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Dear Colleagues,

It is a great pleasure to introduce you to this new version of the newsletter re-looked by our colleagues Socrates Tzartos and his team. You will find new categories, such as scientific and medical news, questions dedicated to patients, or presentation of the teams participating to the network. It is also a pleasure to give you some information about the scientific network on myasthenia gravis "MYASTAID". Please send us your feedback on this newsletter to help improving it.

It's also time to think about our next EuroMyasthenia Meeting. The Cyprus MG Patients Association proposes to organize it in Cyprus, together with their meeting for patients and clinicians. It will be held in Nicosia on December 5 and 6, 2008.

Meanwhile, I hope you are all going to take some vacation to rest and regain a lot of energy for our next academic year.

Sonia Berrih-Aknin, Coordinator

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Presentation of the EuroMyasthenia network partners

We promised in the first Newsletter to present the Euromyasthenia participating groups. Starting from this issue we will present in each issue 1-2 research or clinical partners and 1-2 MG Associations. We are starting with the presentation of the Romanian MG Association and the partner from the Hellenic Pasteur Institute.

Romanian National Myasthenia Gravis Association

Str Gral Macarovici nr 7, s6 Bucharest, 060142 Romania (www.miastenie.ro)

Members of the board: President and founder: Nadia Radulescu, Vice-president and founder: Mihaela Baragan, Simona Dimian, Fund raiser and founder: Gabriela Nutu, Founder and volunteer: Camelia Mihai, Secretary: Adriana Harja

Brief history of the Association: **Asociatia Nationala Miastenia Gravis Romania** is a non profit, humanitarian organization founded in November 2005 by patients suffering from this disease. Our mission is to help myasthenic patients to improve their lives through programs of patient services, public information, medical research, professional education, advocacy and patient care.

In May 2008 the association had 70 active members, supporters and health care professionals who offer services to over 420 MG patients. The Directory Council in Bucharest coordinates all the activities from the national office. We have 6 local chapters and contact persons in the majority of the cities. We have no staff, all the workers are volunteers.

Activities and goals: The primary activity of our association is patient services, including provision of literature on the disorder and treatment, available through the national office and the website (www.miastenie.ro). We translated, published and disseminated the first Euromyasthenia brochure: "Myasthenia Gravis-information for patients and families". Other activities include support groups for patients and their families, psychological and legal counseling, help in purchasing medication and other medical supplies, programs for public information about the specific problems of the myasthenic people.

Problems: Since the main problem for MG patients in Romania is the chronic shortage of specific medication Mestinon®, the association's most important goal is to help patients with medication supply. We fight with the authorities to get the legal status for drug importation, build our own network for collecting information on medication's needs and have our own buffer stocks of medication from donations.

Collaborations with other organizations: We are collaborating partners at Euromyasthenia and one of the initiators of the future European MG Association. We collaborate with other organizations of neurological and rare diseases, organizations of people with disabilities and volunteers.

Funding: The money comes from private sponsorships, donations from the members or other persons and through the national campaign (2%).



From the left :Nadia Radulescu, Simona Dimian, Gabriela Nutu, Mihaela Baragan, Camelia Mihai

**Laboratory of Molecular Neurobiology and Immunology, Department of Biochemistry,
Hellenic Pasteur Institute,
127, Vas. Sofias ave., Athens, 11521 Greece (www.pasteur.gr)**

Members of the group:

Head of the lab: Socrates Tzartos (tzartos@pasteur.gr),
Researchers: Kalliopi Kostelidou, Konstantinos Poulas, Paraskevi Zisimopoulou.
Other permanent personnel: Anna Kokla (research assistant), Nikolaos Trakas (research associate), Elissavet Tzartou (secretary).
Post-docs: Petros Giastas, Konstantinos Lazaridis, Alexandros Sotiriadis.
PhD students: Kalliopi Bitzopoulou, Stamatina Koutroumpi, Eirini Papadaki, Christos Stergiou, Marios Zouridakis.



PATRAS-From left: K.Poulas, G.Kordas, S.Tzartos, D.Kalamida, A.Niarchos, M.Georgostathi and G.Lagoumintzis



ATHENS-From left (seated): S.Tzartos, E.Papadaki, K.Bitzopoulou, P.Zisimopoulou, K.Lazaridis, M.Zouridakis
From left (standing): N.Trakas, K.Kostelidou, A.Kokla, C.Stergiou, E.Tzartou, P.Giastas, A.Sotiriadis, S.Koutroumpi

We also have a sister lab (Laboratory of Molecular Biology and Immunology) at the Department of Pharmacy, University of Patras (200 Km from Athens).
Members: Socrates Tzartos, Konstantinos Poulas (Lecturer), Dimitra Kalamida and Giorgos Lagoumintzis (Post-docs), Asimina Georgostathi, Grigoris Kordas and Athanasios Niarchos (PhD students).

Description of research work related to MG: The major area of research in our laboratory is the main autoantigen in myasthenia gravis (MG), the nicotinic acetylcholine receptor (AChR) and its interactions with the pathogenic autoantibodies. The main aims of our group concern:

- The development of a novel, antigen-specific therapeutic approach against MG, which involves the selective removal of the pathogenic anti-AChR autoantibodies from the blood of MG patients, in contrast to plasmapheresis which unfortunately eliminates important components from patient's blood, as well. This is performed by the construction of "immunoabsorbent" columns, which consist of immobilized recombinant parts of the AChR in sepharose beads. The concept is that the patients' blood will pass through these columns, which selectively attract and remove the autoantibodies, while the "clean" blood will be returned to the patient. We hope that pilot clinical trials will start before long.
- The study of the pathogenicity of MG autoantibodies. Autoantibodies against various AChR subunits are isolated from MG sera and their pathogenicity is tested in laboratory animals in order to further understand the pathogenic mechanisms of MG.
- The structure of muscle and neuronal AChRs and their complexes with antibodies is another area of our interest, for further understanding of the pathophysiological roles of these AChRs.
- The diagnosis of MG and the development of a much more sensitive anti-AChR assay. Our laboratory is the diagnostic center for MG in Greece, with about 2000 diagnosed anti-AChR and 55 anti-MuSK patients.

Other involvements related to MG: We have strong interactions with the Greek MG patients' community and have recently assisted in creating the Hellenic MG Association hosted in our Institute, with great expectations. We also collaborate with the MDA-Hellas.

Participations in networks, organisations, etc: We participate in European networks, in addition to EuroMG including the Myastaid project (described in this Newsletter), and the large NeuroCypres network of FP7 devoted to the study of the structure and function of the AChR superfamily, as well as in Greek networks. We collaborate with several research groups including the laboratories of Profs Marc Debaets, Sonia Berrih-Aknin, Sara Fuchs, E. Eliopoulos, N. Oikonomakos, G. Spyroulias and several others.

3 relevant recent publications

- Kostelidou, K., Trakas, N. and Tzartos, S.J. (2007) Extracellular domains of the β , γ and ϵ subunits of the human acetylcholine receptor as immunoabsorbents for myasthenic autoantibodies: a combination of immunoabsorbents results in increased efficiency. *J. Neuroimmunol.* 190:44-52.
- Bitzopoulou, K., Kostelidou, K., Poulas, K. and Tzartos, S.J. (2008) Mutant forms of the extracellular domain of the human acetylcholine receptor γ -subunit with improved solubility and enhanced antigenicity. *Biochem. Biophys. Acta*, May 6. [Epub ahead of print]
- Zisimopoulou, P., Lagoumintzis, G., Poulas, K. and Tzartos, S.J. (2008). Antigen-specific apheresis of human anti-acetylcholine receptor autoantibodies from myasthenia gravis patients' sera using Escherichia coli-expressed receptor domains. *J. Neuroimmunol.* July 4. [Epub ahead of print].

Myastaid network

MYASTAID (www.myastaid.org) is an ongoing project (10/2006-9/2009) supported by the FP6 program of EC which focuses on Myasthenia Gravis, under the coordination of **Sonia Berrih-Aknin**. It attempts to develop therapies for MG and has several specific objectives:

1. To elucidate mechanisms of pathogenicity in MG (e.g. to answer why is the thymus remodeling in young MG patients? what are the consequences of the autoimmune attack on the muscle organization? why is the prevalence of the females in early onset MG higher?).
2. To develop new diagnostic and monitoring assays for MG.
3. To develop new and specific therapies for MG.

The participants believe that a better knowledge of the pathogenic mechanisms will help in devising new therapeutics approaches. Under MYASTAID, four academic teams and six industrial partners, aim at developing *in-vivo* and *in-vitro* models to further understand the disease and progress towards better diagnosis, follow-up, and therapy of patients with MG, with an ultimate aim to generate appropriate pharmaceutical products to be made commercially available. The academic groups are: the Université Paris Sud (UPS; Leader: **S. Berrih Aknin**), the University of Maastricht (UM; Leader: **M. deBaets**), the Hellenic Pasteur Institute (HPI; Leader: **S. Tzartos**) and the Weizmann Institute of Science (WIS; Leaders: **S. Fuchs** and **M. Souroujon**) and the industrial partners are: **BKT, EBIOT, GENMAB, IDT, OMRIX and REGULON**.

Last May (4 & 5), the mid-term evaluation meeting of the project was held in Paris for the presentation and evaluation of the up-to-now results. **Catherine Berens**, our EC project officer, and **Abdel Saudi**, invited by the network as external advisory member, also participated and contributed with invaluable suggestions. In a relaxed environment the participants exchanged data and presented recent research achievements. The UPS group presented data about the pathogenic mechanisms in the MG thymus and muscle, explained the difference between male and female MG thymuses and the role of estrogens in the susceptibility to MG. The UM group presented data about a new engineered antibody with therapeutic potential (in close collaboration with GENMAB) and also new results on Rapsyn gene therapy for experimental MG. The WIS group presented results on their attempts to treat MG with inhibitors of phosphodiesterases or inhibitors of chemokines and chemokine receptors. The group also presented data on the identification of the immunosuppressive factors in IVIG studied in experimental MG. The HPI group explained their attempts to develop an antigen specific therapy for MG, by the development of anti-AChR immunoadsorbents and also an improved diagnostic assay. The industrial partners presented parts of their efforts to develop innovative therapies, which are targeting different pathways of MG development.



Current treatments for MG

Dr. S. Sathasivam, MRCP, a consultant in Neurology at the Walton centre for Neurology and Neurosurgery in Liverpool, has recently published a review about steroids and immunosuppressant drugs in myasthenia gravis (*Nature Clinical Practice*, 2008, 4:317-326). In this review, Dr Sathasivam summarizes the mechanisms of action of steroids and other immunosuppressants, and presents randomized and nonrandomized evidence of their efficacy in generalized MG.

It is known that in chronic autoimmune diseases like MG, long-term immunosuppression is usually necessary. The mechanisms of action of immunosuppressant drugs in MG are complex and incompletely understood and are divided in three main categories: inhibition of the cell cycle (azathioprine, cyclophosphamide, methotrexate and mycophenolate mofetil), immunosuppression of T cells (steroids, ciclosporin and tacrolimus) and B cell depletion (rituximab). After presenting the data and evidence for the efficacy of each drug, Dr Sathasivam concludes that treatment vary between different countries and physicians, due to the lack of good randomized controlled treatment trials. However, according to clinical experience, observational studies and expert opinion, the recommended first-line therapy but only for short-term use is the oral prednisolone. It is usually started at a low dose and is gradually increased. Azathioprine is the first-choice drug for long-term immunosuppression and has been associated with relatively few treatment failures. Methotrexate, mycophenolate mofetil or tacrolimus should be considered in patients who are intolerant of or unresponsive to azathioprine. On the other hand, ciclosporin and cyclophosphamide due to the serious adverse effects should be only considered if all the other immunosuppressants fail. As far as the use of rituximab in MG is concerned, the evidence that exists so far is inadequate, but there are several promising case reports.

It is fundamental in the future better-designed randomized controlled treatment trials to be performed in order to establish more-definitive best-practice guidelines in generalized MG.

Interesting research findings

Diagnosis

The majority of the 'seronegative' MG patients are no longer seronegative: In myasthenia gravis, antibodies against the muscle acetylcholine receptor are detected in around 80% of the patients. From the remaining 20% of the MG patients, a small percentage exhibits antibodies against the muscle specific kinase (MuSK) and the rest of them have neither anti-AChR nor anti-MuSK detectable antibodies and they are defined as 'seronegative'. Angela Vincent and her group recently showed (Leite et al, Brain, 131: 1940-1952, 2008) that the majority of the 'seronegative' MG patients do have antibodies against the AChR, but they probably bind with low-affinity to AChR. They developed an assay which detects low-affinity antibodies to AChR, by co-transfecting a non-muscle cell line with human muscle AChR and the cytoskeletal clustering protein rapsyn. By this way the AChRs are clustered on the surface of the transfected cells and compensate for the low affinity of the autoantibodies.

Treatment

The current treatments of MG are relatively effective, but have adverse side effects, due to their limited immune specificity. Several promising experimental therapeutic strategies are being evaluated for MG, including:

1) Treatment of refractory MG by "rebooting" the immune system: MG is unmanageable for a small proportion of patients, mainly due to failure of response to current conventional treatments, or due to unacceptable adverse side effects. Daniel Drachman and his group (Ann. N. Y. Acad. Sci., 1132: 305-314, 2008) developed and studied a new treatment strategy, which is called "rebooting the immune system". In all the 12 patients non-responding to current treatments, that were followed for 1-9 years, high dose cyclophosphamide were administered, which eliminates the mature immune system without damaging the hematopoietic stem cells. 11 of the 12 patients showed significant improvement within 3 weeks to 3 months, while some of them became responsive to immunosuppressive drugs. The results from this study verified that high dose cyclophosphamide is a harmless and effective therapeutic approach for the myasthenic patients that do not respond to the conventional immunosuppression treatment. However, the improvement is not permanent for some patients, so the researchers suggest that the treatment with high dose cyclophosphamide must be followed with maintenance immunotherapy.

2) New treatment strategy for antibody-mediated diseases by depletion of antibody-forming cells: In antibody-mediated diseases like myasthenia gravis, autoimmune haemolytic anemia and systemic lupus erythematosus, long-lived plasma cells producing autoantibodies resist current therapeutic and experimental approaches. Therefore, the elimination of plasma cells producing pathogenic autoantibodies represents a new therapeutic approach for the treatment of antibody-mediated diseases. Kirsten Neubert and her group recently published a research paper (Nature Medicine, doi:10.1038/nm1763, 2008) in which they present that the proteasome inhibitor bortezomib depletes both short- and long-lived plasma cells by activation of the terminal unfolded protein response. This is the first therapeutic approach that can practically reduce plasma cells without causing toxic effects in mice. Hence, proteasome inhibition might represent a novel, highly efficient treatment strategy for antibody-mediated diseases. However, well-organized clinical studies should be initiated in order to evaluate this therapeutic approach in antibody-mediated diseases.

3) EN101, a new therapeutic approach: MG has been shown to be associated with the production of a rapid-acting variant of acetylcholinesterase (AChE), the enzyme that degrades acetylcholine, Drs Hermona Soreq, Jon Sussman and their colleagues constructed and used an antisense oligonucleotide (Monarsen, EN101) which selectively destroys this variant of AChE, thereby enhancing the action of the acetylcholine at the neuromuscular junction. Sixteen MG patients were treated with Monarsen and fourteen of them experienced clinically significant improvement. Monarsen is now undergoing Phase II clinical trials and appears to be the first oral antisense therapeutic approach to be successful in humans (Ann. N. Y. Acad. Sci., 1132: 283-290).

Other

Case Report: Dysgeusia and MG: Dysgeusia is the inability to interpret the basic tastes. Researchers from the Saitama Medical University (Saitama, Japan) reported the case of a 38-years-old patient with myasthenia gravis who became unable to discern sweet taste. The rest of the basic tastes were unaffected. Taste disorder rarely occurs in myasthenia gravis, however 6 similar cases have been reported before by other groups, and thymoma is associated in all cases. Taste disorder in all cases improved with therapy for myasthenia gravis (Nakazato *et al*, Inter Med 47: 877-878). 2008).

EUROMYASTHENIA WEBSITE

Visit the EuroMyasthenia Website (www.euromyasthenia.org) to see its new postings and add your contributions

MG Events and News

Worldwalk

WORLDWALK is an exciting series of events within the UK and Ireland to walk 24,902 miles – the equivalent of walking around the world. It is expected that over £100,000 (about £4 per mile) will be raised for supporting people with MG.

Sit/Walk/Run-a-Thon

The first annual MGFA (Myasthenia Gravis Foundation of America) **Sit/Walk/Run-a-Thon**, which helped raise awareness and funds for MGFA, took place on June 13 in Milwaukee, US. It involved asking your friends, co-workers or family to sponsor you whether you walk, run or sit at home.

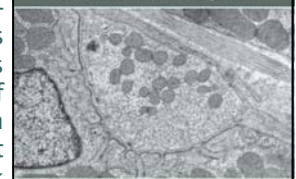
MG Awareness Month

MGFA has requested President Bush to issue a Presidential Proclamation declaring June 2008 as National Myasthenia Gravis Awareness Month. In this context MGFA has printed and distributed a poster "The many faces of MG-Together we are stronger".

Myasthenia Gravis and Related Disorders (Book)

Following the XIth International Conference for Myasthenia Gravis and Related disorders in May 2007, in Chicago, the book *Myasthenia Gravis and Related Disorders* is now published by the series of the Annals of the New York Academy of Sciences (volume: 1132). The book is edited by Henry J. Kaminski (St. Louis University School of Medicine) and Richard Barohn (University of Kansas Medical Center) and released in June 2008. This volume contains reports from basic and clinical researchers about new findings in myasthenia gravis and related diseases. The main topics of the book deal with advances in basic and clinical science, and novel therapies and treatment approaches. It is a must for every researcher and clinician involved with MG.

Myasthenia Gravis and Related Disorders 11th International Conference



edited by
Henry J. Kaminski
Richard J. Barohn

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Forthcoming meetings

- ◆ **MGFA Scientific Session 2008, 20 September, Utah, USA** (http://www.myasthenia.org/mgprogram_scientificsession.cfm)
- ◆ **2nd World Congress on Controversies in Neurology, 2008, 23-26 October, Athens, Greece** (<http://www.comtecmed.com>)
- ◆ **9th International Congress of Neuroimmunology 2008, 26-30 October, Texas, USA** (<http://www.isniweb.org>)
- ◆ **Society for Neuroscience 2008, 15-19 November, Washington, USA** (<http://www.sfn.org>)
- ◆ **Euromyasthenia Meeting 2008, 5-6 December, Cyprus** (later in: <http://www.euromyasthenia.org>)

Ask the Doctor

Below are some FAQs chosen from the EuroMyasthenia booklet for MG patients. Translations of the whole booklet in several languages will become soon available in the EuroMyasthenia web site.

1. What should MG patients keep in mind during the summer?

Increasing body temperature can increase weakness. The nerve and muscle communicate better with cooling (to a point), but generally there are many other effects of weather on a person's well-being. Generally, MG patients are advised to avoid exposure to sun for prolonged periods of time. Also, patients on pyridostigmine (Mestinon®) and related drugs face a higher risk of dehydration and should therefore maintain adequate water intake to compensate for water loss caused by sweating and other causes.

2. Are MG patients at risk when coming in contact with insecticides?

Some of the most commonly used insecticides, organophosphates and carbamates, work similarly to pyridostigmine; they are cholinesterase inhibitors intended to disrupt the nervous system of an insect by attacking enzymes, which are also found in the neuromuscular junction of a human. MG patients, especially those on anticholinesterase medication, are therefore likely to be more susceptible to these pesticides and experience cholinergic crisis upon excessive exposure. There are two insecticides that MG patients have to be cautious about: N,N-diethyl-m-toluamide, now called diethyl-3-methylbenzamide (DEET) and permethrin.

3. What is a cholinergic crisis?

A cholinergic crisis is a chemically induced overdose of medication or overexposure to an anticholinesterase agent. The symptoms of a cholinergic crisis can include muscle weakness, muscle twitching, sweating, excessive salivation, and constricted pupils. It is frequently difficult to tell the difference between cholinergic and myasthenic crises. The latter occurs when the muscles that control breathing are affected. This can create a medical emergency requiring a respirator to help breathing or measures to prevent taking too much air into the lungs. Treatment for cholinergic crisis is to decrease cholinesterase inhibitors, and treatment for myasthenic crisis is to increase cholinesterase inhibitors. Therefore, it is vital that only a specialist decides for the proper treatment.