



2007 Scientific Session of the Myasthenia Gravis Foundation
Robert L. Ruff, MD, PhD
MGFA Medical/Scientific Advisory Board

The annual scientific session of the Myasthenia Gravis Foundation of America (MGFA) was held on October 6, 2007 in Washington, DC at the Wardman Park Marriott Hotel. The session was organized by MGFA Medical/Scientific Advisory Board members Drs. Matthew N. Meriggioli and Gil Wolfe.

The meeting included discussion of the ongoing international clinical trial on thymectomy. There were nine presentations from around the world. Many of the presentations related to clinical treatment studies. Brief reviews of the role of the immune system in MG and seronegative MG are provided to enhance understanding of the presentations.

The Role of the Immune System in MG

MG is an auto-immune disease involving the site of communication between nerve and muscle, the neuromuscular junction. In MG, the body's immune system malfunctions and attacks specific proteins at the neuromuscular junction. The most common target of the autoimmune attack is the acetylcholine receptor (AChR). Lymphocytes (one type of "white blood cells" in your blood) are a class of immune cells that are divided into two large groups: Thymus-derived lymphocytes (T-cells) and B-cell lymphocytes. T-cells are involved in cell-mediated immune responses in which cells attack immune targets. B-cells are responsible for producing antibodies that target specific parts of proteins called epitopes. Cells that process and present potential immune targets (also called antigens) to the immune system are called antigen presenting cells (APCs). T-cells can modulate the activity of B-cells. MG is T-cell dependent antibody mediated disease in which T-cells modulate the activity of B-cells that produce antibodies that are directed primarily against the AChR and secondarily against other sites (epitopes) at the neuromuscular junction. The antibodies binding to the AChR trigger a complement-mediated cellular immune attack against the neuromuscular junction. Complement is an immune-mediated chemical cascade that destroys proteins and injures or destroys cells. Cytokines are proteins produced by the body that modulate the immune system. Interleukins (IL) are a class of cytokines that are secreted by lymphocytes. IL regulate the activities of different classes of immune system cells. Interferons (IFN) are a different class of immune regulatory proteins. DAF is a recently recognized factor that can alter the immune response seen in different tissues. Tolerance refers to a process in which an immune response to a specific agent is suppressed. One mechanism of tolerance is for T-cells to become insensitive to a disease-inciting antigen.

Triggering the Immune Response in Myasthenia Gravis

Authors: Robert H. Fairclough, Lan-Anh Nguyen and Chih-Te Wu (**MGFA Post-Doctoral Fellowship Awardee**). Department of Neurology, University of California at Davis

Why does the immune system target AChR in MG? What triggers the immune system to “forget” that the AChR is part of our body and not a foreign protein? These are key questions to understanding why MG develops and how to reverse the aberrant functioning of the immune system in MG. This paper examined a specific region of the AChR that lies within the part of the AChR called the Main Immunogenic Region (MIR), which is the part of the AChR where most antibodies bind. The specific region that was investigated was amino acids number 192 and 193 (amino acids are the building blocks that are linked together to form a protein). The amino acid at positions 192 and 193 is cysteine. Cysteine is an unusual amino acid because it contains sulfur. There are chemicals present in cells that react with sulfur. The idea of this study is that if the sulfurs in amino acids 192 and 193 are modified the AChR may be sufficiently changed so that the immune system no longer correctly recognizes the AChR. This study is still in progress and there are plans to evaluate human serum from people with MG to determine if antibodies bind more strongly to AChR that has been modified the amino acids containing sulfur.

Distinct Roles of Dendritic Cell and B Cell-mediated *in vivo* Antigen Presentation to Autoreactive CD4+ T cells in Experimental Autoimmune Myasthenia Gravis

Authors: Windy Allman (**MGFA Student Fellowship Awardee**), Shamsheer S. Saini, Huibin Qi, Jing Li and Premkumar Christadoss*. Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, TX 77555-1070

Dendritic cells are immune cells with long, tentacle-like branches called dendrites. Dendritic cells (DC), macrophages and B cells are capable of presenting antigen (serving as antigen presenting cells) and activating a subset of T cells called CD4+ T cells. Activation of AChR-specific CD4+ T cells provides signals to B cells to secrete antibodies that are able to activate complement. It is unknown which antigen presenting cell in experimental autoimmune myasthenia gravis (EAMG) activates AChR-specific T cells. To further characterize the role of antigen presenting cells, the authors induced MG by immunized mice with AChR emulsified in complete Freund's adjuvant. They isolated DCs and B cells at various times within the first week after immunization to evaluate their ability to activate purified CD4+ cells. Cells taken from animals that had been immunized against the AChR were called activated cells. Naïve B cells, obtained from rats that had not been immunized were poor APCs while naïve splenic DCs were able to induce CD4+ proliferation. In contrast, activated DCs had decreased ability to activate CD4+ T cells whereas activated B cells had increased ability to activate CD4+ T cells. These results suggest that B cells may amplify and perpetuate T cell responses in MG. The activation process leads to a proliferation of B cells which likely contributes to

the ability of B cells to sustain T cell activation in MG, whereas dendritic cells may play a larger role in the development or initiation of T cell activation in MG.

Immunoregulation of Experimental Myasthenia Gravis

Authors: Matthew N. Meriggioli*, Jian Rong Sheng (**MGFA Fellowship Awardee**), Liang Cheng Li, PhD, and Bellur S. Prabhakar, University of Illinois, Chicago, IL

Recently, dendritic cells (DCs) and regulatory T-cells (Tregs) have been shown to possess potent capabilities to tolerize T-cells in an antigen-specific manner. Strategies manipulating specific subsets of DCs and Tregs have shown promise in experimental models of T-cell mediated autoimmune disease. We have observed that the selective activation of particular subsets of DCs utilizing granulocyte-macrophage colony-stimulating factor (GM-CSF) suppressed the induction of experimental autoimmune myasthenia gravis (EAMG), and that GM-CSF treatment significantly ameliorated well-established, chronic EAMG, effectively suppressing anti-AChR T cell *and* pathogenic antibody responses. In response to the autoimmune attack against AChR in EAMG, there may be a compensatory increase in expression of acetylcholine receptor (AChR) protein in damaged muscle cells as if the muscle is trying to compensate for the autoimmune attack. The increased AChR expression is reduced in GM-CSF-treated animals. Interestingly, they also showed that this increased AChR expression appears to be aberrant in that the expressed protein does not form functional AChRs. These latter findings raise intriguing possibilities with regard to compensatory mechanisms that in MG and perhaps why the body is not able to fully recover after repeated attacks of MG. In addition, if the response to MG is to produce altered AChR proteins, the response may serve to perpetuate MG because the altered proteins may be immunogenic and continue to trigger the production of antibodies against AChR.

Myasthenia Gravis Triggered by Statins: Presentation of 11 Cases

Authors: Rugiero Marcelo MD, Mazia Claudio MD, Myasthenia Gravis Association of Argentina

This study reports the ability of a commonly used class of medications, statins, to induce MG in a small subset of people receiving these medications. Statins are lipid lowering agents, drugs that are used to treat elevated blood levels of cholesterol and triglycerides. Commonly used statins include: simvastatin, atorvastatin, fluvastatin, pravastatin and rosuvastatin. Side effects of statins include muscle weakness and pain. The authors presented 11 patients who developed MG after the onset of the treatment with a statin. The 11 patients developed MG between 1999 and 2005. The male to female ratio of the 11 subjects was 1:2.6. The mean age at the time of diagnosis was 65 years old (range 54-82 yrs). The authors do not know if the statins actually caused MG or the possible mechanism by which the statins may have induced the subjects to develop MG. The authors also do not know what fraction of the people in Argentina who received a statin developed MG.

Thymomatous MG: Paraneoplastic Dysautonomia and Small Fiber Neuropathy.

Authors: David S. Younger, New York University School of Medicine, Paul Magda and Lindsey Lair, St. Vincent's Catholic Medical Center, NY, NY; Shera Scott and Saika Sharmeen, Hunter College, NY, NY.

This presentation discussed a person who developed MG along with impaired function of the autonomic nervous system (the part of the nervous system that regulates blood pressure, heart rate, sweating, pupil size and bladder and bowel function) and damage to peripheral nerve fibers. The syndrome appeared to be triggered by tumor of the thymus gland (thymoma). The syndrome developed 5 months after the thymoma was removed. It appears as if the thymoma induced the production of antibodies that triggered the development of MG and other problems. Thymomas have been linked to MG. Usually the MG improves when the thymoma is removed. It is not clear why the symptom onset was delayed.

Clinical Studies about MG

Mycophenolate Mofetil in MG – It's Not the End of the Road Yet!

Author: Zaeem A. Siddiqi*, Division of Neurology, University of Alberta, Edmonton Canada

Recently, two of the largest trials conducted on the efficacy of Mycophenolate mofetil (MMF; Cellcept) in MG failed to show clinical efficacy of MMF as an adjunctive or steroid sparing agent in MG. These results carry significant implications for the MG patients who are already on MMF as well as for the treating physicians. Based on the results of the negative trials, MMF was discontinued in four patients who were in pharmacological remission on a combination regimen of low dose steroids and MMF. The steroid and pyridostigmine doses were kept unchanged during the observation period. In two out of the four patients myasthenic weakness relapsed within a few months of discontinuation of MMF. One patient had a moderately severe relapse that required rescue therapy with intravenous immunoglobulin, along with an increase in steroid dose and re-institution of a steroid sparing agent. The other has had a mild relapse after three months of withdrawal of MMF that has not required any major alteration in his medications so far. The third patient remains clinically unchanged six months after the discontinuation of MMF. Based on this small "withdrawal trial" of MMF it is possible that MMF may have a beneficial effect in certain MG patients.

Mycophenolate Treatment for Myasthenia Gravis

Authors: Sabine Mueller, Dena B Dubal, Andrew Josephson and John W. Engstrom*, Department of Neurology, University of California San Francisco, San Francisco CA 94143

Mycophenolate mofetil (MMF; Cellcept) is a widely used, oral immunosuppressive agent that is considered to have few adverse effects compared to similar drugs. The authors reported the case of a patient who developed a lymphoma-like cancer following chronic treatment with MMF and prednisone for myasthenia gravis. A 69 year-old woman with myasthenia gravis, on MMF and prednisone for six years, developed two lesions that began as ulcer-like sores and grew to several centimeters over two months. Concurrently, the patient lost ten pounds and experienced intermittent cough with low-grade fever. Her baseline symptoms of double vision, ptosis, and dysphagia due to myasthenia gravis were stable since beginning immunosuppressive treatment. Laboratory investigations revealed mild reduction in the blood lymphocyte counts. Biopsy revealed a lymphoproliferative (lymphoma-like) condition involving T cells. MMF was titrated off and replaced with pulse intravenous immunoglobulin therapy for management of myasthenia gravis. Chemotherapy was not instituted since the lesions regressed and resolved after four months following cessation of MMF. This case, combined with outcomes from other rare reports describing MMF-associated B-cell lymphomas, suggest that malignancy may be initially treated with drug cessation.

Background for presentation on Seronegative MG

Antibodies are proteins produced by the immune system that bind to and target substances for destruction by the immune system. Antibodies are designed to target foreign substances such as bacteria. In patients with MG, antibody production is disturbed and antibodies are produced against self (i.e., antibodies target normal body proteins). The most common and the major pathogenic autoantibodies in MG are directed against the AChR on skeletal muscle. Patients with antibodies against the AChR are considered to have seropositive MG. A fraction of patients with MG, about 20% of patients with MG, do not have anti-AChR antibodies. The MG in these patients is referred to as seronegative. Many people with seronegative MG have autoantibodies against other components at the neuromuscular junction. The autoantibodies cause additional problems for patients with MG. Serum from patients with seronegative MG can be injected into animals and cause a MG-like condition. Immunoglobulins from seronegative patients bind to muscle cells, but not to AChR. The most common target for antibodies in patients with seronegative MG appears to be a muscle protein called MuSK (muscle-specific receptor tyrosine kinase). MuSK is a muscle-specific protein that regulates how AChRs are incorporated into the neuromuscular junction. About 30-50% of seronegative MG patients have antibodies directed against MuSK. The seronegative patients who have antibodies against MuSK are sometimes referred to in studies as MuSK+ or MuSK-MG. For comparison, patients with antibodies against the AChR are sometimes referred to as having AChR-MG.

Myasthenia Gravis with Muscle Specific Tyrosine Kinase (MuSK) Receptor Antibodies Treated with Rituximab: Two Case Reports

Authors: Jonathan Goldstein*, Huned Patwa, Steven Novella, Jessica L. Johnson, New Haven, CT

Rituximab is a monoclonal antibody treatment for some lymphomas and autoimmune diseases, such as rheumatoid arthritis. Rituximab targets normal and malignant B-cell lymphocytes. MuSK+ seronegative MG may be resistant to typical MG treatments, and may have a particularly severe course. This study reported two patients who had MuSK+ MG treated with Rituximab. Rituximab treatment resulted in a reduction in the dose of immunosuppressants and/or the use of plasmapheresis in both patients. No adverse effects have occurred to date. While encouraging, more work needs to be done to determine if and how Rituximab should be used to treat seronegative MG or the usual form of MG.

MGTX Recruitment in a Surgical Clinical Trial

Authors: Gary Cutter, Birmingham, AL; Henry J. Kaminski* (**MGFA Post Doctoral Fellowship Awardee**), St. Louis, MO; Gil I. Wolfe* , Dallas, TX; Alfred Jaretzki III*, New York, NY; Chichi Aban, Birmingham, AL; Greg Minisman, Birmingham, AL; Robin Conwit, Bethesda, MD; John Newsom-Davis*, Oxford, UK

Thymectomy is the most commonly employed surgical treatment for MG. There has been an ongoing debate for many years about whether the procedure is effective in inducing remission of MG or reducing disease severity. MGTX is an NINDS (National Institute of Neurological Diseases and Stroke – a branch of NIH). cooperative multinational, multicenter, two-arm trial enrolling patients with non-thymomatous MG. The study aims to determine if extended transsternal thymectomy (ETTX) plus prednisone compared to prednisone alone results in a greater improvement in myasthenic weakness, a lower total dose of prednisone, and an enhanced quality of life by reducing adverse events and symptoms. The trial is ongoing, and eighty-five centers worldwide are currently participating or are being certified. This project has been in development since 2000. The MGFA is supporting a section of this study to evaluate pathology in the thymus and to compare pathology pattern with clinical state. Most of the study work is being funded by the NIH. MGFA provided early support to permit the investigators to get together to work out the initial version of the protocol. Note that people who have a thymoma need to have a thymectomy to remove the thymus tumor, which can compromise heart function and breathing. Therefore, people who have a thymoma should be considered for thymectomy independent of whether they have MG. This is an important study that will evaluate the benefits and risks of thymectomy and help to determine when this surgery should and should not be done. Additional information that will be examined are: 1) are there biomarkers (specific forms of proteins that can be detected by simple tests) that correlate with clinical course or response to treatment – this could help to determine the best treatment for an individual and 2) are there patterns of pathology in the removed thymus that can be used to predict disease severity and response to treatment. To date 55% of

those eligible to participate have agreed to participate. To date they have screened over 2750 patients; 98% are ineligible mainly due to age restrictions and disease duration. Among eligible subjects, leading reasons not to enroll are refusal to undergo thymectomy (57%), distaste for corticosteroids (17%), and family advising against participation (17%). Fourteen centers have now randomized a total of 27 patients, and the rate of enrollment is increasing as we incorporate newly certified centers. Individuals who have been diagnosed with MG in the past 3 years, who have not received steroid-sparing medications and who are willing to consider thymectomy as treatment for their MG should discuss participating in this study with their doctors.

A memorial tribute to Dr. John Newsom-Davis

Donald B. Sanders, MD*

At the end of the scientific session Donald B. Sanders provided a memorial tribute to Dr. John Newsom-Davis who recently died in a motor vehicle accident while traveling in Romania where he was trying to set up another study site for the thymectomy trial. Dr. Newsom-Davis was an enormous contributor to MG clinical treatment and related research.

* Member of MGFA's Medical/Scientific Advisory Board