

Presence of Mono-Oligoclonal Rearrangement of V δ -J δ TCR Genes in Myasthenia Gravis

Grazyna Michalowska-Wender, Jerzy Nowak and Mieczyslaw Wender

Neuroimmunological Unit, Medical Research Center and Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland

Abstract

Rearrangement of T cell receptor (TCR) V δ -J δ genes has been analyzed in 20 myasthenia gravis (MG) cases and 17 controls (blood donors) using the seminested polymerase chain reaction (PCR). Unlike the healthy subjects the majority of patients demonstrated a mono- or oligoclonal type of rearrangement of TCR V δ 1-6-J δ 1 genes. It is suggested that T lymphocytes may undergo clonal expansion in myasthenia gravis due to stimulation by an autoantigen related to myasthenia pathogenesis. The results strongly argue for a significant role of the γ / δ T cells.

Key words: rearrangement, TCR genes, myasthenia gravis.

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Myasthenia gravis is a chronic autoimmune disease mediated by serum antibodies directed against the acetylcholine receptor (AChR) of muscles which leads to depletion of these receptors within the motor- end plates. The problem of its pathogenesis is complicated by the fact that molecular mimicry, based on comparable peptide sequences may provoke an autoimmune T- cell reaction which then drives the anti AChR- response. Schalke and Wekerle (1983) have generated several different AChR-specific T- cell lines most of which, if not all, contain subsets involved in a delayed type hypersensitivity as well as T cells interacting with antibody producing B lymphocytes. Handa et al. (1994) demonstrated a case of pure red cell aplasia, associated with malignant thymoma and myasthenia gravis with a clonal proliferation of T cells within the thymus, as demonstrated by a T cell antigen receptor delta chain rearrangement.

Recent reports suggest a role for α/β TCR in the pathogenesis of autoimmune diseases. In addition to α/β TCR also T cells expressing γ/δ TCR may be involved in the pathogenesis of MG. The repertoire of the T cell receptor delta chain is generated by rearrangement of six V, three D and four J gene segments [2, 4]. The aim of our study was to evaluate the TCR δ chain repertoire in the peripheral blood of myasthenia gravis patients by PCR (polymerase chain reaction) analysis of the V δ -J δ segment rearrangements.

Material and Methods

Peripheral blood was obtained from 20 patients with myasthenia gravis and 17 healthy blood donors. The population of myasthenia patients consisted of 16 females

(mean age 26 years) and 4 males (mean age 54 years). The healthy control group consisted of 7 males (mean age 49 years) and 10 females (mean age 32 years). Genomic DNA was extracted from peripheral blood according to the standard method described by Sambrook et al. (1989). TCR gene rearrangement was studied by PCR analysis using oligonucleotide primers specific for V δ 1-V δ 6 and for J δ 1 external (J δ 1e-bac) in the first round of the PCR. For the second round of the PCR J δ 1 internal primer (J δ 1i-bac) was used. The different sequences of the primers are presented in Table 1. The PCR2 analysis was performed in a thermocycler (Biometra) using 500 ng of genomic DNA, 1.5 U of Taq DNA polymerase (Perkin-Elmer Corp.), and 25 pmoles of each primer. The reaction mixture was heated at 94°C for 3 min followed by 1 min at 60°C. Afterwards, 35 cycles were performed for 1 min at 94°C, 1 min at 56°C and 1 min at 72°C. In the second round of PCR 25 cycles were performed (1 min at 94°C, 1 min at 58°C and 1 min at 72°C). The PCR products were afterwards analyzed by electrophoresis in 2.5% agarose gel and also in 5% - 12% polyacrylamide gel stained by the silver method described in detail elsewhere [11].

Results

The TCR δ gene repertoire in the myasthenia gravis patients was studied by two round- PCR (seminested) with oligonucleotide primers specific for each of six TCR delta segments and two primers (external and internal) for the J δ 1 region. A monoclonal type of rearrangement V δ 1-J δ 1 (400 bp) was found in 4 patients, V δ 2-J δ 1 (400 bp) in 5 cases, V δ 3-J δ 1 (230 bp) in 3 cases, V δ 4-J δ 1 (290 bp) in 4

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Table 1. Sequences of TCR delta gene primers used for PCR amplification.

Vδ1-for	5' - ACT CAA GCC CAG TCA TCA GT
Vδ2-for	5' - GAGTCA TGT CAG CCA TTG AG
Vδ3-for	5' - ACA GCA GAT CAG AAG GTG CA
Vδ4-for	5' - CCA GTG ATC CCA GTT ATG GTC
Vδ5-for	5' - CTG AAG GTC CTA CAT TCC TG
Vδ6-for	5' - TAT CAT GGA TTC CCA GCC TG
Jδ1e-bac	5' - AAA TGC TAG CTA TTT CAC CCA
Jδ1i-bac	5' - GAGTTA CTT ACT TGG TTC CAC

patients, Vδ5-Jδ1 (270 bp) in 2 cases and Vδ6-Jδ1 (130 bp) in 3 myasthenia patients. Since it is difficult to distinguish between one single band and two closely spaced bands it would be more accurate to talk about a mono-oligoclonal type of rearrangements as opposed to a clearly distinct polyclonal pattern. In several other cases of MG the oligo-clonal type of rearrangement (2-4 bands) was found. In a

few cases the polyclonal pattern of TCR 5 rearrangement was noted. By contrast to the MG group, almost all the healthy individuals produced a smear-like or multi-band patterns of Vδ1-Jδ1 and Vδ2-Jδ1. The electrophoresis results from selected MG patients and healthy individuals are presented in Figures 1-6. In the figures the same MG patients (numbers 11-18) are shown in all six gels along with the same three healthy individuals which served as a control group.

A summary of the results obtained from the rearrangement of Vδ1-Vδ6 to Jδ1 is presented in Table 2.

Discussion

T lymphocytes express two types of TCR's, either α/β or γ/δ . Unlike the α/β T cells a minority of the T cells express γ/δ TCR. The genes encoding the TCR γ and δ chains are composed of V, D, J and C regions that undergo rearrangement during development [9]. γ/δ T cells may play a significant role in some diseases, mainly in autoimmune processes. A very constant single-band pat-

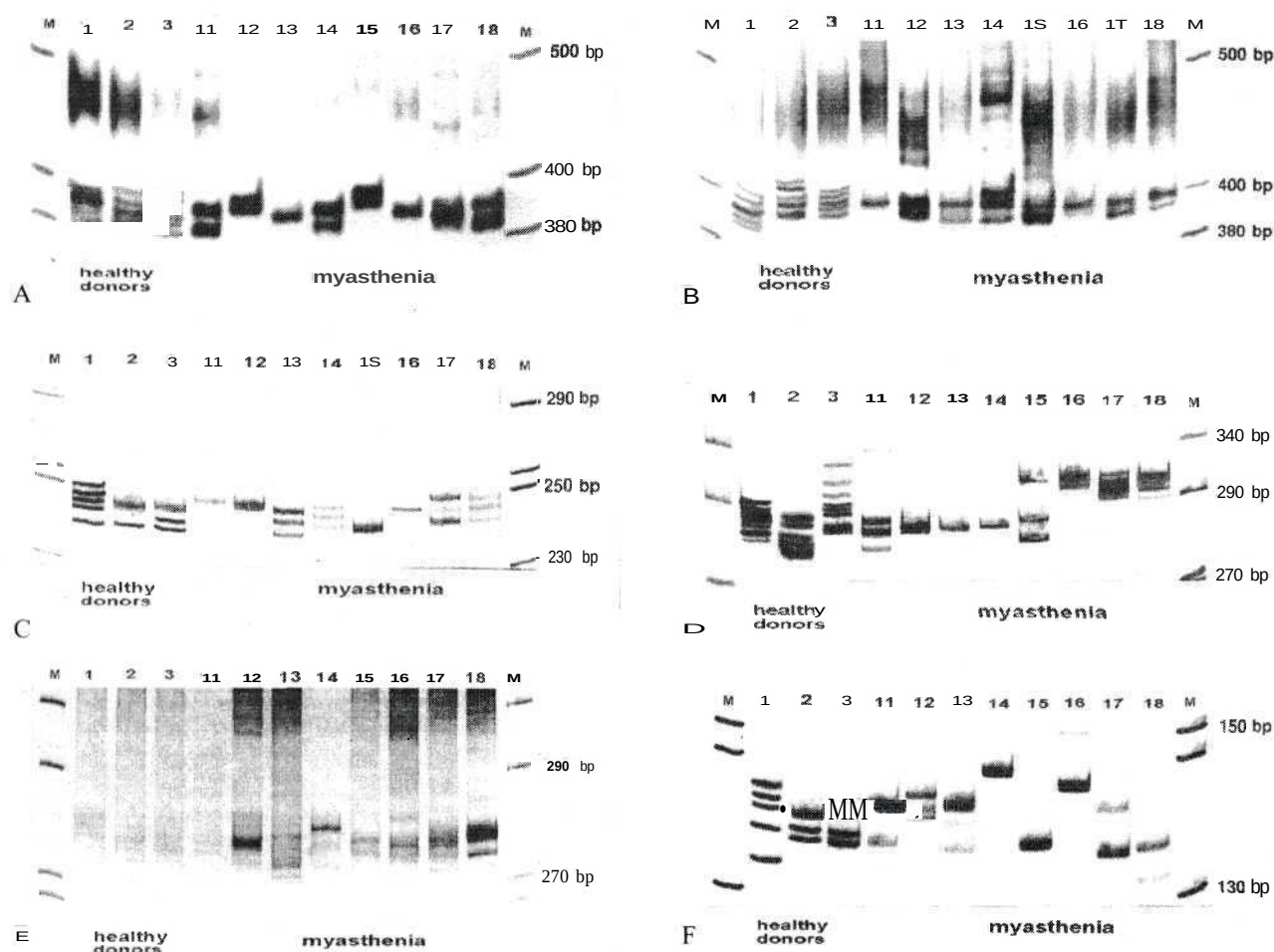


Figure 1. Silver stained PCR products of Vδ1-Jδ1 (A), Vδ2-Jδ1 (B), Vδ3-Jδ1 (C), Vδ4-Jδ1 (D), Vδ5-Jδ1 (E) and Vδ6-Jδ1 (F) rearrangements analysed by PAGE. M- KBL size marker. Bands 1-3- healthy donor controls. Bands 11-18- myasthenia gravis patients.

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Table 2. Pattern of TCR V δ -J δ gene rearrangement in peripheral blood lymphocytes of myasthenia gravis patients (MG; n = 20) and healthy individuals (H; n = 17). The figures represent the number of patients showing the specific rearrangement patterns.

	Monoclonal		Oligoclonal		Polyclonal	
	MO	H	MG	H	MG	H
V81-J61	4	1	13	-	3	16
V82-J81	5	1	12	-	3	16
V83-J81	3	1	13	3	4	14
V84-J81	4	-	12	3	4	14
V85-J81	2	-	12	3	6	14
V86-J81	3	-	14	3	3	14

tern of the V85-J81 rearrangement was observed in almost all multiple sclerosis patients by Nowak et al. (1996). The clonal nature of this rearrangement, as proved by PAGE and sequencing analysis, could indicate on antigen-driven expansion of γ/δ T cells, which may play significant role in MS pathogenesis [6].

Myasthenia gravis and multiple sclerosis are polygenic autoimmune diseases, probably triggered by environmental factors. The problem is open as to the delineation of the hierarchy of the numerous genes that control the onset of myasthenia gravis in interaction with environmental factors [13]. The role of TCR genes has been investigated by studying TCR polymorphism. No conclusive results were obtained for either α or β chains using a hybridization technique [5, 8, 12]. Another approach is to study V gene usage by analyzing the distribution of those lymphocytes expressing the various V genes. Grunewald et al. (1991) using a series of anti-V δ monoclonal antibodies found some repertoire distortion among α/β T cells. In our experiments we observed one or two band-patterns in the electrophoresis analysis of most of MG patients. One band on the silver stained gel of the PAGE was considered as a monoclonal pattern, 2-4 bands as oligoclonal and more than 4 bands as polyclonal. In our studies using the semi-nested polymerase chain reaction we established that the majority of myasthenia gravis patients demonstrated mono- or oligoclonal rearrangements of T cell receptor V8-J8 genes. The majority of the healthy controls showed a polyclonal pattern of V81-J81, V82-J81, V83-J81, V84-J81, V85-J81 and V86-J81 rearrangements (Table 2). It can be suggested that in myasthenia gravis VS T lymphocytes undergo clonal expansion due to stimulation by some autoantigen, not definitely identified, but clearly related to myasthenia pathogenesis. The results strongly argue for a significant role of γ/δ T cell subpopulation in the immunological response in myasthenia gravis and similarly in multiple sclerosis. Our results are consistent with the observation of Oksenberg et al. that specific TCR Va and Ca alleles are associated with myasthenia gravis [9]. In an-

other neurological disease e.g. multiple sclerosis clonally expanded α/β and γ/δ T lymphocytes have been documented (Oksenberg [10], Wucherpfennig [16]). It is also possible that the known correlation between HLA and specific TCR found in MS and diabetes mellitus [9] may also exist in MG.

Address correspondence to:

Dr Grazyna Michalowska-Wender, Neuroimmunological Unit, 60-355 Poznań, 49 Przybyszewski Str., Poland.

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