Journal of Neuroimmunology 236 (2011) 118–122

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Concomitant autoimmunity in myasthenia gravis — Lack of association with IgA deficiency

Ryan Ramanujam [a], Fredrik Piehl [b], Ritva Pirskanen [b], Peter K. Gregersen [c], Lennart Hammarström [a,*]

[a] Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden
[b] Department of Neurology, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden
[c] The Feinstein Institute for Medical Research, Manhasset, New York, 11030, USA

ARTICLE INFO

Article history:
Received 16 March 2011
Received in revised form 14 May 2011
Accepted 14 May 2011

Keywords:
Myasthenia gravis
IgA deficiency
Concomitant autoimmunity

ABSTRACT

A marked increase in concomitant autoimmune diseases has previously been noted in patients with myasthenia gravis (MG). We show that these diseases occur both before and after the onset of MG and that the process is not influenced by thymectomy.
IgA deficiency (IgAD), which is strongly associated with the same HLA haplotype as early onset MG, has recently been suggested to be an autoimmune disease. However, there was no increase in the prevalence of IgAD in a large cohort of Swedish MG patients.

1. Introduction

Myasthenia gravis (MG) is an antibody mediated autoimmune disorder, characterized by auto-antibodies against the nicotinic acetylcholine receptor (anti-AChR antibodies), situated on the muscle end-plate, that impair transmission of nerve impulses to the muscle. MG occurs in approximately 1 in 10,000 individuals in European populations and has a concordance rate of 30–40% in monozygotic twins (Lisak, 1994), indicating a strong genetic component. Several genetic loci have previously been shown to be associated with MG, including PTPN22, IL-1, TNF-α, and the MHC, in particular the B8, DR3 haplotype in early onset MG and the B7, DR2 haplotype in late onset MG (Giraud et al., 2008).

MG often occurs concomitantly with other autoimmune disorders, including rheumatoid arthritis (RA) (Christensen et al., 1995), Type I diabetes (TID) (Toth et al., 2006), thyroid disorders (Graves' disease) (Sahay et al., 1965; Monden et al., 1986; Christensen et al., 1995; Marinó et al., 1997; Kanazawa et al., 2007) and systemic lupus erythematosus (SLE) (Vaiopoulos et al., 1994; Mevorach et al., 1995; Hrycek, 2009; Omar et al., 2010). In a long term study in Denmark, autoimmune disorders were concomitant with MG in 14% of cases, the most common being RA and thyroid disorders (Christensen et al., 1995). SLE has been reported in over 50 MG cases in the literature (Bhinder et al., 2006). Similar genetic associations, overlapping with other autoimmune conditions, including the HLA-B8, DR3 haplotype and the R620W variant of PTPN22, suggest that common mechanisms may exist which predispose MG patients for additional autoimmune disorders. However, it cannot be excluded that immunosuppressive treatment in MG, including thymectomy, disturbs the balance of immunity, leading to additional disorder(s) (Zonana et al., 2002).

Selective IgA deficiency (IgAD) is defined as serum IgA levels less than 0.07 g/l with normal levels of IgM and IgG. Its prevalence of 1 in 600 in European populations makes it the most common form of primary immunodeficiency (Hammarström and Smith, 2007). A high rate of familial incidence and a high relative risk for siblings indicate a strong genetic predisposition. The extended HLA-A1, B8, DR3, DQ2 haplotype has been shown to be strongly associated with IgAD, occurring in 45% of patients in Sweden (Mohammadi et al., 2008). This haplotype has also been shown to be associated with several autoimmune diseases, including TID (Smith et al., 1978), celiac disease (CD) (Congia et al., 1994), SLE (Skarsvåg et al., 1992) and Graves' disease (Farid et al., 1976), and an overrepresentation of IgAD among patients with these disorders has previously been observed (Ammann and Hong, 1971; Ryser et al., 1988; Jorgensen et al., 2009). Recently, it has even been suggested that IgAD might itself be autoimmune in nature (Ferreira et al., 2010).

Although MG is associated with the B8, DR3, DQ2 haplotype, previous studies, in small sized materials (<110 patients), have not shown an increase in the prevalence of IgAD in MG patients (Bramis et al., 1976; Lisak and Zweiman, 1976; Wetherall et al., 1976; Liblau et al., 1992). The single large scale study published to date screened 333 patients, and found only one to be IgAD (Liblau et al., 1992). However, another study did observe a decrease in serum IgA in 13 of 51 MG patients as compared to 4 of 51 controls (Behan et al., 1976).

In view of the inconclusive data obtained so far, we sought to determine if an increased incidence of IgAD occurs in MG by testing

* Corresponding author. Tel.: +46 8 5248 3586; fax: +46 8 5248 3588.
E-mail address: Lennart.Hammarstrom@ki.se (L. Hammarström).

0165-5728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2011.05.008

our large cohort of Swedish patients for serum levels of IgA. We furthermore attempted to determine if HLA allele contribution could explain the overlap with IgAD, or lack thereof, by determining HLA alleles in our MG and IgAD patients.

2. Materials and methods

2.1. Patients and controls

Five hundred and forty-seven Swedish Caucasian MG patients were included in the study. The diagnosis of myasthenia gravis was made as described previously (Drachman, 1994) and clinical information was documented by the neurologist. Information on concomitant autoimmune disorders was also collected.

Five hundred and thirty-two Swedish IgAD patients were also included in the study. These consist of patients who were referred from their primary physician after infectious symptoms and diagnosis of IgA deficiency by the stated criterion (serum IgA<0.07 g/l measured by nephelometry), and blood donors routinely screened for IgAD.

Ethical permission was obtained from the Regional Ethical Review Board for use of the patient materials.

Anonymized control data for HLA alleles was obtained from the Swedish volunteer bone marrow registry (www.tobiasregistret.se). The data consists of 40,789 participants, HLA typed for HLA-B ($n=40{,}789$), HLA-DR ($n=23{,}609$) and HLA-DQ ($n=1193$). HLA-A data has not been collected in this database, and therefore, the frequency of HLA-A alleles in Sweden from a previous publication using 252 unrelated Swedish individuals was used (Johansson et al., 2008).

2.2. HLA typing

HLA typing of patients for A, B, DR and DQ was obtained through the following methods. First, as part of the IMAGEN project (Rioux et al., 2009), most MG samples were typed at 1288 SNPs, 1230 of which were tag SNPs across the MHC (Rioux et al., 2009). Using this information, two digit imputations of HLA-A, B, DR and DQ were obtained using a previously validated method shown to have a 95% accuracy in imputing 4 digit types in European populations (de Bakker et al., 2006; Leslie et al., 2008). In order to improve the accuracy further, alleles with mismatches between imputation and previous typing via PCR/serology, such as HLA-A3101, B3501, B4002, DR401, DR403 DR701, and DR1401 (Rioux et al., 2009), were subject to PCR-SSP in order to determine the correct alleles.

PCR-SSP was used to genotype samples at the HLA-A, B, DR and/or DQ loci (Olerup et al., 1993). The kits used in this study included the HLA-A low resolution (26F, 62G), the HLA-B low resolution (90F, 63G), the HLA-DR low resolution (40E, 83F), the HLA-DQ low resolution (41E, 91F) and the HLA-DQ-DR SSP Combi Tray (M84) from Olerup SSP AB, Saltsjöbaden, Sweden.

2.3. IgA measurement

Serum levels of IgG, IgA and IgM were measured by nephelometry at the Karolinska University Hospital Clinical Chemistry Laboratory. Samples with an IgA concentration below 0.07 g/l with normal levels of IgG and IgM were considered to be IgAD.

2.4. Statistical analysis

The Chi square test was used to compare the allele frequencies of HLA alleles between MG/IgAD patients and controls. For these measurements, a p-value below 0.05 was considered to indicate statistical significance. Estimates of HLA haplotype structure were constructed using PHASE 2.1.1 (Stephens et al., 2001).

2.5. Patient subgrouping

Subgrouping of MG patients was based on clinical criteria where patients who had an early onset of the disease (age of onset<40 years; EOMG) were separated from those who had late onset of the disease (age of onset>50 years; LOMG). Some MG patients ($n=60$) were negative for anti-AChR antibodies, a large proportion of which were EOMG patients ($n=35$). Due to a North–South European gradient present in muscle-specific kinase (MuSK) antibody positivity (Vincent et al., 2008), very few anti-AChR antibody negative patients were positive for anti-MuSK antibodies (4 of 18 tested), and therefore a subgroup based on anti-MuSK antibodies was not analyzed. Furthermore, it has been suggested that a majority of such seronegative patients are positive for low affinity anti-AChR antibodies (Leite et al., 2008); therefore, these patients were included in the MG cohort. Patients with thymoma and those with purely ocular symptoms were examined separately with regard to presence of IgAD.

2.6. SNP analysis

In order to visualize potential differences between MG patients, IgAD patients and controls, a map was constructed using genotyping data for 1719 SNPs across the MHC from upstream of HLA-A to downstream of HLA-DP. These data consisted of 1116 SNPs extracted from an Illumina custom array panel described previously (de Bakker et al., 2006), which were merged with 897 SNPs across the same region typed using the Illumina HumanHap300 (317 K) genotyping BeadChip. Swedish MG patients, homozygous for the HLA-B8, DR3, DQ2 haplotype ($n=5$) and with normal IgA levels, were subsequently compared to population based controls ($n=6$) and IgAD patients ($n=10$) homozygous for this haplotype with normal IgA levels to determine if associated regions differ between the affected individuals. The SNP map was previously investigated in B8, DR3, DQ2 homozygous IgAD patients ($n=18$) and controls ($n=9$) (Mohammadi et al., 2010).

3. Results

MG patients in our cohort displayed concomitant autoimmune disorders at a rate greater than that of the general population. Of the 547 MG patients, autoimmunity was diagnosed in 87 (15.9%). Fig. 1a illustrates the incidence of concomitant disorders relative to the onset of MG in years. Several patients had more than one additional autoimmune disorder, including one patient with both RA and Crohn's disease, one with IgAD, polymyositis, Sjögren's syndrome and SLE, one with thyroiditis and MS, one with both psoriasis and polyserositis, two with both RA and thyroiditis and one with both thyroidism and polymyositis. Patients with documented onset before/after MG where the exact time was not available were denoted +/−5 years for graphical representation. Included is one patient who developed SLE, polymyositis and Sjögren's syndrome after MG.

Fig. 1b presents a similar view of the time of onset of additional disorders in thymectomized patients relative to surgery. Included are two patients who developed one disorder both before and after thymectomy (Fig. 1b). Of the 87 patients with concomitant autoimmunity, 60 were thymectomized and 27 were not, compared with 263 thymectomized and 193 not thymectomized in the MG cohort without an additional autoimmune disorder ($p=0.049$). EOMG was slightly overrepresented in the patients with concomitant autoimmunity (57.5%) compared with patients without additional disorder (s) (48.2%) ($p=0.11$). Similarly, thymoma was present in 13 of the former patients (14.9%) compared with 46 patients without additional autoimmunity (10.2%) ($p=0.20$).

HLA imputation was conducted on a total of 428 MG patients, with the vast majority being previously typed using either PCR or serological testing. Alleles which have previously been shown to display frequent mismatches were then retyped by PCR-SSP, which was conducted on