytes, to modulate T-cell receptor signaling in thymocytes, and to target several thymocyte proteins with an established or presumed role in central tolerance. Our results imply that the two gain-of-function variants of PTPN22 and CTLA4 might synergize to elicit MG in thymoma patients through central tolerance failure.

11. Microarrays Reveal Distinct Gene Signatures in the Thymus of Seropositive and Seronegative MG Patients

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The central role of the thymus in the disease process of SP and SN MG, we compared the thymic transcriptome of non-MG adults to those of SP patients with a low or high degree of hyperplasia or SN patients. A common gene expression profile related to the immune response was identified in all MG subgroups tested, including an overexpression of MHC class II, Ig and B cell marker genes and of the B-cell chemoattractant CXCL13, possibly leading to generalized B-cell infiltration. However, we also found differences between SP and SN thymuses, notably a specific overexpression of CCL21 in hyperplastic SP thymuses in correlation with the development of germinal centers (GC), suggesting a role for CCL21 in the thymic ectopic GC formation. Interestingly, SN patients present a specific signature with an abnormal expression of genes involved in muscle development and synaptic transmission, which could correspond to potential autoantigenic targets, but also of genes implicated in host responses, suggesting that viral infection might be related to the etiology of SN MG. Altogether, these results underline differential pathogenic mechanisms in the thymus of SP and SN MG and propose new research areas to further understand the disease.
12. The Chemokin IP-10/CXCR3 Pathway is Involved in the Pathogenesis of MG

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

The chemokine IP-10 and its receptor CXCR3, were found, among other EAMG-associated deregulated genes, to be over-expressed in LNC and muscles of EAMG rats and in thymuses and muscles of MG patients. CXCR3 was up-regulated in CD4+ T cells of MG patients. In view of these results, we have initiated a study on the potential of inhibitors of IP-10/CXCR3 signaling as modulators of EAMG. Recent experiments indicate that treatment of EAMG in rats either by a CXCR3 inhibitor or by IP-10-specific antibodies are beneficial in attenuating disease symptoms along with a reduction in anti-AChR titers and in T cell proliferation. These observations in EAMG suggest that inhibitors of IP-10/CXCR3 signaling might be considered as a treatment modality in MG as well.

13. Muscle Gene Expression in MG

Muscle Gene Expression in Myasthenia Gravis

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In order to investigate molecular changes in the muscle, leading to or resulting from the autoimmune attack in myasthenia, we have compared data of DNA microarray analyses of MG and EAMG muscles. To do this, we used the transcriptomic approach in muscle biopsies from seropositive MG patients and myasthenic rats as compared to their relevant controls. Data mining revealed two major categories 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 coding for the chaperone protein category including several heat shock proteins. Real-time PCR validated the above results. There was no over-representation of inflammation-associated deregulated genes in MG or EAMG. Several signaling pathways common to human and rat were also revealed including signaling by IL-6, Nitric Oxide and IGF-1. We have further demonstrated that IL-6 and its receptor IL-6R are deregulated in myasthenic rats and that sera from MG patients affect the mRNA expression of IL-6 and IL-6R in C2 cells suggesting that the changes in IL-6/IL-6R expression observed by the DNA microarrays in MG and EAMG result from the autoimmune attack on the muscle.

Our results show that the pathological mechanisms taking place in the muscle of MG patients and EAMG rats are essentially similar. Since muscle genes are clearly deregulated as a result of the autoimmune attack, modulating these genes could represent a new therapeutic approach for myasthenia.

14. Suppression of EAMG by Combined Treatment with Solumedrol and Pentoxifylline at Suboptimal Doses

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Corticosteroids are frequently employed for the treatment of myasthenia gravis (MG); however, continuous treatment with steroids may result in severe adverse effects. This leads to a search for steroid sparing agents that would enable to lower their dosage. We have previously shown that pentoxifylline (PTX), a phosphodiesterase (PDE) inhibitor, effectively inhibits the progression of experimental autoimmune MG (EAMG) in rats. In the present study we investigated the therapeutic potential of a combined treatment with suboptimal doses of methylprednisolone (solumedrol) and PTX in rats with EAMG. This combined treatment resulted in a pronounced suppressive effect on EAMG and was far more effective than any of these drugs separately. Moreover, when using such a combination, the doses of steroids employed could be reduced by ten folds, as compared to the doses used in steroid treatment alone. The suppressive effect
of the combined treatment on EAMG was accompanied by decreased cellular and humoral responses to AChR and down-regulation of Th1 cytokines and IL-10 in LNC. The expression of PDE-4 and cathepsin-L, a marker for muscle wasting, decreased in the muscle.

This study demonstrates the effectiveness of PTX as a steroid sparing agent when used in combination with low dose steroids in the management of myasthenia.

15. Pathogenic Effects of Anti-MuSK Antibodies on a Muscle Cell Line

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Clinical and EMG signs of muscle atrophy are found in most MG patients with anti-MuSK (MuSK+) antibodies. To explore the pathogenic role of these Abs and the mechanism by which they affect neuromuscular transmission (NMT), we analyzed the functional effects of MuSK+ MG sera on TE671 cell muscle cell morphology, proliferation and gene regulation as compared to controls (healthy individuals; MG patients with anti-acetylcholine receptor antibodies (SPMG); MG patients without defined antibodies (SNMG)). Our results showed that approximately 50% of the MuSK+ MG sera changed TE671 cell morphology towards elongated, multinucleated myotube-like cells. Cell proliferation experiments revealed that these changes in cell morphology were accompanied by time- and dose-dependent perturbation of cell proliferation, correlating with their exit from the cell cycle and their ensuring differentiation. Measurement of atrogin mRNA expression showed muscle cell-specific overexpression of this marker of muscle atrophy. In addition, we found that MuSK+ sera induced downregulation of the expression of postsynaptic genes such as AChR subunit, rapsyn, Rho A, and cdc42 genes. These effects correlated with disease severity and with anti-MuSK Abs titers and disappeared after plasmapheresis. The same effects were seen using anti human polyclonal anti-MuSK Ab, supporting a direct pathogenic role of anti-MuSK antibodies; none of the control, SPMG, SNMG sera had any effect on morphology and growth of TE671 cells. Altogether, these results present strong evidence that anti-MuSK Abs play a major role in the pathogenesis of the MuSK+ form of MG. They cause dysregulation of the postsynaptic genes necessary for the NMT stability and function and contribute to muscle atrophy frequently observed in MuSK+ patients.

16. A DNA Vaccine Encoding the AChR Treats the EAMG Model of MG

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Objective: To develop a novel treatment modality for myasthenia gravis using a DNA vaccine.

Background: We are developing tolerizing DNA vaccines as a novel antigen-specific method of treatment of human autoimmune disease. Current therapies for myasthenia gravis (MG) and other autoimmune diseases, are marginally effective and largely non-antigen specific. DNA vaccines are a powerful tool for eliciting immune responses, and were initially developed as protective vaccines against infection. More recently, DNA vaccines have proven to be an extremely effective method of treatment of autoimmune disease in animal models. Tolerizing DNA vaccines encoding autoantigens specifically turn off pathogenic autoimmune responses, leaving the global immune system intact. We have begun clinical trials using DNA vaccines for two other autoimmune diseases, multiple sclerosis and type 1 diabetes, and are currently exploring the utility of DNA vaccines for MG.

Design/Methods: We have used the murine experimental autoimmune myasthenia gravis (EAMG) model of MG to demonstrate the efficacy of a DNA vaccine encoding the acetylcholine receptor (AChR). In this model we administer the DNA vaccine intramuscularly on a once every week schedule. The treatment is begun only after the onset of symptoms of disease. The animals are evaluated both for clinical parameters of disease severity as well as for titers of antibodies against acetylcholine receptor.

Results: We have demonstrated that with the antigen-specific DNA vaccine we are able to reverse several parameters of EAMG severity in already established disease compared to saline (PBS) injections. There was a reduction in overall mean disease severity which reached a significance when comparing the disease scores at the end of the study to first day of treatment (p = 0.003, Mann Whitney test). There was also a reduction in the percentage of mice with no disease at the end of the study (83% with DNA versus 63% with PBS). Antibodies titers against AChR were measured in the study, but there was no significant correlation to disease outcome.

Conclusions: Our initial results demonstrate that a DNA vaccine encoding acetylcholine receptor is potentially an effective therapy for MG as shown by disease reversal in the EAMG model. We are further
exploring the mechanism of action of this effect given the negative correlation with anti-AChR.

17. Differential Response of Muscle Genomic Profiles to EAMG

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Myasthenia gravis (MG) demonstrates a distinct predilection for involvement of certain muscle groups; however, the reasons for this are poorly characterized. As an initial step to further evaluate the differential response of skeletal muscles to MG, we took an unbiased approach to analyze gene expression profiles. We used cDNA microarray to analyze the gene expression profile in diaphragm (DIA), extensor digitorum longus (EDL) and extraocular (EOM) muscles of rats with actively induced experimentally acquired MG (EAMG) that had developed mild to moderate weakness. We found 359 probes (1.16%) greater than 2 fold changes in expression in 7 of 9 series pairwise comparisons between the experiment and control groups from 31,090 probes. Profiles showed the following alterations in gene expression: DIA 147 (85 genes were up-regulated, 62 genes down-regulated), EDL 205 (100 genes up-regulated, 105 genes down-regulated) and EOM 116 (85 genes up-regulated, 31 genes down-regulated). The three muscles shared altered expression in 22 genes, and EDL and DIA shared 45 gene expression pattern changes, while EOM shared expression pattern alteration with EDL in 8 genes and DIA in 11 genes. Functional annotation found that these muscles shared alterations in genes related to stress response and free radical metabolism. A number of nuclear receptors target genes involved in lipid and glucose metabolism were altered as well as dexamethasone target genes activated. Insulin response pathways were inhibited. Protein degradation pathways were induced. Metabolic alterations were consistent effects of weight loss that were observed in the animals. The studies show unique gene expression profiles of muscles to EAMG and these may ultimately serve to explain the differences in clinical involvement of muscle by human MG. The work also suggests secondary effects of EAMG on muscle which could further compromise muscle function through protein degradation and free radical generation. Supported by grants from the National Institutes of Health R24EY014837 (HJK), R01EY013238 (HJK) and P30 EY11370.

18. Heart Cell Culture: An Effect of MG Sera?

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Myasthenia gravis (MG) is an autoimmune disorder where the patients experience weakness in striated muscle and increased fatigue. The disease is associated with thymoma in 10-15% of all MG cases. 85% of patients have autoantibodies targeting the acetylcholine receptor (AChR). It has been reported that 48% of all MG patients, and 97% of all patients with thymoma associated MG, have antibodies towards heart muscle. Impaired diastolic function, decreased global heart ejection fraction, giant cell myocarditis and ECG changes have been reported in MG patients.

Taken together, these observations clearly suggest a link between MG and cardiac disease. We have previously shown that MG sera cause morphological effects and are cytotoxic to human skeletal myoblasts in vitro. We are now establishing a cardiomyocyte cell culture model using non-beating HL-1 cells (rat) and cardiomyocytes isolated from the atria and ventricles of pigs. We are planning also to culture heart cells from human biopsies. Preliminary results indicate that MG sera are capable of inducing morphological changes and are cytotoxic to HL-1 cardiomyocytes, the effects ranging from mild to severe. The relevance of these results remains to be determined. Further work will include the setup and maintenance of a reliable cell culture system, testing MG sera on heart cells from different species including human, and investigation of the potential mechanisms of cell death, using morphological and biochemical methods and gene arraying.

19. Mycophenolate Mofetil Suppresses the Onset of EAMG

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Mycophenolate mofetil (MMF) is known for years to operate with an extremely high efficacy in patients undergoing organ transplantation. However, the therapeutic effect of MMF has not been confirmed in a large multicenter trial in myasthenia gravis, therefore encouraging further examination of its mechanism using the chronic animal model experimental autoimmune myasthenia gravis (EAMG).
Eight-weeks-old female Lewis rats were immunized with 10 µg of Torpedo californica acetylcholine receptor (AChR) and were treated for 21 days with 30 mg/kg/day of MMF or with vehicle, starting from the day of immunization. Body weight and clinical scoring were determined twice weekly, while blood samples were collected once a week. Eight weeks after the induction of EAMG, rats were euthanized and tibialis anterior muscles were removed.

At the end of the experiment, 3 out of 10 vehicle-treated EAMG rats were clinically ill, of which 2 lost weight by about 12%. Average body weight of MMF-treated animals was not significantly different from vehicle-treated EAMG rats. Anti-AChR-autoantibody titers of vehicle-treated EAMG rats reached a plateau phase 5 weeks after immunization (5.9 ± 4.7 nmol/L). In contrast, anti-AChR-antibody titers of MMF-treated EAMG rats could be completely blocked for 5 weeks, while increasing only slightly thereafter (< 1 nmol/L). Eight weeks after immunization, total muscle membrane AChR concentrations were similar in both untreated (27 ± 8) and MMF-treated EAMG rats (31 ± 7 fmol/g).

These results point out that 3 weeks of MMF administration is effective in suppressing the onset of EAMG, although long-term treatment might be necessary for complete immunosuppression.

20. A Highly Standardized, Sensitive and Specific Assay for the Determination of AChR Autoantibodies in MG
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The most useful laboratory test to confirm a diagnosis of myasthenia gravis (MG) is the identification of autoantibodies directed against acetylcholine receptors (ARAb) in symptomatic patients. We describe a commercial radio receptor assay (RRA) for the determination of ARAb in human serum and plasma.

The RRA depends on the ability of ARAb to bind 125I-bungarotoxin labeled acetylcholine receptors prepared from skeletal muscle of human amputations. Assay analytical and functional sensitivity is 0.01 nmol/L and 0.07 nmol/L, respectively. Precision studies indicate inter-assay coefficients of variation (CVs; n = 15) between 3.8 and 11.1% for concentrations ranging from 0.2 to 6.1 nmol/L. RRA kit between-lot CVs (n = 38) are <12% in the range 0.3 - 34.5 nmol/L.

Three hundred fifteen (92.9%) of 339 sera from MG patients, 10 (1.5%) of 658 sera from healthy blood donors and 5 (3.9%) of 128 sera from autoantibody-positive non-MG patients were tested positive in the ARAb RRA.

Measurement of all 75 serum samples delivered by the UK National External Quality Assessment Service (UKNEQAS; Sheffield) for ARAb from May 2000 to August 2006 showed a total qualitative agreement and a high quantitative correlation (r = 0.956, target value = 0.89 RRA + 1.1) between the RRA results and the UKNEQAS target values.

In summary, the ARAb RRA is a highly standardized assay for a reliable serological diagnosis and management of patients with myasthenia gravis.

21. The Effect of DAF On T-Cell Activation and Endplate Destruction in Experimental MG
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A large body of research on MG patients and on experimental autoimmune MG (EAMG) in animals has shown that the disease is Ab-mediated, producing loss of or compromised function of skeletal muscle nicotinic acetylcholine receptors (AChR’s). Overwhelming evidence indicates that damage to the neuromuscular junction is mediated primarily by the complement system. Complement components can be identified at myasthenic neuromuscular junctions both in humans and in animals with experimental autoimmune MG (EAMG). Decay-accelerating factor (Daf) is a ubiquitously expressed intrinsic complement-regulatory protein whose function is to limit local C3a/C5a production and C3b/C5b-initiated progression of the cascade. Recently, it was identified that Daf modulates T cell immunity by controlling T cell- and APC-induced alternative pathway C3 activation during cognate interactions. EAMG was induced by multiple administrations of purified Torpedo acetylcholine receptor to 8-12 week old mice deficient in Daf 1a gene (Daf 1a/-, H-2b) and Daf +/+ littermates. Animals were assessed for grip strength, weight loss, neuromuscular destruction, acetylcholine receptor antibody production and T cell activation. Daf -/- mice demonstrated greater C9 deposition and significant injury at the neuromuscular junctions compared to wildtype controls. All animals produced acetylcholine receptor antibody, total AChR IgG, IgG1 and IgG2a at comparable levels. IgG2b levels were higher in Daf -/- mice compared to...
wildtype. As assessed by interferon gamma and IL-2 ELISPOT, T cell activity was elevated in Da-f/- mice in response to AChR. This study confirms the role of complement in EAMG and further demonstrates the novel interaction of Da in T cell activation. Therefore, complement inhibition may be an important target in the treatment of human MG.

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22. Isolation and Characterization of AChR Anti-Subunit Autoantibodies: in vitro and in vivo Studies of Their Function in MG

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Autoantibodies against muscle acetylcholine receptor (AChR) play a key role in the pathology of most myasthenia gravis (MG) cases by causing loss of functional AChR molecules, leading to muscle weakness and fatigability. Recently, we have successfully expressed soluble extracellular domains of α, β, γ and ε AChR subunits in the yeast Pichia pastoris and used them to isolate and characterize the anti-AChR autoantibodies. Purified recombinant extracellular domains, immobilized on Sepharose beads, can immunoadsorb the autoantibodies from MG plasmas. The activity of the isolated anti-subunit antibodies and of the antibody-depleted plasmas was studied i) in vitro, by investigating their ability to cause antigenic modulation of AChR in human TE671 cells, and ii) in vivo, by investigating their ability to cause passive transfer of experimental MG. In the in vitro studies, we found that the anti-α subunit antibodies were much more efficient than the anti-β subunit antibodies, whereas the antibody-depleted plasmas were free of modulating activity. Similarly, the in vivo studies showed that pure anti-α subunit antibodies from two plasmas were efficient in inducing severe MG-like symptoms to Lewis rats whereas the depleted fraction did not induce MG-like symptoms, suggesting that the anti-AChR antibodies are the only pathogenic factor in MG plasmas. Elucidation of the function of the anti-AChR autoantibodies will contribute to the development of new therapeutic approaches.

23. AQP4 Antibodies in MG Patients Who Developed Neuromyelitis Optica

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Myasthenia gravis (MG) can be associated with other autoimmune disorders. One such disorder is neuromyelitis optica (NMO), a severe inflammatory demyelinating disease of the CNS. The discovery that antibodies in the sera of up to 65% of patients with NMO bind to the water channel aquaporin-4 (AQP4; Lennon et al 2005) led us to develop two new assays in order to determine the levels of AQP4 antibodies in patients’ sera.

After confirming that we can detect NMO-IgG by indirect immunofluorescence on mouse brain slices (Jarius et al 2007), we transfected AQP4 into HEK cells and showed that we can detect specific binding of antibodies in patients’ sera to the cells. We then extracted the AQP4 from the cells and demonstrated that we can precipitate it with sera from NMO or LETM patients, giving a quantitative readout. The results show high sensitivity and specificity for NMO, with 11/15 NMO sera positive and 0/14 MS sera positive. The results suggest that both of these assays will be as specific and may be more sensitive than indirect immunofluorescence.

The immunoprecipitation assay enabled us to measure accurately the AQP4 antibody levels and to correlate with clinical manifestations in three who developed NMO after thymectomy for MG.

24. Antibodies to Clustered AChR in Seronegative MG

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Patients with myasthenia gravis (MG) who are negative for AChR and MuSK antibodies by standard assays (seronegative MG, SNMG) often present with similar clinical manifestations and treatment responses to those patients with AChR antibodies, and differ from those with MuSK antibodies. We hypothesized that SNMG patients have antibodies that only bind to AChR but only when the AChR is present at high density, as at the neuromuscular junction. We studied serum/plasma samples from 27 patients with generalized SNMG and from 9 patients with ocular SNMG (all with at least 2 years of follow-up) and from controls (other autoimmune diseases, and healthy individuals). We first expressed AChRs in a mammalian, non-muscle, cell line and found only weak binding
to IgG by immunofluorescence. However, when we clustered the AChR by co-transfecting with the intracellular protein rapsyn, which anchors the AChR at the neuromuscular junction, there was strong binding of IgG in 15/27 generalised and in 4/9 ocular SNMG.

These findings provide evidence of IgG antibodies to AChR in a proportion of MG patients previously classified as seronegative. Therefore, the majority of SNMG patients may share pathogenic mechanisms with AChR positive MG patients.

25. Autoimmunization in Thymus in MG Patients Without Detectable AChR or MuSK Autoantibodies

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The thymus is implicated in the pathogenesis of early-onset myasthenia gravis (EOMG) associated with AChR autoantibodies (AChRAb+). It shows hyperplasia of medullary thymic epithelial cells (mTEC) and lymph node-like infiltrates with germinal centres (GC). AChR is expressed by the rare muscle-like thymic myoid cells, and isolated AChR subunits by TEC. In MuSKAb+ and in SNMG, the thymus is usually reported as ‘normal-for-age’ or atrophic, and clinical benefits of thymectomy are unproven. We have re-assessed thymic samples from 30 seronegative MG (SNMG) and 14 MuSKAb+ patients, comparing them with 23 EOMG (AChRAb+) and 11 non-myasthenic adults by immunohistochemistry and double immunofluorescence staining. Overall, we found few abnormalities in the MuSKAb+ thymus, only 4/14 showing small infiltrates. In ~70% of the SNMG samples, however, we found substantial infiltrates (cytokeratin− areas in thymic medulla), though GC (CD20+/CD35+CD21+) numbers and sizes were modest (Leite et al 2005). We now find that in AChRAb+ and nearly 50% of the SNMG thymi, there are myoid cells exposed to the infiltrates. In both groups, some mTECs and many ‘exposed’ myoid cells, particularly near GCs, show deposits of activated C3b (and sometimes C9) complement components. These results further implicate both mTECs in priming specific helper T cells and myoid cells in GC formation with autoantibody diversification/perpetuation. The similarities between AChRAb+ and SNMG thymi strongly suggest that they belong to the same spectrum.

26. Major Role of the Chemokine CCL21 in Thymic Hyperplasia in MG

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Early-onset Myasthenia Gravis (MG) with anti-AChR antibodies is commonly associated with thymic hyperplasia, characterized by the presence of germinal centers (GC) containing B cells producing pathogenic antibodies.

We demonstrate a specific and strikingly increased expression of the chemokine CCL21 in the hyperplastic thymus of MG patients. CCL21 is commonly considered as a chemokine dedicated to the recruitment of naïve T cells and sensitized dendritic cells necessary for lymphoid follicle development. By immunohistochemistry, we show that CCL21 over-expression was localized to specific endothelial vessels. We also observed, surrounding ectopic GC, the presence of numerous high endothelial venules (HEV) known to participate in T and B cell recruitment in secondary lymphoid organs. However, we showed that in hyperplastic thymuses, HEV were not responsible for CCL21 over-expression and the phenotype of the CCL21-positive endothelial cells is currently under investigation. The analysis of the chemotactic properties of CCL21 revealed that, at increasing concentrations, CCL21 was very powerful in recruiting B cells compared to the well known B-cell chemokine, CXCL13, whose expression is also increased in MG thymuses, stressing a novel property of CCL21.

Altogether, these results point to a crucial role played by CCL21 in triggering thymic hyperplasia by recruiting both peripheral T and B cells.

27. Modulation of Thymocyte Fate by Myoid Cells

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Thymic myoid cells correspond to a muscle-like cell population present in the thymic medulla. Up to now, researchers have mainly been interested in these cells because they are suspected to be involved in the pathogenesis of Myasthenia Gravis. However, their biological role is not known. Using a myoid cell line derived from normal human thymus, in co-culture with thymocytes, we demonstrate that myoid cells protect thymocytes from apoptosis as evidenced by a strong decrease in annexin-V-FITC positive thymocytes. This effect was (1) specific of myoid cells compared to thymic epithelial cells, (2) dependent on direct
cell-to-cell contacts, and (3) triggered rapidly from two hours in co-culture. This protective phenomenon was due to the activation of pro-survival mechanisms. Indeed, co-culture with myoid cells induced the activation of extracellular-regulated kinases (ERK1/2) and Akt in thymocytes.

Myoid cells also influence thymocyte maturation. We observed an increase in CD4+ and a decrease in CD8+ single positive (SP) thymocytes when co-cultured with myoid cells, without an increased CD8+SP death or CD4+SP over-proliferation, suggesting that myoid cells may favor CD4+ cell differentiation. We also observed a strong decrease in HLA-DR expression on thymocytes following cell-cell contacts in our co-culture model.

Altogether, these results suggest that, thymic myoid cells present a physiological role in the thymus by protecting thymocytes from apoptosis and modulating their differentiation process and their potential involvement in thymic selection.

28. Inhibitory IGG Receptor FcγRIIB Potentiates Murine MG Pathogenesis by Augmenting IL-6 and IL-10 Production
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IgG receptor FcγRIIB has inhibitory functions in various autoimmune disorders. To investigate the role of FcγRIIB in the development of antibody and complement mediated experimental autoimmune myasthenia gravis (EAMG), we immunized FcγRIIB knockout (KO) and wild type (WT) C57BL/6 mice with 20 µg Torpedo acetylcholine receptor (AChR) in CFA on day 0, 30 and 60. The FcγRIIB KO mice had less severe (p < 0.05) disease, reduced AChR loss (p < 0.05), and significantly elevated anti-mouse muscle AChR (MMR) IgG1 and IgM levels (p < 0.05). However, no significant differences were observed in serum IgG, IgG2b, IgG2c, C3 and C3-immune complex levels between WT and FcγRIIB KO mice. There were significantly more splenic germinal centers (p < 0.05) in FcγRIIB KO mice. The AChR-immune lymph node cells of FcγRIIB KO mice secreted more IL-2 and IFN-γ and less IL-6 and IL-10. Unlike other autoimmune disorders, FcγRIIB augments EAMG susceptibility by enhancing the production of IL-6 and IL-10, the cytokines critical for EAMG pathogenesis. However, FcγRIIB inhibits anti-AChR IgM and IgG1 and these isotypes either do not play a dominant role in EAMG pathogenesis or are involved in the suppression of EAMG.

Therefore, FcγRIIB plays a dual (inhibitory and stimulatory) role in antibody and complement mediated EAMG by inhibiting anti-AChR IgM and IgG1 production, and stimulating the production of IL-6 and IL-10, and the latter potentiate EAMG pathogenesis.

29. Monovalent Anti-AChR Antibodies for Prevention of AChR Internalization
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MG is caused by complement mediated damage of the neuromuscular junction and the reduction of AChR by cross-linking antibodies (antigenic modulation). Monovalent anti-AChR antibodies can potentially protect the AChR by competing with cross-linking pathogenic auto-antibodies. A recombinant human monovalent antibody directed against the main immunogenic region of the AChR was produced by deletion of the CDR3 region in one of the heavy chains of a MG patient autoantibody. Additionally a histidine tag (His) was added in the C-Terminal end of the monovalent antibody to allow its purification. Competition of monovalent antibody with MG patient antibody for binding to the AChR and antigenic modulation were studied in TE 671 cells.

30. Passive Transfer of a Human IgG4 Anti-AChR Antibody Prevents MG in Rhesus Monkeys
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Myasthenia gravis (MG) is an autoimmune disease of the human skeletal muscles. Autoantibodies against acetylcholine receptor (AChR) play a prominent role in the initiation and pathology of the disease and have been suggested to induce episodes of muscle weakness by mediating destruction of muscle endplates or down-regulation of AChR. In a rhesus monkey model, we show that MG-like disease induced by passive transfer of a recombinant antibody against AChR derived from a MG patient can be prevented with an anti-AChR of the IgG4 subclass. Indeed, the IgG4 completely prevented clinical symptoms of muscle weakness and no changes in neuromuscular transmission as observed by electromyography, or endplate structure and integrity
as observed by electron microscopy were detected. Our study suggests a novel approach for the development of an antigen-specific immunotherapeutic treatment of patients suffering from myasthenia gravis. In addition, this concept may also apply to other antibody-mediated autoimmune disorders.

31. Serum Matrix Metalloproteinase (MMP-3) Levels are Elevated in MG
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Matrix metalloproteinase 3 (MMP-3) has been implicated in the pathogenesis of autoimmune diseases including systemic lupus erythematous (SLE) and rheumatoid arthritis (RA), both of which are observed to occur with a higher incidence in myasthenia gravis patients. MMP-3 is capable of degrading agrin, which, through its interaction with muscle specific kinase (MuSK), mediates AChR clustering and therefore the correct formation and function of the neuromuscular junction. MMP-3 mediated destruction of agrin could therefore reduce the safety factor for neuromuscular transmission and contribute to muscle weakness in MG patients.

We therefore examined MMP-3 levels in seronegative and seropositive MG patients and compared this to healthy controls. An elevated MMP-3 level was defined as any value above the mean of the healthy controls + 2SD. Between 10-15% of MG patients (seronegative and seropositive) exhibited an abnormally high level of MMP-3 in their sera, significantly higher than that seen in controls (p < 0.01).

These data indicate that a non-autoantibody factor, MMP-3, may play an important role in the pathogenesis of MG. MMP-3 may therefore be a possible diagnostic factor and therapeutic target in this subset of patients. Furthermore, a common pathogenic mechanism may occur in MG, SLE and RA, leading to increased MMP-3.

32. Antigenic Structure of the Main Immunogenic Region (MIR) of Muscle AChR α1 Subunits and the Effects of the α1 MIR on the Assembly and Acetylcholine Sensitivity of Chimeric α1/α7 ACHRs
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The MIR is a conformation-dependent region on the extracellular tip of α1 subunits of muscle nicotinic acetylcholine receptor (AChR) that is responsible for provoking half or more of the autoantibodies to muscle AChRs in human and canine MG and rat EAMG. By making chimeras of α1 subunits with α7 subunits, the MIR epitopes recognized by rat mAbs to the MIR were determined. Our results showed that two sequences which were not immediately contiguous but which were adjacent in the native conformation formed the rat MIR epitopes. This explains conformation dependence of the MIR. The MIR epitopes recognized by these mAbs were not recognized by MG patient autoantibodies. The MIR epitopes recognized by MG patient antibodies, which must be nearby, are being investigated. The presence of the α1 MIR sequences in α1/α7 chimeras greatly promotes AChR expression. The most antigenic MIR chimera was 10-fold more sensitive to ACh than α7, while another chimera containing shorter α1 sequences exhibited a 13-fold reduction in sensitivity. Thus, the MIR may play important roles in subunit conformational maturation or assembly as well as in the conformational changes associated with AChR activation.

33. Overexpression of Rapsyn in Rat Muscle Increases AChR Levels in Chronic EAMG
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The primary autoantigen in myasthenia gravis, the acetylcholine receptor (AChR), is clustered and anchored in the postsynaptic membrane of the neuromuscular junction by rapsyn. Previously, we found that overexpression of rapsyn by cDNA transfection protects AChRs in rat muscles from antibody-mediated loss in passive transfer experimental autoimmune myasthenia gravis (EAMG). Here, we determined whether rapsyn overexpression can reduce or even reverse AChR loss in muscles that are already damaged by chronic EAMG, which mimics the human disease. Active immunization against purified AChR was performed in female Lewis rats. Rapsyn overexpression resulted in an increase in total muscle membrane AChR levels, with some AChR at neuromuscular junctions but much of it in extrasynaptic membrane
regions. At the ultrastructural level, most endplates in rapsyn-treated chronic EAMG muscles showed increased damage to the postsynaptic membrane. Although rapsyn overexpression stabilized AChRs in intact or mildly damaged endplates, the rapsyn-induced increase of membrane AChR enhanced autoantibody binding and membrane damage in severe ongoing disease. Thus, these results show the complexity of synaptic stabilization of AChR during the autoantibody attack. They also indicate that the expression of receptor-associated proteins may determine the severity of autoimmune diseases caused by anti-receptor antibodies.

34. Rapsyn Determines the Susceptibility to EAMG in Rats
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Age, sex and strain dependant differences in susceptibility of rats to experimental autoimmune myasthenia gravis (EAMG) suggest the existence of risk factors that modulate disease severity. In previous studies, immunological factors (antibody titers, isotype distribution and specificity, upregulation of complement regulatory proteins) have been excluded as a possible cause of the age and sex related differences. Conversely, changes in the composition of the neuromuscular junction have been correlated to the age-related resistance of female rats. In this study we used quantitative immunohistochemistry in order to test if the levels of rapsyn and the acetylcholine receptor at the endplate are correlated to susceptibility to EAMG. Both proteins were strongly increased in EAMG resistant aged rats. The results suggest that the plasticity of the adult neuromuscular junction contributes to increased stabilization of the postsynaptic anchoring of the acetylcholine receptors.

35. Silencing Rapsyn in vivo Decreases Levels of AChR and Causes Hypertrophy of Secondary Postsynaptic Membrane Folding
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The receptor associated protein of the synapse (rapsyn) is required for anchoring and stabilizing the nicotinic acetylcholine receptor (AChR) in the postsynaptic membrane of the neuromuscular junction (NMJ) during development. Here we studied the role of rapsyn in the maintenance of the adult NMJ by reducing rapsyn expression levels with short interfering RNA (siRNA). Silencing rapsyn led to the average reduction of the protein levels of rapsyn (40% loss) and AChR (30% loss) at the NMJ within 2 weeks. Concomitantly, the amount of secondary folds of the postsynaptic membrane was significantly increased in silenced muscles. The neuromuscular transmission in rapsyn-silenced muscles was impaired. Our results suggest that rapsyn determines the AChR concentration at the adult NMJ and support the hypothesis that functional AChR and rapsyn clusters negatively regulate postsynaptic folding. The results are also suggestive of a very dynamic subsynaptic machinery that can rapidly produce new postsynaptic folds in the adult NMJ.

36. MG and Musk: Relation Between Antigen-Specific IgG Subclasses and Disease Severity
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In myasthenia gravis with anti-MuSK antibodies (MuSK MG), disease severity is broadly correlated with antibody-titers measured by radioimmunoprecipitation. We studied the longitudinal relation between disease severity and titers of MuSK Ab-specific IgG1 and IgG4.

Six patients were included from whom 55 samples had been collected during 2.5-13.4 years. Disease severity was scored retrospectively on a semi-continuous scale by 2 neurologists blinded for antibody titers. MuSK Ab-specific total-IgG, IgG1 and IgG4 titers were determined by ELISA and expressed as arbitrary units compared to an internal standard. The ratio of MuSK Ab-specific total-IgG versus subclass IgG in sera that contained nearly exclusively one subclass yielded a factor that was used to compare MuSK Ab-specific IgG1 and IgG4 titers.

Slopes of MuSK Ab-specific IgG4 correlated with clinical state (β-coefficients varying from 0.01 - 0.15), except for one patient (β = -0.003). Overall, IgG4 titers were significantly associated with disease severity in a
linear mixed effect model ($p = 0.043$). IgG1 was unrelated to disease severity. The average IgG4/IgG1 ratio was 17 (range 1.3-50.6). The one patient with a ratio of 1.3 had high IgG4 titers only when she presented with severe oculobulbar symptoms (IgG4/IgG1 ratio 16.1) and made a class-switch to IgG1 when she went into complete clinical remission (ratio 0.2). We conclude that in MuSK MG, autoantibodies are mainly IgG4 and MuSK Ab-specific IgG4 titers are significantly correlated with disease severity.

37. Identification of a Novel MuSK Splicing Variant
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Muscle-specific kinase (MuSK) is a transmembrane receptor kinase which colocalizes with acetylcholine receptor (AChR) at the neuromuscular junction (NMJ) and is essential for agrin-induced AChR clustering. MuSK was recently found to be involved in a new autoimmune disease, similar to myasthenia gravis.

In the course of cloning MuSK cDNA from adult mouse skeletal muscle, we detected two isoforms of MuSK. Their cDNAs contain extra DNA sequences (30 and 60 nucleotides) in frame compared to the published Gen Bank sequence for mouse MuSK cDNA. We found these extra sequences in the intron 5-6 of the mouse MuSK gene. The presence of a splice acceptor, a donor and possible branch sites followed by pyrimidine-rich sequence in the surrounding sequence indicated that these isoforms of MuSK are splicing variants. We named these new exons as exon 5a and exon 5b. One variant contained exon 5b (MuSK 30), comprised of multiple charged residues and another contained both exons 5a and 5b (MuSK 60) in the interval region between the DNA sequences encoding Ig-like domains 2 and 3. MuSK 30 was identical to the MuSK isoform reported by Valenzuela et al. MuSK 60 has not been reported previously. We identified equivalent exons in human and rat MuSK genes in the Genome Database. PCR analysis of muscle cDNA showed the expression of these variants in human as well, and demonstrated that MuSK 60 is expressed more abundantly than MuSK 30 in mouse muscle. When the N-terminal extracellular domain of MuSK and MuSK 60 were fused to a secretion signal peptide and expressed heterogeneously, MuSK 60 was secreted more efficiently and showed more stability against proteolysis compared to MuSK which lacks these new exons. Biological characterization and functional studies of MuSK 60 are in progress.

38. Serum BAFF Levels in MG
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Autoimmune myasthenia gravis is a B-cell mediated disease. BAFF (B-cell activating factor) is a newly identified molecule that is part of the tumor necrosis factor superfamily. It is a potent B-cell survival factor and it regulates peripheral immune tolerance of B-cells. BAFF is an important molecule within the germinal center, and it may play a role in the survival and maturation of autoimmune B-cells in myasthenia gravis (MG). Serum BAFF levels were measured in 39 patients with MG who were not receiving immunomodulatory therapy. MG patients were compared to 47 non-myasthenic control subjects [healthy subjects: $n = 33$; untreated patients with multiple sclerosis (MS): $n = 14$]. BAFF levels were measured in duplicate by an enzyme-linked immunosorbent assay (ELISA). Serum BAFF levels (mean ± SD) were 1.92 ± 0.9 for MG patients, and 1.52 ± 0.4 for non-myasthenic control subjects. Mean BAFF levels for patients with MG were approximately 25% higher than those of non-myasthenic controls. The difference between the two groups was statistically significant. These data suggest that BAFF is likely to be an important molecule in the pathogenesis of autoimmune MG. BAFF and its receptors may be suitable targets for therapy in patients with MG.

39. Clinical and Immunological Studies of Seronegative MG
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Autoantibodies against radiolabelled acetylcholine receptor-ligand complex (AChR-abs) can be demonstrated in about 90% of patients with generalized symptoms and in about 70% of patients with purely ocular disease. A small number of patients do not have these antibodies despite typical symptoms. This condition has been named seronegative MG and it is often claimed to be a distinct disease entity. Some of these patients have antibodies against other structures at the endplate such as antibodies directed against muscle specific receptor tyrosine kinase. The autoantibody species specific for MG also encompass antibodies that bind to the ligand binding site, antibodies that bear the same idiotypes as AChR- abs, and anti-idiotypic antibodies. The aim of this study was to find out whether the seronegative MG patients differ from the
seropositive patients regarding clinical and immunological parameters. Of 481 patients with MG, 36 were permanently seronegative. The seronegative patients more often had purely ocular myasthenia and less severe disease and less often had thymic hyperplasia and/or thymomas. Seronegative patients were less often treated with immunosuppressive drugs. There were no differences in the prevalence of HLA-Class I (B7, B8) or Class II (DR2, DR3, DR4) antigens between seronegative and seropositive patients, whereas variants of the b2 adrenoceptor gene and the gene for IL-10 were associated to the presence of AChR-antibodies. The spectra of myasthenia-related autoantibodies were similar in both seronegative and seropositive patients, although all antibody species were less frequent in the seronegative patients. Thus, seronegative patients had less severe disease and more often had normal thymic histopathology, whereas the qualitative autoantibody repertoires were similar, with the exception of antibodies binding to the labelled toxin-receptor complex.

40. **Sequencing of PD-1, CTLA-4, TNF-α and CHRNA-1 Genes in MG Patients Yields Target SNPS for in Tandem Genotype Analysis**


DNA Sequencing of full length genes PD-1, CTLA-4, TNF-α and CHRNA-1 was conducted for 12 Myasthenia Gravis patients – 6 with thymic hyperplasia and 6 with thymoma – to locate disease specific polymorphisms. Sequencing was done by PCR amplification of overlapping sequences approximately 600 bp and Sanger chemistry using an ABI 3730 capillary sequencer. The results were aligned by fragment using Staden package and compared to the reference sequence. Analysis of aligned sequenced yielded approximately 35 polymorphisms on total patient sequences with high allelic frequency (> 20%) on the four genes. Of these, 17 were unique to Myasthenia patients; that is, they are not recorded as known SNPs with minor allele frequency > 1% on NCBI. PD-1 has the greatest number of polymorphisms within MG patients (14 of 35). Notably a unique T/C polymorphism was found on 100% of thymoma patients sequenced and 83% of hyperplasia patients, next to a known SNP with a minor allele present in 83% of thymoma and 66% of hyperplasia patients, suggesting a strong disease correlation at these loci. Amplification of PD-1 was incomplete due to a suspected deletion >1 kb in intron 5 present only in patient samples. Investigation is underway to determine the exact region of this deletion.

The target polymorphisms will be genotyped for 293 Myasthenia patients and 275 sex and ethnically matched control samples from Sweden using a Sequenom mass array (MALDI-TOF method with 28-plex). All 35 genotypes will be analyzed in tandem using a support vector machine model in order to discriminate important genotype combinations across the four measured genes required to confer predisposition to Myasthenia Gravis.

41. **Immunological Effects of IVIgG as Complementary Treatment in Autoimmune/Inflammatory Disorders**

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Intravenous IgG is used in patients with Myasthenia Gravis. In our experience, about 25% of patients show a clear beneficial clinical effect. IVIgG is also used in inflammatory vasculopathies, such as Wegener's Granulomatosis, with beneficial effects in about 50%. This study was undertaken to elucidate the mechanism by which IVIgG exerts its effect. Eighteen patients were treated with IVIgG, 1G/kg/month. The concentration of auto-antibodies in serum, the expression of activity and co-stimulatory markers on PBMC and the concentration of soluble T cell cofactors in serum were tested before each infusion of IVIgG.

The concentration of auto-antibodies decreased in all patients. There was also a decreased expression of CD25 on T cells. Twelve patients had expanded T cell populations with abnormally low expression of CD28 and high expression of CTLA-4, and there was a temporary normalization of these values in all patients. The levels of soluble CD28 and CD86 decreased in all patients.

Therefore, IVIgG is an attractive treatment for autoimmune/inflammatory disorders and has profound effects on several parameters of immune function.

42. **The Effect of Dexamethasone and MuSK Antibodies on Muscle Atrophy Pathways**

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Patients with myasthenia gravis (MG) who are positive for MuSK antibodies have usually more severe
clinical symptoms. Muscle atrophy is common, particularly in patients with a long duration of disease and possibly associated with corticosteroid treatment. We have already demonstrated that dexamethasone or MuSK antibodies upregulate the atrophy-related gene product MuRF1 in C2C12 cells, implicating the possible role of downstream signaling in the pathogenesis of muscle atrophy. Here we tried to determine the signaling pathways that might be involved. We looked at the effect of dexamethasone and MuSK antibodies on intracellular pathways involving AKT, pAKT, p38, pp38, IKKbeta and pIKKalpha/beta. Cultured C2C12 cells were incubated with either increasing doses of dexamethasone or control PBS, for up to 5 days. The cell diameter was reduced up to 20% and the concentration of proteins in the cell extracts was reduced up to 40% in the cells with dexamethasone. However, western blotting did not show any significant changes in AKT, p38 and or IKKbeta expression or phosphorylation at times between 12hours and 5days. However, in masseter muscle from mice immunized with MuSK, there was a modest decrease in AKT expression. To see if this change can be induced in vitro, we are studying the effects of MuSK antibodies on the C2C12 cells.

43. Novel Complement Inhibitor (rEV576) Ameliorates EAMG

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Production of acetylcholine receptor autoantibodies associated with deposition of complement at the neuromuscular junction (NMJ) significantly contributes to the pathogenesis of myasthenia gravis (MG). In this study the active and passive induction of experimentally acquired MG (EAMG) were used to investigate the prophylactic and therapeutic role of a complement inhibitor derived from tick salivary glands designated as rEV576. Prophylactic application of rEV576 (gift of Evolutec, Reading, U.K.) in passive transfer protected rats from EAMG. All rats in the treated group had 100% survival rate and a low disease severity score. Active EAMG rats developed weight loss by week 4 post immunization and could be divided into mild and moderate disease severity groups. Treatment with rEV576 for 10 days reduced the severity of weakness and prevented additional weight loss. Serum complement activity (CH50) in severe and mild EAMG was reduced to undetectable levels during treatment. Treatment with rEV576 resulted in reduction of toxicity of serum from severe and mild EAMG. C9 deposition at the NMJ was reduced. Levels of total AChR IgG, IgG1 and IgG2a antibodies were similar. Unexpectedly, the concentration of complement fixing IgG2b antibodies were slightly higher in mild EAMG animals without treatment suggesting an effect of rEV576 on cellular immunity. Inhibition of complement activity significantly reduced the weakness in EAMG produced by administration of AChR antibody and immunization with purified acetylcholine receptor. The data demonstrate that C5 inhibition could prove to be of significant therapeutic value in human MG. Supported by grants from the National Institutes of Health R24EY014837 (HJK) and P30 EY11370.

44. Regulatory T Cells Generated ex vivo as an Approach for Immunotherapy of MG

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Myasthenia gravis (MG) and its animal model experimental autoimmune MG (EAMG) are antibody-mediated, T cell-regulated autoimmune disorders in which the acetylcholine receptor (AChR) is the major autoantigen. MG patients were reported to have lower numbers or functionally impaired CD4+CD25+ T regulatory (Treg) cells that are considered key players in immune tolerance. We have shown that PBL from myasthenic rats also contain decreased numbers of CD4+CD25+FoxP3+ cells as compared with healthy controls. These observations have led us to test the suppressive effect of Treg cells from healthy donors in EAMG. Since the number of naturally occurring Treg cells is low we used an approach for large-scale ex vivo generation of Treg cells. Functional Treg cells were differentiated in vitro from naive CD4+ splenocytes by stimulation with anti-CD3 and anti-CD28 antibodies in the presence of TGF-beta and IL-2. Such cells expressed high levels of CD25, CTLA-4 and FoxP3, and were capable of suppressing proliferation of AChR-specific T cells in vitro. Administration of ex vivo-generated Treg cells to myasthenic rats inhibited the progression of EAMG and resulted in down-regulation of humoral AChR-specific responses, as well as of IL-18 and IL-10 expression. The number of CD4+CD25+ cells in the spleen of treated rats remained unchanged, whereas the sub-population of CD4+CD25+ cells expressing FoxP3 (CD4+CD25+FoxP3+) was significantly elevated. Our
data suggest that Treg cells have a therapeutic potential for MG.

45. Identification and Characterization of Novel Autoantigens Associated with MG
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Myasthenia Gravis (MG) is characterized by antibodies directed against the nicotinic acetylcholine receptor (AChR) in 80-90% of patients, but only 54% of those with pure ocular MG have detectable anti-AChR antibody in serum. Furthermore, Ab against the muscle-specific receptor tyrosine kinase (MuSK) were detected in 38-54% of patients with AChR-A negative (seronegative) MG but not in pure ocular MG. Therefore, identification of novel autoantigens associated with early stage MG is still demanded for improving current diagnosis.

Here, in combination of affinity technology and mass spectrometry, we have developed an integrated proteomics platform (patented) to systematically and efficiently identify autoantigens. After screening 16 MG patients, we have identified 2 promising candidate autoantigens, MG1 and MG2, for early detection. We have purified these two candidate antigens and validated them with enzyme-linked immunosorbent assays (ELISA). The clinical samples applied for validation are sera collected from 54 control individuals and 209 MG patients with different stages. 76 patients had type I MG; 81 had type II A; 38 had Type II B, and 14 patients had either Type III or Type IV.

Our results indicate that combination of these two novel markers improve the detection of the early stage MG from 50% to 98%.

46. An Experimental Model of MuSK MG: Active Immunisation Without Adjuvant
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Muscle specific kinase (MuSK) antibodies are predominantly of the human IgG4 subclass. There is growing evidence that these antibodies are directly pathogenic but it remains unclear how such antibodies cause disease. It is our hypothesis that such antibodies cause disease by modifying function of the AChR rather than by reducing receptor numbers.

We immunised three different strains of mice (C57BL/6, SJL and AJ) with either 10 µg human extracellular MuSK or placebo administered intraperitoneally without adjuvant. The mice received 4 further boosts over a 12 week period. MuSK antibodies were detected using both a radio-immunoprecipitation and an immunofluorescence assay. We looked for objective signs of weakness before recording miniature endplate potentials (MEPPs) and endplate potentials (EPPs) from diaphragm-phrenic nerve preparations. We assessed AChR numbers using 125I α-bungarotoxin labelling of the diaphragm and the structure of the neuromuscular junction using immunofluorescence staining.

Our results suggest that active immunisation with MuSK without adjuvant can generate a modest antibody response and subtle changes in endplate morphology. However AChR numbers were unaffected. These murine MuSK antibodies are predominantly of the IgG1 (non-complement fixing) subclass in comparison to the IgG2a/b (complement fixing) subclass which are usually generated by immunisation with adjuvant.

47. Analysis of Native AChR-Associated Proteins in Muscle Extracts
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A proportion of patients with myasthenia gravis (MG) neither have antibodies to the acetylcholine receptor (AChR), nor to the muscle specific kinase (MuSK). The hypothetically neuromuscular antigen of these patients is still unknown.

To identify important AChR-associated proteins of the neuromuscular junction the TE 671 cell line and rat muscle membrane extracts were used. Cell lysates were subjected to AChR precipitation using α-bungarotoxin-biotin. After the precipitation, AChR and associated proteins were analyzed by SDS-page and immunoblotting. Moreover 2D gel electrophoresis was performed, followed by silverstaining to visualize the co-precipitated proteins. Finally, mass spectrometry was performed to identify the AChR-associated proteins.
48. CD4+ T Cell Epitope Repertoire in C57BL/6 Mice Presented by HLA Class II Molecules Relevant for Development of Human MG
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Anti-AChR CD4+ T cells trigger and modulate the synthesis of the high affinity anti-acetylcholine receptor (AChR) Ab that cause neuromuscular junction failure in myasthenia gravis (MG). CD4+ T cells recognize epitope peptides associated with MHC class II molecules. Expression of the DR3, DQ8 and DR4 HLA class II molecules is increased among MG patients, suggesting that they facilitate MG development. Expression of DQ6 is negatively associated with MG. We examined the role of the DR2, DR3, DR4, DQ6 and DQ8 molecules in the development of MG using C56Bl/10 mice transgenic for each of these molecules, immunized with individual recombinant human AChR (HAcHR) α, β, γ, δ and ε subunits, or pools of overlapping synthetic peptides spanning the different subunit sequences (“subunit peptide pools”). We used a microproliferation assay to assess the subunit ability to sensitize CD4+ T cells, and to identify CD4+ T cell epitopes presented by the human class II molecules transgenically expressed in the mice. The responses to the subunit used for immunization were strongest in the β subunit immunized DR3, DQ6, DQ8 mice and in the α subunit immunized DR2 mice: their average stimulation indexes (SI) were 20 or higher. The α and γ subunit immunized DR4, DQ4, DQ8 mice, the β subunit immunized DR4 mice, and the δ subunit immunized DQ6 and DQ8 mice also had substantial proliferative responses to the immunizing subunits (SI of 10-20). In contrast, α and γ subunit immunized DR3 mice, δ subunit immunized DR2, DR3 and DR4 mice, and ε subunit immunized DR3, DQ6 and DQ8 mice had weak responses (SI of 2.5-10).

After α subunit immunization, all HLA-transgenic mouse strains recognized peptide α215-235; all but DQ6 also recognized α101-120. After immunization with the α subunit peptide pool, all the DR transgenic mice recognized the sequences α48-67, α91-120 and α173-235, whereas the DQ transgenic mice recognized fewer and different peptides, such as α387-400. After β subunit immunization, DR3 mice recognized the sequences β31-50 and β256-275, DR4 mice recognized β211-230 and β286-305, and DQ8 mice recognized β16-35, β256-275 and β391-410. DQ6 mice did not recognize any peptides. After immunization with the β subunit peptide pool, DR transgenic mice recognized β181-200, and DQ transgenic mice recognized β16-35 and β76-93. All mice immunized to the γ subunit had strong proliferative responses to several γ subunit peptide sequences. DR3 mice recognized the sequence region γ105-124, DR4 mice γ180-199 and γ321-340, and DQ6 and DQ8 mice γ190-209. DQ8 mice recognized also γ120-139. Mice immunized with the γ subunit peptide pool recognized the same peptides recognized after immunization with the recombinant γ subunit. After immunization with the δ subunit, all DR transgenic mice recognized the sequence δ211-230. DQ6 mice recognized δ46-65, DQ8 mice δ76-95. After immunization with the δ subunit peptide pool, DR mice recognized δ211-230 and δ53-377. DR2 and DR4 mice recognized also δ181-200. DQ mice recognized δ91-110 and δ401-420, although their proliferative responses were much weaker than those of DR mice. After ε subunit immunization, DR mice did not recognize any peptide sequence, whereas DQ mice recognized ε161-180. After immunization with the ε subunit peptide pool, DR4, DQ6 and DQ8 recognized ε11-30. DQ mice recognized also ε161-180. DR3 did not recognize any peptide. In conclusion, sensitization of mouse CD4+ T cells to the HAcHR when presented by human class II molecules may involve all but the ε subunit of the HAcHR. The HAcHR contains also a number of cryptic CD4+ T cell epitopes, some of which are on the ε subunit. The complexity of the CD4+ epitope repertoire recognized by HLA transgenic mice suggests that the development of specific immunosuppressive therapy targeted on the CD4+ epitopes in EAMG may be challenging.

49. Transgenic Mice Expressing Human HLA Molecules are Susceptible to EAMG Induced with Recombinant Human ACHR α, β, γ, δ and ε Subunits
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Epidemiological studies suggested a positive correlation between the expression of DR3, DR4 and DQ8
and the presence of MG, whereas the expression of DQ6 correlated negatively. Mice transgenic for human HLA molecules (HLA-Tg) are useful to study the role of different HLA molecules in the susceptibility or resistance to an autoimmune disorder. We used C56Bl/10 mice transgenic for DR2, DR3, DR4, DQ6 and DQ8 immunized with recombinant human acetylcholine receptor (HACHR) α, β, γ, δ and ε subunits, to determine the ability of each subunit to induce Experimental Autoimmune MG (EAMG) when presented in combination with a particular HLA class II gene product. Mice were immunized three times, four weeks apart, with one of the HACHR subunits (20g/mouse) in Freund’s Adjuvant. We considered a strain to be susceptible when more than 57% of the mice developed EAMG manifestations. All the HACHR subunits induced EAMG, although to different degrees. DR4-Tg mice were the most susceptible strain, regardless of the subunit used for the immunization (57%, 100%, 75%, 83% and 100% of the mice developed EAMG after α, β, γ, δ and ε immunization, respectively). DQ6-Tg mice developed EAMG only after immunization with the α subunit, DQ8-Tg mice only after immunization with the β and ε subunits. Only a few DR3-Tg mice developed EAMG after immunization with the α (40%) and the δ (33%) subunits. None of the DR2-Tg mice developed EAMG, regardless of the subunit used in the immunization. We measured the anti-HACHR subunits antibody (Ab) in the mouse sera collected 12 weeks after the first immunization. DR3-Tg mice developed specific Ab only after immunizations with the α and δ subunits, which were the only subunits able to induce EAMG in this strain. Although DR4-Tg mice were very susceptible to EAMG induction, their Ab response was mostly represented by the IgG1 isotype: they did not have anti-α, β and ε subunit IgG2c and anti-δ subunit IgG2b and 2c. All the DQ6-Tg and DQ8-Tg mice (with the exception of DQ6-Tg mice immunized to the α subunit) had serum Ab against the subunit used in the immunization. DR2-Tg mice had high levels of serum Ab against the HACHR subunits Ab used for the immunization, in spite of their resistance to EAMG development. We also measured the serum Ab cross-reacting with mouse AChR, which are responsible for the EAMG manifestations in mice. Mice immunized with the α HACHR subunit had the highest levels of anti-mouse AChR Ab. Anti-mouse AChR Ab were detectable in the sera of mice immunized with the β HACHR subunit, but they were absent or present in very low concentrations in mice immunized with the δ, γ and ε HACHR subunits. We conclude that DR4 and DR2 expression correlates with susceptibility and resistance to EAMG respectively. Moreover, as observed in MG patients, there was no correlation between the anti-AChR Ab titers and the severity of the myasthenic weakness.

50. Characterisation of a Mouse Model of the Slow Channel Syndrome
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In a subset of congenital myasthenic syndromes, known as the slow channel syndrome, activation of acetylcholine receptors (AChR) is prolonged which results in fatigable muscle weakness and muscle wasting. In order to visualise AChR at the neuromuscular junction an EGFP fluorescent tag was attached to the slow channel mutant AChR (heL221F-EGFP) and expression of the mutant AChR was driven by the AChR β-subunit gene promoter. Mice expressing the mutant ACHR ε-subunit were bred with ε-subunit knock-out heterozygous mice (mε+/−). Visualisation of the fluorescently tagged mutant receptor at the neuromuscular junction of heL221F-EGFP+/− mε−/− mice shows incorporation into the machinery of neurotransmission. Tests for fatigable muscle weakness over a 6 month period demonstrate increasing deficit in heL221F-EGFP+/+ mε−/− compared to mε+/- mice. At 10 weeks, in the diaphragm muscle, amplitude of spontaneous endplate potentials was unchanged compared to mε+/- mice. However, decay time of both spontaneous and evoked endplate currents was 11.6 and 13.6 fold longer, respectively. When heL221F-EGFP+/+ mε−/− diaphragms were subjected to high frequency stimulation, decrement of endplate current occurred sooner and to a greater extent. By the 5th stimuli in a 50 Hz train, current amplitude had decreased to 47.1 ± 9% of initial amplitude (n = 8 endplates) compared with 87.2 ± 3% (n = 12 endplates) in mε+/- mice.

This animal model displays many of the hallmarks of the slow channel syndrome (progressive weakness and prolonged endplate currents with decrement) and therefore should prove a useful tool for investigating the pathogenic mechanisms in this disease and evaluating novel treatment strategies. This work was supported by grants from MDC/MGA and MRC UK.

51. Ocular Myasthenia Gravis (OMG): Diagnostic Utility of Facial RNS, SFEMG, AND AChR AB Testing in Patients with Nonfatigable vs. Fatigable PTOSIS
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Objective: To assess the diagnostic utility of facial repetitive nerve stimulation (RNS), frontalis single
fiber EMG, and acetylcholine receptor antibody testing (AB) in patients presenting with nonfatigable vs. fatigable ptosis. We evaluated 28 patients; 26 patients had ptosis and two had isolated diplopia. Of the 26 with ptosis, 20 had nonfatigable ptosis, while 6 had fatigable ptosis and/or demonstrable weakness of the orbicularis oculi (OO). Eventually, 11 patients were diagnosed with OMG, based on + AB and/or frontalis SFEMG.

Results: In patients with nonfatigable ptosis, yields were: RNS 0%, SFEMG 13%, and AB 9%. In patients with fatigable ptosis and/or OO weakness, yields were: RNS 22%, SFEMG 80%, and AB 56%.

Conclusion: Compared to patients with fatigable ptosis and/or demonstrable OO weakness, patients with nonfatigable ptosis have a significantly lower diagnostic yield for OMG. Screening these patients with AB should be sufficient. SFEMG may be used to clarify borderline results, but RNS is not likely to help the diagnosis.

52. Prognosis and Diagnostic Studies in Late Onset Ocular MG
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Late onset ocular myasthenia gravis (OMG) patients traditionally have a poorer outcome. OMG progresses to generalized MG (GMG) in 50-85% of patients. OMG’s with immunomodulation Rx less commonly develop GMG. We evaluated all MG patients with initial symptom onset > 69 years at Lahey 1980-2006; i.e. senior MG (SMG) as to symptoms, Rx response, and prognosis. 43/102 SMG cases presented with OMG; GMG developed in 12 (27.9%) during average 4-year follow-up. No OMG/SMG patient, who later developed GMG, required ventilator assistance or a feeding tube. All 10 OMG/SMG patients presenting in their ninth decade had had an excellent Rx response. Gender, endplate and skeletal muscle antibodies, repetitive nerve stimulation (RNS), single fiber EMG (SFEMG), & chest CT were compared in SMG patients who remained as OMG and those developing GMG. Late onset OMG patients do not have a worse prognosis than younger individuals. Although specific endplate antibodies, SFEMG, and RNS, are useful for SMG diagnosis none predict a risk for GMG.

53. Is There an Increasing Incidence of Late-Onset ACHR-AB Positive MG?
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An unexpectedly high incidence of MG in aged people has been recently reported (Aragones JM. Neurology, 2003). We retrospectively reviewed MG diagnosis at our Institution to investigate whether the trend in MG age at onset is changing. Out of 135 patients with MG diagnosed since 1987, 97 patients were anti-AChR+$. Age at onset and year of onset correlated in anti-AChR+ patients ($r = 0.37$, $p < 0.001$). Changes in age at onset over time showed a significant trend to seeing older patients. The average age has been going up about 1 year on the age of the patient per year over the duration. Sixty years or older patients were 24% before 2000 and 56% since 2000. While the number of patients over 60 yrs linearly increased over the observation period ($r = 0.67$; $p < 0.05$), the total number of patients $\leq$ 60 remained stable over time and the increase in the number of AChR+ patients seen was due to those $> 60$. Higher frequency of men, ocular symptoms at onset, fewer with generalized onset, approximately the same bulbar onset and normal chest CT scans were the clinical characteristics in the late-onset AChR+ MG. Clinical outcome was similar in early- and late onset MG.

54. Complete Remission and Autoantibody Patterns in MG: A Large Population Study
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Outcome evaluation using Kaplan-Meyer analysis has not been performed on large series of myasthenia gravis (MG) patients and the chance of reaching true remission has not been evaluated in patients grouped according to their autoantibody specificity. MG patients can be divided into three subgroups: AChR+$, MuSK+ and seronegative (AChR-/$\mu$MuSK-). We evaluated the occurrence of complete stable remission (CSR), as well as other epidemiological and clinical parameteres, in a large series of 677 MG patients, including 55 MuSK+ (8%), 105 seronegative (16%), and 517 AChR+ (76%) patients. All patients were followed-up by the same group of physicians; disease duration was at least 6 years for each group. CSR was reached in 3.6% of MuSK+ compared with 22% in both the
AChR\(^+\) and seronegative groups. MuSK\(^+\) patients were characterized by increased incidence of bulbar symptoms and respiratory insufficiency at onset and maximal worsening. Immunomodulating treatments (i.e. plasmapheresis or intravenous immunoglobulins) were used more frequently in MuSK\(^+\) patients, with a positive clinical response not different from that observed in AChR\(^+\) and seronegative groups. The outcome of seronegative patients was not worse compared with AChR\(^+\) patients. Our study indicates that anti-MuSK antibody-associated MG shows a worse outcome and deserves early intensive immunosuppression and immunomodulation. Treatment protocols specific for MuSK\(^+\) MG should be designed.

55. HLA Allele Frequency in MuSK-Positive MG Italian Patients

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Association between anti-MuSK positive (+) MG and certain class II HLA alleles has been reported. Our aim was to compare the frequencies of class II HLA alleles in Italian patients with anti-MuSK+ MG compared with normal controls. We studied 20 subjects with anti-MuSK+ generalized MG. They were all Caucasians, with grandparents of Italian origin. The control group consisted of 380 unrelated individuals representative of central Italian population. Genomic DNA was extracted from PBL; HLA-DQA1, -DQB1 and -DRB1 loci were typed by PCR followed by a reverse line blot using labeled sequence-specific oligonucleotide probes (PCR-SSO). High resolution HLA-DQA1 and DQB1 typing and low resolution DRB1 typing were performed (Innogenetics, Italy). Statistical analysis was carried out by two-sided Fisher exact test and Odds ratios (ORs) with 95% CIs. The following alleles were increased in patients in comparison with controls: DQA1*0104: 15% versus 3.9% (OR 4.2941, 95% CI 1.6747-11.0102, \(p < 0.01\)); DQB1*0502: 17.5% versus 4.6% (OR 4.3939, 95% CI 1.8165-10.6286 \(p < 0.005\)); DQB1*0503: 12.5% versus 4.8% (OR 2.873, 95% CI 1.0622-7.771 \(p < 0.05\)); DRB1*14: 12.5% versus 7% (OR 1.9057, 95% CI 0.7168-5.0662, \(p = 0.20\)); DRB1*16: 15% versus 2.2% (OR 7.7128, 95% CI 2.8597-20.802, \(p < 0.001\)). Serological DQ5 allele frequency was 40% in patients and 21.3% in controls (OR 2.4609, 95% CI 1.277-4.7422, \(p < 0.02\)). Twelve out of 20 patients carried either DRB1*14 or DRB1*16 allele or both. Our data confirm and extend a previous report on an increased frequency of DQ5 and DR14 alleles in anti-MuSK+ MG, showing that the increased frequency of DQ5 allele was due to both DQB1*0502 and DQB1*0503 alleles. Moreover, in our population, an increased frequency of DRB1*16 allele was found.

56. Modulation of Acetylcholinesterase Molecular Forms in EAMG: Response to Treatment with Specific Antisense

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Recent studies indicate that the nicotinic acetylcholine receptor (nAChR) and acetylcholinesterase (AChE) function in the neuromuscular junction (NMJ) are intimately related. In addition, we previously found that treatment of EAMG with antisense oligodeoxynucleotides suppressing AChE biosynthesis (EN101), improved muscle activity and clinical symptoms. To determine the effect of EN101 treatment on muscle AChE, EDL muscles of EAMG, Lewis rats were isolated. And AChE concentration and isoform composition were determined. Although differences in AChE concentrations (per protein) between healthy, EAMG and disease-treated rats were not evident, changes were observed in isoform composition. Healthy rat EDL contained globular (G\(_{1+2}\), G\(_4\)) and asymmetric (A\(_8\), A\(_{12}\)) isoforms. G\(_{1+2}\)-AChE significantly decreased in EAMG muscles while G\(_4\) and A\(_{8,12}\)-AChE remained unchanged. EN101 treatment showed recovery in G\(_{1+2}\) but a reduction in A\(_{12}\)-AChE. In addition, by double-labeling AChE and AChR with fluorescent-toxin probes and quantitative confocal fluorescence imaging the AChE/AChR ratio was determined. Our results emphasize the tight connection between AChR and AChE at the myasthenic NMJ and its importance in maintaining the cholinergic balance. Supported by AFM (TB), ISF (LA).

57. Correlation of Quality-of-Life with Regional Symptoms and Signs in MG: Muscle Study Group Cellcept Trial Experience

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Goal: To develop a user-friendly, symptom-based evaluative QOL instrument for myasthenia gravis (MG) by determining the relative weights of various regional symptoms and signs.
**Methods:** We analyzed data from 80 patients with MG enrolled in the MSG CellCept trial. Data at trial entry for each domain (double vision, eyelid droop, dysarthria, eating weakness, upper extremity weakness, and lower extremity weakness) were extracted from the QMG, MG-ADL and MG-MMT. The worst score of the three assessments of each domain was regressed against a disease-specific 15-item QOL measure (MGQOL-15) and the SF-36.

**Results:** Ocular features were more often moderately or severely affected than other regional domains. Low vital capacity (VC) and dyspnea were much less likely to be moderately or severely affected. The MGQOL-15 correlated with the SF-36 total score and with each SF-36 subscore. MGQOL-15 correlated (p < 0.05) with: diplopia, trouble talking, dyspnea, arm weakness and leg weakness. Dysfunction of each of these domains affected the MGQOL-15 to a similar degree. MGQOL-15 did not correlate with eating dysfunction or VC score. All regional domains except diplopia correlated with the SF-36 physical role subscore. Dyspnea, arm weakness and leg weakness correlated with the SF-36 physical component and physical functioning subcores.

**Conclusions:** In these MG patients, diplopia, trouble talking, dyspnea, arm weakness and leg weakness correlated significantly and to a similar degree with the MGQOL-15. Regional domains correlated better with the disease-specific MGQOL-15 than to the SF-36 total score and SF-36 subscores. Further analysis will be performed for data from different time-points during the CellCept study.

**58. Cognitive Function in Patients with MG**

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Association between MG and CNS dysfunction, especially in learning and memory, has been occasionally reported. A possible central effect of anti-AChR abs has been hypothesized. Our aim was to evaluate cognitive functions in a series of elderly MG patients.

One hundred consecutive MG patients (58M/42F) older than 65 at inclusion and 31 healthy subjects (15M/16F), matched for age, gender and educational level, underwent a standardized test battery exploring multiple cognitive domains. Statistical analysis was performed by One Way MANOVAs and Pearson’s Chi Square; clinical variables entered into a factorial analysis in relation to the diagnosis of cognitive impairment.

Overall, no significant differences between MG patients and controls were found. Patients with severe MG were significantly impaired in divided attention tasks, constructional praxis and frontal control tasks (Stroop test, Go No-Go) when compared with patients with mild/moderate disease. Corticosteroid treatment was associated with better performances in an attention time dependant task (p < .03), verbal learning memory tasks and frontal abilities. No effects of anticholinesterase therapy, thymus pathology, presence of anti-AChR abs, and MG duration were observed. The separate analysis of associated diseases showed that this variable correlated with low performances in attention time-dependant task, verbal fluency and frontal tasks. A principal component factorial analysis showed that diabetes and dysthyroidism were the variables most closely related to the presence of cognitive impairment.

Our study does not support the hypothesis of a CNS cholinergic involvement in MG, rather it shows that the impairment of attention, memory and control tasks in these patients is due to both a general visual motor slowness and the concomitant presence of other chronic diseases.

**59. Development of a New Oculobulbar Facial Respiratory Score in the Assessment of Bulbar MG**

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It is often difficult to objectively quantitate bulbar weakness in neuromuscular patients with bulbar muscle involvement as sometimes occurs with myasthenia gravis. The Myasthenia Gravis Foundation of America (MGFA) classification of disease simply attributes a patient’s clinical status into a particular class and subgroup. The Quantitative Myasthenia Gravis score (MGFA-QMG), however, is a scoring system that includes some bulbar features, although it is often incomplete in ascertaining the level of bulbar dysfunction in a patient. We developed a number of measures that provide an oculobulbar facial respiratory score (OBFR) to help us assess in detail patients with MuSK antibody positive myasthenia gravis, who often had predominant bulbar disease. We observed that these patients scored rather well on the MGFA-QMG, MGFA-ADL (activities of daily living) and the Manual Muscle Test (MMT), which did not help to distinguish disability among these patients. The OBFR
score ranges from 0 (minimal disability) to 21 (maximal disability) and consists of five clinical items:

1. Facial muscle strength for orbicularis oculi and oris, buccinator, corrugator supercillii and frontalis (grades 0 to 10, with each muscle scoring 0 if normal, 1 if mildly weak and 2 if severely weak or non-contraceptive);
2. Swallow time for 100mls of water (graded 0 to 3);
3. Tongue appearance (graded 0 = normal, 1 = lateral thinning, 2 = central atrophy, 3 = triple furrowing);
4. Soft palate contractility (graded 0 = normal, 1 = mildly weak and 2 = movement is virtually absent)
5. FVC standing (graded 0 to 3, based on the QMG scoring system).

In a recent study (Farrugia et al., 2006), we established that the new OBFR score bears a positive correlation with the extent of fatty replacement in the tongue as measured on MRI T1W sequences in both MuSK-MG and AChR-MG patients (p = 0.004). Furthermore, the higher the extent of muscle atrophy as established on MRI, the higher the likely the OBFR score (p < 0.0001).

60. Thyromoly in the Management of MG: The West of Scotland Experience

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Thymectomy in the west of Scotland (UK) has been performed by a single operator (JCF) using a “limited” trans-ternal technique since January 1992. Services cover a population of circa 3.5 million and although a formal database of myasthenia gravis (MG) patients is not kept to date, it is estimated that there are approximately 400 MG patients in the west of Scotland. We wanted to ascertain the morbidity and mortality associated with this procedure, the extent of patients’ improvement in clinical outcome as determined by MGFA clinical score and their long-term requirement for immunosuppressive treatment.

We retrospectively studied a consecutive series of 66 patients with MG, who underwent thymectomy between January 1992 and March 2006. Patients’ medical records were studied by a single neurologist (MEF). Patients’ demographics, initial symptoms and MGFA were recorded, the latter at distinct time points (at presentation, maximal before thymectomy, maximal MGFA score following thymectomy and final recorded MGFA score post-thymectomy). In our cohort, 44 patients were female and 52 patients were positive for acetylcholine receptor antibodies. The mean duration of disease pre-thymectomy was 2.5 years, the mean age at thymectomy was 41.7 years and the mean duration of follow-up post-thymectomy was 4.5 years. The procedure was associated with minimal complications and there was no associated mortality. Thymoma was present in 23% of cases. Post-operatively, 59% of patients achieved remission and the MGFA score at the final recorded end-point was significantly lower than the maximal MGFA score pre-thymectomy (p < 0.0001). Post-thymectomy, 26% of MG patients were on corticosteroids, while 47% were on azathioprine. Our study confirms that the “limited” transternal thymectomy is associated with a low rate of post-operative complications and a favourable outcome in the clinical status of MG patients.

61. Tongue Muscle Atrophy in MuSK Antibody Positive MG

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Myasthenia gravis (MG) patients with antibodies to MuSK (muscle specific tyrosine kinase) often develop tongue muscle atrophy.

We studied 15 MuSK antibody positive (MuSK-MG) patients and 15 MG patients positive for acetylcholine receptor antibodies (AChR-MG), similar in age, sex ratio, duration of disease and current bulbar weakness. We performed MRI of the tongue on these patients (12 healthy controls for comparison). For the in vitro studies, we quantitased surface AChRs in mouse myotubes (C2C12) using 125I-α-bungarotoxin, AChR subunit mRNA using RT-PCR and AChR clusters using tetramethylrhodamine α-Bungarotoxin. Eight MuSK-MG patients in contrast to 3 AChR-MG patients had tongue atrophy in the form of lateral thinning, central or triple furrowing. On MRI, the intrinsic tongue muscle areas were smaller in the MuSK-MG patients compared with healthy controls. A larger extent of high signal replacement in the tongue was noted in MuSK-MG patients (p = 0.009 on ANOVA). This high signal was confirmed to be secondary to fatty change on cUTE sequences. The area of high signal performance is not necessary for the effective treatment of patients. However, it is important to monitor the response to therapy and to alert the patient to the risk of aspiration and respiratory complications.
correlated significantly with duration of treatment with high-dose steroids (p = 0.006). In C2C12 myotubes, MuSK-MG sera caused a reduction in agrin-induced clusters, but there was no significant effect on total surface AChR numbers or AChR subunit mRNA.

Tongue muscle atrophy is relatively common in MuSK-MG. In vitro studies demonstrate little effect of MuSK antibodies on AChR expression but there is evidence that these antibodies up-regulate atrophy-related genes. MuSK antibodies may act selectively on specific muscle fibre types or fibres of a particular embryological origin. Facial and tongue muscles appear especially vulnerable to such an action, and corticosteroids at high doses may exacerbate this effect.

62. Effectiveness and Safety of Low Dose Tacrolimus in a Cohort of 178 Thymectomized Patients with MG
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Thymectomy is a standard treatment for myasthenia gravis (MG). Immunomodulating agents are frequently given during the post-thymectomy latency period until complete remission is fully consolidated. We describe the clinical results obtained in an ongoing cohort of 178 consecutive patients (59 men, 114 women) with MG treated with low dose tacrolimus (initial dose 0.1 mg/kg b.i.d.) immediately after transternal extended thymectomy. The mean (SD) follow-up was 48 (16.8) months (range 0–72 months). The mean age at thymectomy was 38.2 years (range 15–70 yrs). Historically, there were 63 cases of thymoma (invasive 30), 84 thymic hyperplasia, and 31 thymic involution. The preoperative MGFA classification included I (n = 2), II-a (n = 1), II-b (n = 3), III-a (n = 18), III-b (n = 65), IV-a (n = 2), IV-b (n = 66), and V (n = 21). During the first post-thymectomy year, blood level of tacrolimus was maintained at 7 ng/mL. According to MGFA postintervention status, complete stable remission was achieved in 28 (15.7%) patients and pharmacologic remission in 120 (67.4%). Adverse effects included hypertension in 2.8% of cases, hyperglycemia in 5.3%, in 2.8%, paresthesias in 5.6%, hypercholesterolemia in 3.3%, infections in 1.4%, tremor in 2.2%, hypomagnesemia in 5.1%, and anemia 1.7%. Twelve patients discontinued tacrolimus, 7 of them for reasons unrelated to the drug and 5 due to adverse events (hallucinations 1, ataxia 2, nephrotoxicity 2). Malignancies occurred in 4 patients (renal cancer after 3 months of treatment in 1 and lung cancer in heavy smokers in 3).

Post-thymectomy administration of low dose of tacrolimus is effective with a low incidence of drug-related side effects. Tacrolimus is a promising drug in the therapeutic armamentarium of MG.

63. Psychiatric Disorders in 201 Patients with MG
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Although it is known that patients with myasthenia gravis (MG) may complain of psychiatric/psychological disorders, there is little information on the characteristics and management of psychiatric diseases in clinical series of patients with MG.

A retrospective analysis of 201 consecutive MG patients undergoing thymectomy between 1990 and 2005 is presented. None of them had family or personal history of psychiatric disorders. Sixty-four percent of patients were women (mean age 45.7 years). Histologically, thymoma was identified in 63 patients, hyperplasia in 97, and thymic involution in 40. All patients were treated with prednisone associated with cyclosporine in 97 patients and with tacrolimus in 67. Currently, patients are being treated with tacrolimus only, with a mean (SD) duration of treatment of 47.4 (25.3) months, except for 36 patients in whom complete stable remission (CSR) was achieved.

Fifty-four (27%) patients required psychopharmacological treatment during the course of MG because of the following diagnoses (DSM-IV): major depression (n = 5), moderate depression (n = 22), and generalized anxiety disorder (n = 27). These conditions were diagnosed after a mean of 26.4 (10.6) months post-thymectomy. Pharmacologic treatment included venlafaxine (n = 7), fluoxetine (n = 4), citalopram (n = 33), escitalopram (n = 9) and duloxetine (n = 1) at standard doses. The mean duration of treatment was 47 (27.8) months.

In none of the patients the use of these medications concomitantly with MG treatment had an unfavorable effect on MG symptoms. All patients are continuing under psychiatric treatment, including 15 patients with CSR. The psychiatric disorder is considered to be in symptomatic remission on treatment in 67% of patients.
64. Correlation of Presence of Lymphoid Follicles in Thymoma with Clinical MG

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Myasthenia gravis (MG) is often associated with thymic pathology, most commonly in the form of lymphofollicular hyperplasia (LFH) or thymic epithelial tumor/thymoma (TET). Approximately 30-45% of patients with TET have or will develop MG. Association of LFH with MG has been established, however the significance of LFH in or outside the tumor in cases of TET has not been well studied and the correlation with MG remains unclear.

We studies 28 cases of TET diagnosed between 1989 and 2004 that were examined histologically for the presence of LFH either within the tumor or adjacent non-neoplastic thymus (NNT). Nine cases (9/28, 32%) had LFH either within the TET or in adjacent NNT. Six of the nine (66%) cases had MG at presentation and 1 developed MG post-thymectomy. In the 19 (19/28, 68%) cases without LFH, normal (non-neoplastic) thymus tissue was identified in 11 (58%), of which 2 (10.5%) had MG at presentation and 1 (5%) developed MG post-thymectomy. MG was seen more frequently in cases with LFH (66% vs 10.5%; p = .005).

Our results suggest that in patients with TET and LFH within the TET or adjacent NNT, there is an increased chance of having clinical MG than those without LFH. Thorough pathological evaluation of TET specimens, including the surrounding NNT should be performed in all thymectomies for TET.

65. Cancer in MG Patients Treated with Oral Immunosuppressants

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Background: Myasthenia gravis is increasingly being managed with oral immunosuppressants including corticosteroids, azathioprine, cyclosporine and mycophenolate mofetil. A potential adverse effect of all immunosuppressants is an increased risk of developing cancer.

Objective: To determine if patients treated for myasthenia gravis with oral immunosuppressants have an increased risk of cancer compared to the general population.

Methods: Patients enrolled in the Duke Myasthenia Gravis Registry completed questionnaires during the years 2004-2005. Information was collected regarding age, gender, past history of cancer and treatment for myasthenia gravis.

Results: Two hundred sixty-seven (267) patients completed the questionnaire. Forty-seven patients (16%) reported a history of cancer (excluding skin cancer). Based upon age and gender risk data from the National Cancer Institute, 17% would have been expected to have had a history of cancer. Subgroup analysis revealed no increased occurrence of cancer for patients taking prednisone, azathioprine, cyclosporine or mycophenolate mofetil.

Conclusion: Based upon this analysis, oral immunosuppressants as used in the treatment of myasthenia gravis do not increase the risk of developing cancer. Further analysis on the effects of using multiple immunosuppressants is underway.

66. Prognosis of Ocular MG in South Korea: A Retrospective Multicenter Study

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Ocular myasthenia gravis (MG) is the most common initial subtype of autoimmune MG and often progresses to a generalized form. Early immunosuppressive therapy in ocular MG is suggested to prevent or delay generalized disease, but an optimal treatment for ocular MG is controversial. Ocular MG is reported to be more prevalent in Oriental populations than in Caucasians, but the risk of secondary generalization is reported to be lower among Orientals. We assessed ocular MG onset, prognosis and current status of treatment in South Korea. From 13 medical college-based
general hospitals, we conducted a retrospective survey of patients 20 years or older at presentation diagnosed with MG from Jan 2000 to Dec 2005. A total of 376 MG patients were identified (M:F = 152:224, mean age 45 years). Nearly half (n = 202, 54%) presented with ocular MG. Patients diagnosed as ocular MG at presentation were divided into two groups based on whether they remained ocular during the follow-up period (OMG-NG; n = 155, 77%), or generalized (OMG-G; n = 47, 23%). Time to the secondary generalization in OMG-G group was 7.9 ± 11.1 months (mean ± S.D.), and the follow-up duration in OMG-NG group was 20.8 ± 21.2 months. Sex and age at presentation were no different, but time to initial presentation was significantly shorter in OMG-G than in OMG-NG group (mean, 5.2 months vs. 16.6 months; p < 0.001). AChR antibody was positive in 70% (n = 135) of ocular patients at presentation (mean level 4.8 ± 3.7 nM). The rate of seropositivity was significantly greater in OMG-G compared with OMG-NG group (93.5% vs. 64.6%; p < 0.001), and AChR antibody titer of the seropositive patients was significantly higher in OMG-G group (6.4 nM vs. 3.8 nM; p = 0.03). Decremental responses in repetitive nerve stimulation test (RNST) were more frequent in OMG-G compare with OMG-NG patients. Chest computed tomography was performed in nearly all ocular MG patients (n = 180) and thymoma found in 33 patients. The proportion with thymoma was higher in OMG-G compared with OMG-NG group (36.2% vs. 12.0%, p < 0.001). Oral corticosteroid was used in 66 patients with ocular MG at presentation (33%) before secondary generalizations occurred, and 22 patients received prednisone plus azathioprine combination therapy. OMG-NG patients were more frequently treated with prednisolone than the OMG-G group (37.4% vs. 17.0%, p = 0.009). Thymectomy in none-thymoma patients was done in 7 patients (4.1%) with ocular MG. For patients with ocular MG at presentation, univariate Cox proportional hazard regression analysis indicates that the seropositivity of AChR antibody and its titer in seropositive cases, and thymoma on chest CT, increased the risk of secondary generalization, whereas the greater time to presentation and prednisolone treatment were associated with decreased risk (p < 0.05). Multivariate analysis using the same regression model yielded significance for prednisolone treatment (Hazard ratio, 0.25; 95% CI, 0.11-0.57), AChR antibody seropositivity (HR, 5.17; 95% CI, 1.55-17.3) and thymoma (HR, 2.23; 95% CI, 1.15-0.57).

In conclusion, the proportion of ocular MG patients is higher in South Korea, but the rate of secondary generalization seems to be lower than reported in Caucasians, indicating that there are ethnic differences in disease manifestation and progression. AChR antibody and its level, abnormality in RNST, and thymoma appear to be associated with increased risk of secondary generalization. Our results are consistent with smaller retrospective studies and suggest that patients with ocular MG at presentation could benefit from early corticosteroid treatment, which decreased the risk of secondary generalization by 75% in our population.

67. Recalculated Modulating Anti-AChR Antibodies are Appropriate Index of Myasthenic Crisis

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Modulating antibodies of myasthenia gravis (MG) have the mechanism of acetylcholine receptor (AChR) degradation at the muscle cell surface. In this study, we purified the IgG from 15 MG sera, and measured the binding type of anti-AChR antibody (binding antibody of purified IgG) and percent of modulation (modulating antibody) using TE671 cell line. Also we measured the binding antibody in sera from these patients.

We recalculated the modulating antibodies from two viewpoints. First was potency of modulating antibody in sera that was calculated by percent of modulation x (binding antibody in sera / binding antibody of purified IgG), and second was index of modulating antibody that was calculated by percent of modulation / logarithm of binding antibody of purified IgG. Although titers of binding antibody in sera, binding antibody of purified IgG and percent modulation did not relate to the severity of the disease, all of 3 patients of ocular type MG showed the low point of the index. In addition, 3 patients out of 4 MG who showed the high point of the potency fell into myasthenic crisis during the course of the disease. This result indicates that we might predict the crisis of MG in patients who showed the high point of the potency of modulating antibody in sera.

68. IgG Subclasses and Complement Activation in Seronegative Myasthenia

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We have demonstrated the presence of antibodies to clustered acetylcholine receptors (AChRs), on transfected cell lines, in previously “seronegative” myasthenia gravis (SNMG) patients (see Leite et al., this meeting). This study was undertaken to characterize the IgG subclasses of these antibodies and look for in-vitro complement activation.

Human non-muscle cell lines were transfected with AChRs and clustered using the intracellular protein rapsym. The cells were incubated with SNMG or AChR positive patient sera and the IgG subclasses determined using monoclonal fluorescent-labeled secondary antibodies. Complement activation was demonstrated using antibodies to C3c. All the AChR antibodies were predominantly of the IgG1 subclass. The serum antibodies activated the classical complement pathway as demonstrated by the deposition of activated C3c on the cell surface, colocalizing with the AChRs.

This study shows that the AChR antibodies, which we have recently described in seronegative myasthenia, are predominantly of the IgG1 subclass and they may contribute to the pathogenesis of SNMG by activating the classical complement pathway.

69. Intensive Therapy Unit Admissions in MG: Why the Fall?
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Greater Manchester, U.K. has a population of approximately 3 million. In 1998 a regional myasthenia clinic was established. Between 1998 and 2004 there was an 80% drop in the number of patients admitted to ITU with myasthenic crisis unassociated with thymectomy. The characteristics of the 23 patients admitted to the intensive therapy units was compared with a cohort of 61 patients attending the clinic in 2004 who had not required ITU admission.

The mean interval between diagnosis and ITU admission was 3½ years. None of the patients admitted to ITU had been receiving immunosuppressive therapies other than prednisolone. Two-thirds of the control group had been receiving azathioprine or mycophenolate in addition to prednisolone. Approximately half of the admissions to ITU were triggered by infection, usually pulmonary. In one third of cases no cause for deterioration could be identified. Our data is consistent with the view that ITU admission can be prevented by immunosuppressive therapies, possibly by preventing the patient’s myasthenia from deteriorating in response to infection or other factors that may influence the immune system or neuromuscular physiology. This supports our current policy of tending towards the early introduction of immunosuppressive agents in patients with myasthenia requiring steroid therapy, even if their recently diagnosed myasthenia appears limited to the ocular muscles.

70. Lambert-Eaton Syndrome (LES) Findings in a Large Cohort
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LES is a rare, autoimmune neuromuscular junction disorder. Clinical and electrophysiological findings of all LES patients evaluated in a university myasthenia gravis clinic were analyzed.

LES diagnosis was based on proximal weakness, autonomic symptoms, hyporeflexia and electrophysiological evidence for abnormal neuromuscular transmission. P/Q-type voltage-gated calcium channel antibodies (VGCC) were measured in 56 patients. 3 Hz repetitive nerve stimulation (RNS) was performed in 76 patients in abductor digitii mani (ADM), abductor pollicis brevis (APB), and extensor digitorum brevis (EDB), and in trapezius in 44 patients. From 1980-2007, 99 patients were diagnosed with LES. Men comprised 48%, and malignancies were seen in 41% (CA-LES). All malignancies were lung primaries with 85% small cell pathology. CA-LES patients were 54% men versus 43% NCA-LES. Mean onset CA-LES was 63.4 years, NCA-LES 48.2 years. VGCC occurred in 78% CA-LES and 76% NCA-LES. Compound muscle action potential (CMAP) amplitude was usually reduced in all muscles, most often ADM (95%). RNS decrement exceeded 10% in ≥ one hand muscle in 99%. Post-exercise facilitation (PEF) was >100% in ADM, APB, and EDB in 37%, but occurred in at least one muscle in 80%. In a large LES cohort, less than half had lung cancer, usually small cell carcinoma, and about one quarter were seronegative. Nearly all had decrementing responses to 3Hz RNS in at least one hand muscle, and CMAP amplitudes were reduced in most tested muscles. The sensitivity of PEF was increased by testing additional hand and foot muscles.

71. Down Regulation of COLQ by RNA Interference as an Alternative Therapy in Myasthenia
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AChE is localised and concentrated at the NMJ by the anchoring protein ColQ. It functions to end signal transmission by cleaving the neurotransmitter ACh; either before it reaches the AChR or as it dissociates. Disorders of the NMJ may be either genetic or autoimmune, and often result in a loss of signal reaching the muscle. AChE is the target for one current therapy in myasthenia. AChE inhibitors aim to increase the amount of time ACh is present at the NMJ, enhancing neuromuscular transmission.

The aim is to increase neuromuscular transmission using siRNAs targeting ColQ mRNA. Partial loss of the ColQ-associated forms of AChE will enhance neuromuscular transmission.

A reporter construct was produced by fusing ColQ cDNA to EGFP, allowing ColQ expression to be followed by fluorescence. The activity of siRNAs targeting ColQ mRNA was investigated in HEK-TSA cells. Cells were transiently transfected with siRNAs and ColQ-EGFP. The activity was followed by fluorescence, western blotting and real-time PCR. ColQ (without EGFP) has also been expressed along with AChE in HEK-TSA cells allowing the effect on ColQ-associated forms of AChE to be followed using sucrose density gradients and Ellman AChE assays.

Three siRNAs were designed against ColQ mRNA, targeting different positions along its length. Downregulation of ColQ mRNA was efficient using two siRNAs. In their presence fluorescence was reduced to 15% of controls. Results from western blotts show an almost complete loss of ColQ expression. Real-time PCR reveals a loss of mRNA levels to 30% of controls.

siRNAs against ColQ mRNA effectively downregulate expression in HEK-TSA cells. Our future work will look at their effects in the AChR deficient mouse model of myasthenia.

72. Lambert Eaton Myasthenic Syndrome (LEMS) in a Patient with MG

Ferah Kizilay, Angela Young Aehong, M.D., Gwen Claussen, Shin j. Oh, Neurology Dept., The University of Alabama at Birmingham, Birmingham, AL.

Myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS) are two autoimmune diseases which affect the neuromuscular junction. MG and LEMS overlap syndrome have been reported in a few cases. We report another case of this syndrome.

A 51-year-old woman was diagnosed with MG when she was 26 years old. Her initial symptoms were ptosis, diplopia and weakness, especially in her legs. Tensilon test was positive. SFEMG was abnormal. Two repetitive nerve stimulation (RNS) tests showed the classical pattern of MG [normal CMAP, 16% decrement at low rate stimulation (LRS), and 0% response at high rate stimulation (HRS)]. Acetylcholine receptor antibody (AChR Ab) was repeatedly negative. She subsequently was treated with prednisone, thymectomy, immunosuppressive therapy, IVIG, and plasma exchange over the years. Her course was relatively stable for 15 years. In 2002, at 46 years of age, she was re-evaluated because of non-responsiveness to treatment and found to have areflexia and post-exercise facilitation in reflex and muscle strength. Repeat RNS showed a classical LEMS pattern (4mV CMAP amplitude, 36% decrement at LRS, 248% increment at HRS). P/Q VGCC antibody was positive. She was started on prednisone, guanidine and pyridostigmine with improvement.

This case raises a question as to whether this patient had MG initially or LEMS from the beginning. On the basis of present diagnostic criteria, she had seronegative MG initially and LEMS subsequently. This case suggests that if a patient with MG has no response to therapies, one should suspect the possibility of LEMS, and the RNS test should be repeated.

73. Autoantibodies in Patients with Small Cell Carcinoma with and without LEMS

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The Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disorder of the neuromuscular junction in which over 90% of patients have autoantibodies to the P/Q-type voltage-gated calcium channel (VGCC). Approximately 40% of these patients also have antibodies to another VGCC subtype, namely the N-type VGCC. Approximately 60% of LEMS patients have an associated small-cell lung carcinoma (SCLC) and it is believed that functional expression of VGCC on the surface of the tumour is central in instigating the disease in the paraneoplastic form of LEMS. However antibodies to VGCC and other autoantibodies have also been found in a small subgroup of SCLC patients without the clinical symptoms of LEMS.

In this study we have investigated the frequency and characteristics of the P/Q- and N-type VGCC and anti-neuronal antibodies in 100 consecutive patients with SCLC with and without neurological symptoms. Anti-VGCC antibodies were detected in 6 patients, 2 of whom had clinical signs of LEMS. Additionally antibodies to Hu (3%), Ri (3%) amphiphysin (2%) and both Hu and Ri (1%) were also detected. The IgG
subclass distribution of the VGCC antibodies were determined and compared with nonparaneoplastic LEMS and LEMS patients with cerebellar ataxia.

74. Thiopurine S-Methyltransferase Gene Polymorphisms in MG Patients

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Pharmacogenetic studies DNA variations that can decrease therapeutic effects, or increase the side effects of drugs. Hereditary changes in thiopurine S-methyltransferase (TPMT) gene could determine a different response to azathioprine (AZA), a chemotherapy drug routinely used in several autoimmune disorders. In about 70% of MG patients AZA is effective as a single drug or in combination with steroids, but in the remaining 30% no response or hepatotoxicity, bone marrow depression, gastrointestinal and idiosyncratic intolerance are the main cause of AZA withdrawal. TPMT is a cytosolic enzyme that catalyzes the S-methylation of aromatic and heterocyclic sulphydryl compounds such as 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and AZA. The aim of our study was to assess the frequency of TPMT gene polymorphisms in MG patients.

We genotyped 101 MG patients (17 AZA intolerant or not responder) and 63 healthy blood donors. AZA functional effect was also tested in vitro on PBLs. The most frequent polymorphism in our patients (84.6%) was that located 101 bp upstream exon 4 (c.460G>A) and 3.5% was observed (c.460G>A) and 3.5% was observed (c.460G>A and c.719A>G). In vitro studies showed that this polymorphism determined no responsiveness to AZA, suggesting that it might alter the protein structure causing enzymatic hyperactivity. In intolerant patients the genotype *3A (c.460G>A and c.719A>G) was the most frequent polymorphism (6.7%), this result was slightly higher than that reported in the literature (4.4%); 6% showed genotype *3B (c.460G>A); 1.8% the genotype *3C (c.719A>G). Altogether our results support the importance of pharmacogenetic testing in clinical practice.

75. Comparison of Single Fiber EMG Studies to Outcome Measures in a Controlled Study of Mycophenolate Mofetil (MMF) in Generalized MG

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We compared jitter measurements (mean MCD, % blocking fibers, % normal fibers) to the concomitant primary outcome measure Quantitative MG score (QMG), and secondary outcome measures Manual Muscle test (MMT) and Activities of Daily Living scale (ADL) in a prospective, multi-center, double-blinded study in which patients received 20 mg prednisone daily and either placebo or MMF 1250 mg BID for 12 weeks, followed by open-label MMF for 24 weeks. SFEMG studies were performed at baseline and 12 weeks in 18 of 80 study subjects and also at 36 weeks in 11 subjects. Using Pearson’s correlation coefficient MCD significantly correlated (p < 0.05) with QMG (r = .30), MMT (r = .45) but not with ADL. The following changes from baseline at weeks 12 & 36 were also correlated (p < 0.05): % change QMG vs % change MCD (r = .47), % change QMG vs change in % normal fibers (r = -.053), % change QMG vs change in % blocking fibers (r = .41), % change in MMT vs % change MCD (r = .58), % change in MMT vs change in % normal fibers (r = -.54), % change in MMT vs change in % blocking fibers (r = .48), % change ADL vs % change MCD (r = .45), and % change ADL vs change in % normal fibers (r = -.53). Conclusions: Changes in jitter measurements significantly correlated with change in QMG, MMT and less so for ADL. These findings indicate that changes in SFEMG jitter values may be useful as an outcome measure in clinical trials of MG.

76. Clinical Response to Rituximab and Long-Term Follow-Up in Treatment-Refractory MG

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We report the results and follow-up of six patients with severe generalized MG who failed to respond to escalating treatment including immunosuppression, plasmapheresis or immuno-adsorption. Three
patients each had antibodies to the acetylcholine receptor (AChR) or the muscle specific tyrosine kinase (MuSK). Patients received a standard course of rituximab (MabThera® 375 mg/m² weekly for four weeks) which was well tolerated. After the first dose CD20+ B-cells dropped and disappeared from circulation for at least 6 months. Serial determination of antibodies to AChR or MuSK showed a lasting reduction of the pretreatment level over at least 9 months. The clinical response was more pronounced in our patients with antibodies to MuSK than in those with antibodies to AChR. Under maintenance of conventional immunosuppression all patients remained in clinical remission according to the QMG-Score and the MGFA postinterventional status for up to 4 years and reported minimal disability.

The depletion of B lymphocytes by rituximab is an off-label treatment option which merits formal clinical investigation and may be considered at specialized centres in patients with severe generalized myasthenia gravis who do not respond to the standard treatment regimen.

77. Prednisolone Treatment Compensates Dysfunctional Regulatory T Cells in Patients with MG

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CD4+CD25hi regulatory T cells (Treg) play a decisive role in the maintenance of peripheral tolerance, and a defect in the suppressive function of these cells has been described in several autoimmune diseases, including myasthenia gravis. Interestingly, patients treated with steroids (prednisolone) showed a much improved regulatory T cell function, although a direct effect of this drug on the expression of the transcription factor FOXP3 (a marker for Tregs) or the suppressive function of regulatory T cells in vitro assays could not be proved. By contrast, prednisolone seems to act on dendritic cells, preventing the upregulation of costimulatory molecules (CD80 and CD86) upon maturation induction, stimulating the production of high amounts of immunomodulatory cytokines such as IL-10 and TGFß, and reducing the production of pro-inflammatory cytokines such as IL-12. A similar effect has been observed when treating monocyte-derived or CD1c+ dendritic cells with the corticosteroids hydrocortisone and dexamethasone, but not with the sex hormone estradiol. We propose that corticosteroid hormones act on antigen presenting cells and render them tolerogenic, potentiating a regulatory network either through cytokines or by giving rise to induced regulatory subsets which are beneficial in the treatment of autoimmune disease.

78. Electromyographic Decrement with Very Slow Stimulation Frequency in Two Children with Congenital Arthrogryposis Multiplex

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Two children were born with congenital arthrogryposis multiplex in two unrelated families. Both parent pairs had already lost a previous child with the same condition, compatible with autosomal recessive inheritance. The children were severely hypotonic at birth and needed artificial ventilation. EMG with repetitive nerve stimulation showed a very pronounced disturbance of neuromuscular transmission. In the first patient, there was a 80% amplitude reduction at 3 Hz, and still a 20% reduction at 0.2 Hz. In the second patient, the amplitude reduction was 70% at 3 Hz and 10% at 0.3 Hz. The abnormalities were confirmed in 2 different muscles for each patient.

There was no increment at fast stimulation frequency. Anti-acetylcholine-receptor-antibodies were absent. In view of the family history the diagnosis was felt to be congenital myasthenia. Unfortunately, the patients did not respond to intravenous neostigmine and died after 3 and 6 weeks of life. Motor endplates of quadriceps and psoas muscles of the first patient showed at the ultrastructural level smallness or sprouting of some nerve terminals while infoldings of the postsynaptic membrane were almost absent. Molecular genetic analysis is pending. To our knowledge, a decrement at stimulation frequencies of 0.2 or 0.3 Hz has not been previously described in congenital myasthenia.

79. Improvement with Plasmapheresis in LES After 17 Years Disease Duration

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In 1988 a 39 year-old female patient was diagnosed with Lambert-Eaton myasthenic syndrome based on proximal weakness and EMG findings. Despite repeat CT-scans no tumor could be found. Voltage gated calcium channel antibodies were undetectable. The
patient had a symptomatic treatment with guanidine 250 mg tid and remained relatively stable for many years. A switch from guanidine to 3,4-diaminopyridine was not tolerated and a course with azathioprine was stopped early because of gastric intolerance. After gradual deterioration the patient became wheelchair bound for most daily activities and a treatment with plasmapheresis was offered in September 2005. EMG of the ADM before the plasmapheresis showed a CMAP amplitude of 2.8 mV, a 27% decrement at 3 Hz and a 200% increment. On the day of the last plasmapheresis CMAP amplitude was 4.1 mV, decrement 20% and increment 125%. Two weeks later, CMAP amplitude was 11.2 mV with a 5% decrement and a 10% increment. One month later, there was no decrement or increment left. There was a parallel clinical improvement: the patient could walk several hundreds of meters and was able to climb stairs. Despite complementary treatment with oral corticosteroids, there was a new gradual decline in December 2005. Cellcept was added in February 2006. The patient developed a severe panniculitis with extensive wounds on the legs and died in June 2006. No tumor was found at autopsy. The course of this patient indicates that the pathology of Lambert-Eaton myasthenic syndrome remains reversible with plasmapheresis even after 17 years disease duration.

80. Clinical Findings in MuSK-Antibody Positive MG

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Several features characteristic of MuSK-Ab positive MG are reported. These include female predominance, prominent bulbar involvement, and greater disease severity. The objective of this study is to report the clinical and electrophysiological features and treatment outcomes of 55 U.S.-based myasthenia gravis (MG) patients with antibodies to muscle-specific tyrosine kinase (MuSK-Ab). Fifty-three MuSK-Ab positive MG patients were retrospectively identified from nine university-based clinics in the U.S. Of 53 cases, 38 were Caucasian; 45 were women (85%). Age of onset was 9 to 79 years, and in 26 cases was before 35 years. At presentation, 26 (49%) were Myasthenia Gravis Foundation of America Class III or IV. Twenty-four patients experienced myasthenic crisis. Tension testing was positive in 21 of 32 (66%) tested cases. Repetitive nerve stimulation (RNS) was abnormal in 39 of 47 (83%) cases and in 31 of 39 (79%) facial muscle recordings. Single fiber EMG on the EDC was abnormal in 19 of 21 (90%). Non-responsiveness to anticholinesterase therapy was observed in 27 patients; only eight had a good response to these agents. A good clinical response was seen in 27 of 46 patients on corticosteroids, 18 of 38 on immunosuppressive agents, 5 of 25 on intravenous gammaglobulin (IVIG), and 17 of 33 with plasma exchange. Thymectomy was considered beneficial in 7 of 18 (25%) patients at three years. Treatment outcome was very favorable in 85% of cases with two patients achieving complete stable remission, seven pharmacologic remission, and 30 minimal manifestation status.

81. Correlation of Outcome Measures and Static Fatigue Testing from a Controlled Study of Mycophenolate Mofetil in Generalized MG

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Fatigue is a clinical manifestation of skeletal muscle weakness in myasthenia gravis (MG). Quantification of the fatigue in MG during isometric exercise may be a useful measurement in clinical research trials. Static Fatigue testing (SFT) was performed as part of the prospective, multi-center, double blinded controlled mycophenolate mofetil (MMF) trial for shoulder abduction (SABD), elbow flexion (EF), elbow extension (EE) and hand grip (HG). This trial was performed to determine if MMF in combination with prednisone provided better control of MG than prednisone alone. The primary outcome measure was the Quantitative MG score QMG score at 3 months. Secondary outcome measures included the MG Activity of Daily Living Profile (MG-ADL), MG Manual...
Muscle Test (MMT) and SFT \{[(Epoch 2-Epoch 4)/Epoch 4]x100\}. Epoch 2 is defined as the time frame from 2-7 seconds and Epoch 4 is defined as the time frame from 25-30 seconds. The measures were collected at baseline and weeks 4, 8 and 12. 8 patients completed the study at our center. The age of the subjects included 28-83 (63±18). Spearman correlation coefficient was used to compare the measures. At baseline, there were no significant correlations seen between SFT (SABD, EF, EE, HG) and QMG scores or MMT. There were no significant correlations between the QMG, MMT and MG-ADL. There was good correlation between MG-ADL and SFT SABD (r = 0.75, p = 0.03). At 4 weeks, the QMG score correlated well with HG SFT (r = 0.89, p = 0.002). The MMT also correlated well with MG-ADL (r = 0.92, p = 0.001). At 8 weeks, there were no significant correlations between any of the outcome measures. At 12 weeks, the QMG scores correlated well with the SFT HG (r = 0.72, p = 0.02) and the MMT correlated well with MG-ADL (r = 0.76, p = 0.02). Although QMG continues to be the primary outcome measure recommended in MG therapy trials, other outcome measures such as SFT can add additional information.

82. MuSK MG in Greece
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Myasthenia gravis (MG) is an autoimmune disease which affects the neuromuscular junction of the skeletal muscles, usually caused by antibodies against the acetylcholine receptor (AChR). In about the 20% of patients with generalized MG such antibodies are not detected (seronegative MG) while in about 20-40% of these seronegative MG patients serum autoantibodies against the muscle specific receptor tyrosine kinase (MuSK) are identified.

In the present work, we studied the epidemiological characteristics of MuSK-MG in Greece. A population based study was carried out and 42 MuSK-positive patients were identified, with first symptoms appearing within the period from 1 January 1986 to 30 June 2006. The average annual incidence was 1.24/million population/year. The point prevalence rate was 3.84/million. The women:men incidence ratio was 4:1 and the prevalence ratio was 5:1. The average age at onset was 46.48 years for the women and 47.52 for the men, respectively.

In a set of 15 MuSK-positive sera tested, the by far predominant Ig subclass of the anti-MuSK antibodies was IgG4. In fact, small percentages of binding to other Ig subclasses seemed to be IgG4 antibodies crossreactive with the other anti-subclass anti-antibodies. None of the sera from 590 AChR-positive MG patients was MuSK-positive.

83. MuSK Myasthenia: A Case of Misleading Electrodiagnostic Findings and Antibody Testing
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A 49-yo man presented in 6/2001 with facial weakness, dysphagia and hoarseness. Examination revealed facial, tongue and sternocleidomastoid muscle weakness, with normal limb strength. AChR and striated muscle antibodies were negative. Repetitive stimulation studies at 3 Hz of the median/ABP, axillary/deltoid, spinal accessory/trapezius and facial/orbicularis oculi were normal. Pyridostigmine was prescribed with no benefit. EMG in 11/2001 revealed abnormal spontaneous activity in cranial, limb and thoracic paraspinal muscles with normal motor unit configurations. There was concern that this represented a diffuse lower motor neuron process such as motor neuron disease. He was followed at an outside facility for 3 years and had variable dysphagia with a gastrostomy tube inserted and later removed. He was seen again in 2/2005 and had weakness of facial, tongue and neck flexor muscles. EMG of the deltoid muscle revealed low amplitude, short duration motor units with early recruitment. CPK was mildly elevated (244, normal<195). Genetic testing for oculopharyngeal muscular dystrophy was negative. Biopsy of the deltoid revealed denervation atrophy. MuSK antibody testing (8/2005, Athena) was borderline. Ophthalmologic evaluation revealed cataracts. Genetic testing for myotonic dystrophy type 1 and 2 was negative. In 6/2006, mild (L) eyelid ptosis was noted and repetitive stimulation of the median/APB showed abnormal decrement (>10%) with 3Hz stimulation. Prednisone 20mg daily was started with dramatic improvement in his speech and improved cranial muscle strength. This case illustrates the propensity for MuSK myasthenia to affect the bulbar muscles. EMG may be misleading with spontaneous activity and “myopathic” motor units. The electrodiagnostic and clinical findings may be confused with muscular dystrophy, inflammatory myopathy or motor neuron disease. Repetitive stimulation (and single fiber EMG) may be normal in MuSk myasthenia,
even in weak muscles. In the proper clinical context, a “borderline” or “equivocal” test for MuSK should not preclude treatment with immunomodulatory agents.

84. MG Early Treatment Trial Proposal
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The role of immunosuppressive treatment of MG other than for relatively short term amelioration of symptoms is unknown. Whether immunosuppressive therapy has any effect on the natural history of the disease after cessation of treatment, or whether it merely suppresses the condition while continued and for an uncertain period of time thereafter, is unknown, but vitally important. If there is no long term effect, MG can be treated as needs be for symptoms, but if there is an improvement in the natural history then aggressive early treatment of MG would be indicated. Retrospective data from patients presenting with ocular MG (OMG) suggests significantly fewer immunosuppressed patients go on to have generalized MG (GMG) at one year. In our study of 262 patients with OMG, at one year of presentation 15 (10%) who had not received immunotherapy and 60 (52%) who had remained ocular. In contrast, those patients that had generalized, 132 (90%) had not received immunotherapy and 55 (48%) had received immunotherapy. However, the most patients remain on treatment at one year thus not discriminating ongoing suppression of MG from alteration of the natural history. A placebo controlled RCT of AChR+ OMG and grade IIa GMG where therapy is not obligated for symptoms is proposed. A one year course of prednisone or placebo is given. Pyridostigmine is available openly. End points are at two years from commencement and include (1) The number of patients in full remission MM-0 status (2) The number of patients with either a deterioration in MGFA Grade or a requirement for open label prednisone to control such a deterioration. At a power of 0.8 and alpha 0.05 the calculated number needed is 97 per arm; allowing 50% safety = 300. This simple trial could be readily performed by an international collaboration and the results would significantly impact on current treatment.

85. Programmed Death-1 (PD-1) in Human Autoimmune MG: A Gene to Protein Analysis
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The co-stimulatory molecule, PD-1 delivers negative signals to T, B cells and monocytes through its ligands PDL1/PDL2. Therefore, PD-1 is pivotal in peripheral tolerance and considered to be a key factor for terminating autoimmunity. Our study focused on analyzing PD-1 at genetic level, at transcript level and at protein level in human Myasthenia Gravis (MG). At genetic level, 4 SNP’s were selected in exon and intron regions of PD-1 gene. Totally, 274 MG patients and 275 normal controls were genotyped by Taqman–Allelic discrimination and RFLP. At transcript level, quantification of mRNA was performed by ABI real-time PCR in 61 MG patients and 45 normal controls. At protein level, cell surface expression of PD-1 on CD4+ T cells and CD8+ T cells and its ligands PDL1/PDL2 on CD19+ B cells and CD14+ monocytes were analyzed in PBMC of 19 MG patients and 15 normal controls. In result, at genetic level, no association of PD-1 gene was found in MG patients. Upon thymic classification of MG patients, the unthymectomized group had a trend for higher frequency of A allele in intron-4. At transcript levels, no significant difference in quantity of PD-1 mRNA, between MG patients and normal control, was found. At protein level, we demonstrated increased cell surface expression of PD-1 and PDL-1 on CD4+ T cells and CD14+ monocytes, respectively, among MG patients. Also, elevated expressions of PD-1 were found in males and the unthymectomized group of patients. In conclusion, increased expression of PD-1 and PDL-1 might reflect natural regulatory mechanisms behind autoimmune MG.

86. The Duke MG Clinic Registry – A Disease-Specific Database Program
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The Duke MG Data Registry is a computer database that has been used to collect and maintain selected information from Duke MG Clinic patients since 1980. A printout from the database summarizing the patient’s history and treatments is generated at each clinic visit and becomes part of the patient’s medical record. Information in the database is updated after each visit. The clinical utility of this reporting function has been a major factor in assuring data entry compliance.

Patients sign informed consent for their data to be kept in the Registry and potentially used for research studies. When Registry information is to be used for research studies, an IRB application specific to these
studies is submitted and data extracted from the Registry for such studies are de-identified. The MG Registry database files are stored on a secure computer on a local area network and can be accessed only by authorized personnel. Security of the database and compliance with HIPAA requirements are certified by the network administrator.

The MG Registry database currently contains information on more than 12,000 clinic visits by more than 1,500 patients seen at Duke University Medical Center since 1980, including over 1,000 patients with acquired MG. Data from the Registry have been the basis for case reports and publications on the epidemiology, diagnosis and treatment of MG.

The structure of the Registry database can be shared with MG Clinics at other medical centers with the goal of collecting comparable data from many sources to compare, among other things, outcomes of different treatment approaches.

87. MuSK-Antibody MG (MMG): Response to Various Treatments in 32 Patients

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Methods: Anti-MuSK antibodies have been found in 32 of 91 patients (35%) with generalized, AChR-Ab negative MG seen in the Duke MG Clinic since January 2001. Treatment was determined by their treating physicians. The MG-Post-Intervention Status (PIS) and MG-Clinical Class were recorded at each clinic visit. The response to each treatment modality was determined by review of clinical records.

Results: Treatment responses are given as number of patients improved/number receiving an adequate trial of each treatment. Edrophonium – 9/16. Pyridostigmine – 16/30 (2 became worse and 6 had profuse fasciculations.) Prednisone (as sole treatment) – 17/22. Azathioprine – 7/13 (5/7 with concomitant prednisone). Cyclosporine – 8/9 (6/6 with prednisone). Mycophenolate mofetil (MMF) – 17/20 (10/10 with prednisone). One patient improved after receiving rituximab while taking concomitant prednisone and MMF. Plasma exchange (PLEX) – 21/24 (usually rapid, dramatic improvement.) IVig – 5/10. 16 had thymectomy - all received immunotherapy before and after surgery. Current PIS: complete stable remission – 1; pharmacologic remission – 3; minimal manifestations – 10; improved – 16; unchanged – 1; 1 has not received immunotherapy.

Conclusions: MMG patients frequently did not improve with cholinesterase inhibitors; some actually became worse or had profuse fasciculations. Most im-

proved with corticosteroids. All but three improved after PLEX, usually rapidly and dramatically. With selected immunosuppression, most achieved and maintain a good response. The effect of thymectomy in MMG remains uncertain.

88. Neoadjuvant Treatment of Primary Unresectable Tymoma with Octreo-Tide Acetate in Microspheres (Sandostatin® LAR®) Reduces Tumor Size Up to Operability

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Long term survival of thymoma patients with and without paraneoplastic myasthenia gravis is often limited by incomplete resectability of the tumor, because RO resection, the most reliable prognostic factor for longtime survival, is no not possible, due to infiltration or close adherence to lung, arch of the aorta, pulmonary vein or artery and cardiac structures. Nearly all thymoma (WHO-classification A, A/B, B 1-3) are positive for the somatostatin receptor (SSR) in the SSR-scintigraphy as well as in immunohistochemistry. In a pilot study neoadjuvant treatment of primary unresectable thymoma with octreotide acetate in microspheres leads to significant volume reduction of the tumor (up to 80%) in approximately 75% of the patients, so surgical removal of the tumor becomes possible, even RO resection. Morphology shows regressive changes including apoptosis and calcification. The exact mechanisms which lead to tumor necrosis mediated by blockade of the SSR by the somatostatin analog octreotide, till now is not known. A prospective, open label, single arm study with Sandostatin® LAR®, 30mg administered i.m. once every 2 weeks in combination with prednisone (0.6mg/kg) is started. The patients will be treated for 12 to 24 weeks. Primary objective is to show that Sandostatin® LAR®, 30mg is effective to induce a decrease in tumor volume of 20% at month 3 as compared to baseline. Secondary objective is to reach operability.

89. Single-Fiber EMG of the Frontalis and Orbicularis Oculi Muscles in MG: A Comparative Study

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Background: SFEMG is commonly done on the frontalis and/or orbicularis oculi (OO) muscles, which
are considered to be more sensitive than the limb muscles for the diagnosis of MG.

Objective: To determine the relative sensitivity of orbicularis oculi and frontalis muscles in patients with MG.

Methods: SFEMG was performed in both frontalis and OO muscles in a random order in 18 patients (nine seropositive) with MG who had ocular symptoms.

Results: Increased jitter was found in 17 of 18 OO (sensitivity: 94%) and 15 of 18 frontalis muscles (sensitivity: 83%). When defective neuromuscular transmission was detected in both muscles, the OO was found to be more severely affected in 12/18 (67%) and frontalis in 6/18 (33%) patients.

Conclusions: Both frontalis and orbicularis muscles are highly likely to show increased jitter in patients with MG though the OO muscle appears to be more sensitive and more severely involved in patients with ocular symptoms.

90. Focal MG in Humans – A Rare Entity
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In addition to the ocular and generalized forms, a “focal” form of myasthenia gravis occurs in animals but the focal form has been rarely, if ever reported in humans. The diagnosis in such rare cases can be problematic, particularly if the disease is seronegative and involves muscle groups that are infrequently studied in electrophysiologic laboratories.

We present two patients with seronegative MG who presented with isolated but persistent right sided ptosis for few years and underwent extensive work up for a number of neurological conditions. Their Tensilon tests were equivocal and repetitive nerve stimulation studies were normal in multiple muscles. MG had been ruled out based on normal SFEMG studies performed on limb and facial muscles on the left side, likely due right handedness of the electromyographer. Subsequent studies on the right frontalis and orbicularis oculi showed increased jitter and initiation of therapy for MG resulted in resolution of symptoms.

SFEMG is usually performed on right sided facial muscles, which are considered to be most sensitive for the diagnosis of MG patients, regardless of the site or muscles involved. Our two cases illustrate a “focal” or “localized” form of MG that an electromyographer should consider in patients who present with restricted left sided weakness. In such rare cases the diagnosis of MG should not be ruled out unless an affected muscle has been studied.

91. Seropositive MG in North Indian Population
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Background: Myasthenia gravis (MG) is an autoimmune disorder in which antibodies against postsynaptic nicotinic acetylcholine receptors (AChRs) are formed resulting in destruction of AChRs at neuromuscular junction (NMJ). The disease is heterogeneous with respect to age at onset and distribution of muscle weakness. MG typically involves ocular muscles in about 80-90% of patients and in about 70-80% of the patients complain of generalized weakness. Aims & Objectives: To assess the incidence and age distribution of seropositive AchR antibodies in samples of north Indian MG patients population from the Neurology Dept. of a tertiary care center.

Methods: Two hundred and fifty patients of MG attending neurology Dept. out patients’ clinic of a tertiary referral center from 2004 to 2006 were recruited in the study after obtaining written informed consent. The anti-nAChR antibody was measured by using radio receptor assay kit (IBL Immuno Biological Laboratories Hamburg, Germany) as per the manufacturer protocol. Acetylcholine receptor (labeled with 125I-α-Bgt) from human muscle was used as antigen in this radio receptor assay.

Results: Blood samples of two hundred and fifty patients with clinical diagnosis of MG were obtained. Out of 250 patients, 161(64.4%) were male and 89 (35.6%) were female (male/female, 1.8/1). The age of onset was 4 to 78 yrs. A total of 149 (≈ 60%) MG patients were seropositive myasthenia gravis (SPMG) with mean age (±SD) of 42.38 (±16.93) years. Among 101 (40.4%) MG patient who were seronegative myasthenia gravis (SNMG) the mean age (±SD) was 37.31 (±17.48) yrs. When we categorizes the age in to two groups (< 40yrs & >40 yrs) to check clinical variation we found that male above to 40 years of age were highly significant (p = 0.001). The association between age and two groups of AchR shows a significant difference (p = 0.023).

Conclusion: Results show that in our patient populations, 60% MG cases are SPMG, male MG patients predominated and were older than female MG patients.

92. Photophobia and Pseudophotophobia on MG Patients
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Abstracts
Patients with myasthenia gravis (MG) present some ocular symptoms such as ptosis, diplopia. Literally, intraocular muscles of MG patients are supposed to be normal. The other hand, some clinicians notice complaints of photophobia or pseudophotophobia, which are pain or unpleasant, irritating feeling caused by light stimulation. We examined clinical frequency of photophobia complaints on 23 MG patients (average 30.1 years old, M: F = 3:20) and 19 healthy volunteers (average 30.4 years old, M: F = 4:15) with questionnaire, and evaluated their light reflex with Irisorder C7364 (Hamamatsu Photonics, Japan), which is video monitoring pupillography. Thirty nine percent patients with MG have or experimented photophobia or pseudophotophobia. Only five percent of healthy volunteers have photophobia symptom. On light reflex examination with Irisorder, we assessed D1 = maximal pupil dilator before light stimulation, D2 = minimum pupil dilator after light stimulation, and rate of change (RC) = D1-D2/ D1. Group of MG patients with photophobia showed RC decrement after repetitive light stimulation, compared that healthy volunteers present increment of RC after repetitive stimulation. Decrement RC phenomenon on MG patients with photophobia could cause clinical symptom of photophobia, and be considered as fatigability of intraocular muscles. Photophobia and pseudophotophobia must be one symptom to discuss on MG patients, and intraocular muscles might be involved in MG.

94. Screening Patients with LEMS for Small Cell Lung Cancer: What to Do?
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In 50% of the patients with the Lambert-Eaton myasthenic syndrome (LEMS) a small cell lung carcinoma (SCLC) can be found. A significant clinical concern is the early detection of these tumors in newly diagnosed LEMS patients. In 51 of 98 patients with LEMS, diagnosed between 1990 and 2006 a SCLC was found. Median age at SCLC diagnosis was 60 years (39 to 77). All 51 tumor patients had a positive smoking history; 86% were still smoking at diagnosis. SCLC was found in 92% of our patients within 3 months and 98% within a year. In 47 patients no tumor was found during a median follow-up of 7.5 years (range 1.5 - 26).

Complete data about screening were available for 48 patients with LEMS and SCLC. In 40 patients (83%) a SCLC was found at first screening by X-ray in 20 patients (50%) and by CT-scan in 39 (98%). In one patient only PET-scan was positive. During follow-up a SCLC was found in 8 other patients, (median 3 months, range 1-42). In three patients SCLC was found by an additional CT-thorax. Two tumors were found by PET scanning. Three patients presented with cerebral metastases, of whom one had retrospectively a positive PET study.

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A decrement of the Compound Muscle Action Potential (CMAP) during low-frequency (1-5Hz) repetitive nerve stimulation (RNS) is a hallmark of both myasthenia gravis (MG) and the Lambert-Eaton myasthenic syndrome (LEMS). An increment at high-frequency RNS (> 10Hz) LEMS of >100% is seen in LEMS, but a lesser but abnormal increment occurs in MG. Finally, CMAP amplitude at rest is low in LEMS, but whether this also holds to a lesser degree for MG is unknown. How results relate to severity is imperfectly known. We retrospectively described patterns of abnormality in relation to severity (MGFA) in 111 RNS tests from 70 patients with AChR-positive MG and 52 tests from 29 LEMS patients. Amplitudes of all ten CMAPs in a train were recorded for the abductor digitii minimi, the nasal and the trapezoid muscle at 1, 3 and 5 Hz stimulation.

The median resting CMAP-amplitude of the hypothenar in MG in MGFA class 0 was 7.1mV, decreasing progressively with increasing MGFA class. MGFA IV showed a 25% decrease. In the nasal muscle a median resting CMAP of 1.6mV in MGFA 0 was found with a gradual decrease per class towards 60% in MGFA IV. In LEMS this result was even more pronounced with a decrease up to 70% in MGFA III for the hypothenar and over 90% in the nasal muscle. The resting CMAP decreases with disease severity. This suggests a long-term block in both LEMS and MG that may explain part of the weakness.
Concluding, in almost all patients (98%) the tumor was found within one year of diagnosis. One or more CT-thorax scans detected the tumor in 88% of the patients. A PET-scan may have additive value in selected cases. Routine brain imaging might be considered in cases with high clinical suspicion.

95. Correlation of Outcome Measures from a Controlled Study of Mycophenolate Mofetil in Generalized MG

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We recently completed a prospective, multi-center, double-blinded, controlled trial to determine if mycophenolate mofetil in combination with prednisone provided better control of MG signs and symptoms than prednisone alone. The primary efficacy measure was the change from baseline in the Quantitative MG score (QMG) at 3 months. Secondary outcome measures included the MG-Activity of Daily Living profile (MG-ADL) and MG Manual Muscle Test (MMT). The measures were collected at baseline and weeks 4, 8, and 12 in the blinded study and at weeks 16, 20, 28 and 36 in an optional open-label extension. Pearson’s correlation coefficient was used to compare the measures for all evaluations collected over the 9-month period. At baseline, the QMG for the 80 subjects displayed excellent correlation with the MG-ADL (r = 0.55, p < 0.01) and the MMT (r = 0.53, p < 0.01). When all 550 study assessments were included, correlation between the QMG and MG-ADL (r = 0.64, p < 0.01) and between the QMG and MMT (r = 0.62, p < 0.01) remained robust. Strong correlations also existed for the change in absolute scores and percentage change from baseline. We conclude that these familiar outcome measures correlate strongly with each other. Although the MGFA task force recommended use of the QMG in all prospective therapy trials in MG, the MMT and MG-ADL are suitable alternatives and offer potential advantages: no special training or equipment is required and they are less time-consuming.

96. A New Mouse Model of Autoimmune Ocular MG

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There is currently no good animal model of oMG or a model in which both oMG and gMG coexist. To study the potential for the H-AChRa subunit to induce MG, we immunized and boosted HLA-DQ8 transgenic, HLA-DR3 transgenic and B6 mice with an E. coli plasmid expressing the recombinant H-AChRa subunit in complete CFA. Immunized HLA-DQ8 transgenic, HLA-DR3 transgenic and C57BL6 mice developed ocular myasthenia gravis with varying disease incidence and severity. HLA-DR3 transgenic mice were relatively resistant to oMG induction. The differences were significant as evaluated by Fisher’s exact test (p < 0.0001 for HLA-DQ8 vs HLA-DR3 and p < 0.01 for B6 vs HLA-DR3). As compared to HLA-DR3 transgenic mice, HLA-DQ8 transgenic mice and B6 mice also had significantly higher serum anti-M-AChR Ab levels and increased amounts of NMJ C3, IgG and MAC deposits at extraocular and limb neuromuscular junctions and droopiness of eyelids. Our findings suggest that oMG pathogenesis in HLA-DQ8 transgenic and C57BL6 mice could be triggered by immunity to human AChR alpha subunit. The ocular signs and pathology in EOM and limb muscles induced by H-AChR alpha subunit immunization makes these HLA-class II transgenic and B6 mouse models ideal ones to study the role of various immune related cells, genes, and molecules in the development oMG and gMG.

97. Retrospective Study of MG in Wuhan, China

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Many MG patients in China present under the age of puberty with ocular MG, which differs from that in Western countries. Some patients have myasthenic crisis (MC) but there have been no long-time retrospective clinical studies of MC in patients from China.

We reviewed the medical files of 3352 patients diagnosed with MG admitted from 1971 to 2005. 2.89%
(97/3352) of MG patients developed MC, 20 of them had 2 episodes. Specific precipitating factors were infections, thymectomy and gentamicin injection. Mean age at MC presentation was 30.59 ± 16.45 years; however, there was a bimodal distribution with 17 patients between 0 and 5 and 30 patients between 25 and 35, only 2 of them were older than 60. The first symptom restricted to ocular muscles was present in 73.7% (14/19) of the children. Thymoma had been detected in 31 patients. There were no significant differences in the duration of MG before MC, the duration of MC, outcomes between thymectomized and non-thymectomized patients, or between patients with and without thymoma (p > 0.05). Over the last 34 years, 31(32%) MC patients died, 46 (47.4%) MC patients improved, and 20 were unchanged. We conclude that myasthenic crisis is not common in Chinese MG but can occur in children as well as adults and carries a high risk of mortality.

98. Association of Telomerase Activity in T Lymphocytes of Patients with MG
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This study is the first one addressing the association of telomerase activity of peripheral blood T lymphocytes with myasthenia gravis. The main objective of this study is to investigate the association of telomerase activity of peripheral blood T lymphocytes with myasthenia gravis.

Total 16 MG patients and 16 healthy controls were recruited. Telomerase activity of lymphocytes was determined by polymerase chain reaction-based enzyme immunoassay (PCR-ELISA) using telomerase repeat amplification protocol (TRAP). The positive rates of telomerase activity of peripheral blood CD4+T lymphocytes are 14/16 and 7/16 for MG patients and controls, respectively (Fisher exact p = 0.023). Mean telomerase activities of peripheral blood CD4+T lymphocytes are 0.44 and 0.21 for MG patients and controls, respectively (Wilcoxon’s p = 0.023). Mean telomerase activities of CD8+T lymphocytes are 0.09 and 0.16 for patients and controls, respectively (Wilcoxon’s p = 0.08).

This study showed that there exists a significant correlation between MG and the telomerase activity of peripheral blood CD4+T lymphocytes. This result also explains why the positive rate of telomerase activity of peripheral blood CD4+T lymphocytes in MG patients is significantly higher than the one in control group. However, no significant correlation was found between MG and the telomerase activity of peripheral blood CD8+T lymphocytes.

99. Recombinant Domains of AChR Subunits as Immunoabsorbsents for the Development of an Antigen-Specific MG Therapy
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The development and implementation of new therapeutic approaches for Myasthenia Gravis (MG) attains particular importance, since the number of identified MG patients increases considerably. So far, treatment of the symptoms is the only medical support to the MG patients, while the pathogenetic mechanisms of the disease are not adequately understood. The fact that the patients generate autoantibodies against the acetylcholine receptor (AChR) which seem to be the major pathogenic factor in MG, presents an attractive target for novel therapeutic approaches. We aim to develop an antigen-specific alternative to plasmapheresis using the binding affinity of the autoantibodies to the immobilized recombinant extracellular domains (ECDs) of human AChR. Therefore, we are exploiting a specific immunoabsorption approach, in order to selectively clear patient’s sera from the anti-AChR autoantibodies. We have expressed the ECDs of all human AChR subunits both in Pichia pastoris and in E. coli. These polypeptides have been immobilized on CNBr-Sepharose and used in immunoabsorption assays with MG sera. We were able to improve our system insofar as to achieve the almost complete depletion of anti-AChR antibodies from patient’s sera tested. The stability of the constructed ECD-columns, the high selectivity to the anti-AChR antibodies and the reproducibility of the assay support the quality and the value of the system. We conclude that these immunoadsorption columns should lead to the development of a new efficient antigen-specific therapy for MG.