Overexpression of Select T Cell Receptor Vβ Gene Families within CD4+ and CD8+ T Cell Subsets of Myasthenia Gravis Patients: A Role for Superantigen(s)?

Dulceaydee Gigliotti,* Ann-Kari Lefvert,[†] Mahmood Jeddi-Tehrani,* Semih Esin,* Vida Hodara,* Ritva Pirskanen,[‡] Hans Wigzell,* and Roland Andersson*

*Microbiology and Tumorbiology Center (MTC), Karolinska Institute, Stockholm, Sweden
[†]Immunological Research Laboratory, Department of Medicine, Karolinska Hospital, Stockholm, Sweden
[‡]Department of Neurology, Söder Hospital, Stockholm, Sweden

ABSTRACT

Background: The principal symptoms of myasthenia gravis (MG), muscle weakness and fatigue due to impaired neuromuscular transmission, are caused by autoantibodies to the muscle nicotinic acetylcholine receptor (AChR). The mechanisms underlying the autoimmune response, however, appear to be initiated by activation of specific HLA class II–restricted CD4+ T lymphocytes. Thus, central to elucidating the causation of MG is determining how T cells are recruited to contribute to misguided immunological assaults on the major autoantigenic target, AChR.

Materials and Methods: By combining a polymerase chain reaction (PCR)–based strategy and Southern blot technique, we have analyzed the frequency of expression of 22 individual T cell receptor (TCR) Vβ gene subfamilies in CD4+ and CD8+ peripheral blood T cell subsets derived from eight MG patients and seven healthy controls. The quantification of relative usage of individual TCR Jβ gene segments was performed by hybridization of PCR-amplified products (specifically Vβ1-Cβ) with a complete panel of ³²P-5′-end-labeled Jβ-specific oligonucleotide probes, followed by scanning analysis of autoradiographs.

Results: Comparisons of data obtained from Vβ analyses of T cells from MG patients with those from healthy individuals established that MG patients significantly overexpressed Vβ1, Vβ13.2, Vβ17, and Vβ20 gene family members within both CD4+ and CD8+ T cell subpopulations. Moreover, analysis of the relative utilization of individual TCR Jβ gene segments in Vβ1+/CD4+ and Vβ1+/CD8+ T lymphocytes revealed distribution patterns in patients indistinguishable from those recorded in the corresponding cell subsets derived from controls.

Conclusions: T lymphocytes from MG patients displayed a biased overexpression of four TCR Vβ gene segments: Vβ1, Vβ13.2, Vβ17, and Vβ20. The relative frequencies of association of individual Vβ1(Dβ)Jβ combinations revealed that Jβ gene usage in the Vβ1-overrepresented T cell subsets had normal distribution patterns. It can thus be deduced that Jβ gene segment products appear not to have a selective effect on the process leading to overexpression of Vβ1 exons in MG patients. Hence, our observations suggest a possible role for superantigen(s) in the T cell activation in MG patients.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disorder of neuromuscular transmission. The presence of high-affinity autoantibodies directed against the muscle nicotinic acetylcholine receptor (AChR) in more than 90% of MG patients suggests the involvement of humoral responses in disease pathogenesis (1,2). In addition, although the precise role of cell-mediated responses in MG remains to be elucidated, several lines of evidence indicate an important contribution by T lymphocytes: (i) frequent incidence of medullary lymphofollicular hyperplasia in MG thymi (3); (ii) amelioration of myasthenic state following thymectomy (3); (iii) presence of T

lymphocytes exhibiting an impaired production of cytokines, such as interleukin-2 (IL-2) and interferon-γ (IFNγ), as measured in vitro (4); (iv) identification of AChR-specific CD4$^+$ T cells derived from peripheral blood of MG patients (5,6); and, interestingly, (v) mitigation of symptoms of disease by treatment with anti-CD4 monoclonal antibody (7).

During early events in thymic maturation processes, T cells acquire functional heterodimeric α/β T cell receptors (TCRs) (8). The actual specificity of these receptors results from somatic recombination of one of the variable (V) and joining (J) exons, and of one of the V, diversity (D), and J exons encoding the variable domains of α- and β-chains, respectively. Depending on the nature of the antigen, interaction with the TCR will involve different parts of the variable domains. A group of protein molecules, defined as superantigens (SA), have the potential of activating a substantial fraction of T cells (5–30%) through interaction with specific target sequences of distinct Vβ gene family products (reviewed in Refs. 9–12). As opposed to conventional presentation of nominal antigen, the powerful T cell activation by SA occurs in the absence of protein processing via binding to monomorphic portions of MHC class II molecules, outside the peptide-binding groove area (13). This polyclonal, albeit selective, activation of T lymphocytes may eventually result in peripheral termination of the state of tolerance to particular self antigens (11,12). A potential connection between SA and autoimmune diseases is thus intriguing because of the microbial origin of many of these antigens and the frequently reported relationship between infection and onset of human autoimmune illnesses (14).

In recent years, several T cell–mediated autoimmune disorders have been studied in an attempt to identify disease-associated diverging distribution patterns of TCR expression (15–18). Analyses of T cell expansions in peripheral blood of MG patients using a panel of Vα and Vβ gene product-specific monoclonal antibodies have been performed by several groups (19), including our own (20). Collectively, these results did not identify an expansion of T cells expressing a particular Vα or Vβ gene segment product consistently present in MG patients.

The present study was designed to scrutinize the relative usage of individual Vβ gene segments in PBL T lymphocytes derived from MG patients, employing semiquantitative reverse transcriptase–polymerase chain reaction (RT-

PCR)–based technology. Analysis of expression of a panel of 22 Vβ exons revealed that the Vβ1, Vβ13.2, Vβ17, and Vβ20 families were constantly overrepresented, in both CD4$^+$ and CD8$^+$ T cell subsets. In addition, a complete pattern of the relative expression of TCR Jβ multigene family members, recombined with Vβ1 exons, was established. The implications of the nonrandom usage of Jβ gene segments in relation to the relative prevalence of expression of rearranged Vβ1 exons associated with MG will be discussed.

MATERIALS AND METHODS

Subjects

Eight MG patients, diagnosed on the basis of clinical features, electromyographic decrement, positive response to acetylcholinesterase inhibitors, and presence of circulating antibodies directed against the AChR, were included in the study. The median age of the patients was 56 years (range: 26–73 years). Clinical stage was determined according to Osserman's classification, modified by Oosterhuis (21). Three patients were in stage IIA (mild, generalized myasthenia: BMR, EiNi, BR), and five were in stage IIB (severe, generalized myasthenia: HA, SB, EN, GD, EJ). One patient (EN) received both corticosteroids and azathioprine, and two patients (HA, EJ) corticosteroids only. At the time of sample collection, all but one patient (EiNi) had active, fluctuating MG. Seven healthy Swedish blood donors (age range: 30–55 years) constituted the control group.

Cells

Peripheral blood mononuclear cells from MG patients and healthy subjects were separated from heparinized blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation. T cells were then fractionated into CD4$^+$ and CD8$^+$ subsets by the use of anti-CD4 or anti-CD8 monoclonal antibody-coupled magnetic beads (Dynal AS, Oslo, Norway).

RNA Preparation and First-Strand cDNA Synthesis

Total RNA was extracted from CD4$^+$ and CD8$^+$ T lymphocytes by using an RNA isolation kit (Stratagene, La Jolla, CA, U.S.A.), based on the guanidine thiocyanate-phenol-chloroform-isoamyl-

alcohol RNA extraction method (22). RNA was denatured at 90°C for 5 min and chilled on ice. First-strand cDNA synthesis was then performed as previously described (23).

PCR-Based Analysis of TCR Vβ Gene Segment Usage

For an estimation of the concentration of the cDNA encoding TCR β-chains, the total cDNA was serially diluted ($\log_2$ intervals) and a given sequence of Cβ amplified. Sequences of the Cβ 3' and Cβ 5' primers (specific for both Cβ1 and Cβ2) used have been previously published (24,25). Resulting PCR products were electrophoresed in 1.6% ethidium bromide-stained agarose gels. To determine the degree of amplification, negative films (Polaroid Film 665) of gels were scanned (2400 Gel Scan XL, Pharmacia-LKB, Uppsala, Sweden) for quantification. Based on individual scan values, total cDNA preparations were adjusted to equal concentrations of TCR β-chain–encoding cDNA material. Amplifications of TCR β-chain–encoding cDNA were then performed using a panel of 22 Vβ-specific 5' primers in conjunction with a Cβ 3' primer (24). PCR mixtures contained 10× concentrated buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, 1 mg/ml gelatine, pH 8.3 [Boehringer Mannheim, Scandinavia AB]); dNTPs at 0.2 mM final concentration (Pharmacia Molecular Biology), 200× diluted Taq polymerase 5 U/μl (Boehringer Mannheim, Scandinavia AB), and 0.5 μM of each primer. Samples were amplified in a Perkin-Elmer thermal cycler for 35 cycles. The temperature profile included a denaturation step at 95°C, an annealing step at 55°C, and an extension step at 72°C, 30 sec for each step. To ensure complete extension of the products, the final step was extended to 9 min in duration. To confirm the expected size of Vβ-Cβ amplified material, PCR products were separated on ethidium bromide-stained 1.6% agarose gels. The profile of amplification was determined to be in its exponential phase between 33 and 36 PCR cycles (data not shown).

Southern Blot Analysis of PCR-Amplified TCRβ Chain-Encoding cDNA

PCR products were subjected to electrophoresis in 1.6% agarose gels. The gels were denatured, neutralized and the products transferred overnight at room temperature onto Hybond-N nylon membranes (Amersham, United Kingdom)

as previously described (26). Membranes were air dried followed by baking for 2 hr at 80°C. After prehybridization for 6 hr at 42°C in a buffer containing 2× SSPE, 5× Denharts and 0.5% sodium duodecyl sulphate (SDS), membranes were hybridized overnight at 42°C with 1 × 10⁶ cpm/ml of ³²P-5'-end-labeled Cβ probe (Cβ reporter) (25). For labeling procedure, see below. Membranes were washed for 10 min at 42°C in a buffer containing 0.2× SSPE and 0.5% SDS, and subsequently exposed to Hyperfilm MP (Amersham) overnight at −70°C. Autoradiographic intensity signals were quantified using scanning technique (2400 Gel Scan XL, Pharmacia-LKB). A computer program was used to convert optical density values to percentages, and thus the results are expressed as relative Vβ usage (23).

TCR Jβ- and Cβ-Specific Oligonucleotide Probes

Jβ1.1–1.6– and Jβ 2.1–2.7–specific oligonucleotide probes had been previously designed (23) and were purchased from Scandinavian Gene Synthesis (Köping, Sweden). The ³²P-5'-end-labeling of probes was performed according to standard protocol (26), resulting in a practically equal labeling of the 13 Jβ probes and the Cβ reporter, with a variation of $< \pm 4\%$ (27).

Quantification of Vβ1(Dβ)Jβ Gene Segment Association Frequencies

Amplified Vβ1-Cβ PCR products were diluted in TE buffer, boiled for 10 min, cooled on ice, and slot blotted in thirteen identical samples onto Hybond-N nylon filters (Amersham), using a Bio-Dot SF microfiltration unit (BioRad, CA, U.S.A.). Filters were then soaked in denaturing and neutralizing buffers, air dried, and baked for 2 hr at 80°C. After prehybridization for 6 hr at 42°C, filters were hybridized overnight with ³²P-5'-end-labeled Jβ-specific probes (26). Stringency washing was performed for 10 min at 42°C in a buffer containing 0.2× SSPE and 0.5% SDS. Washed filters were exposed to Hyperfilm MP (Amersham) at −70°C. The signal of individual slots on the film was quantified by scanning, and the resulting scan values were used to calculate the relative expression (percentage) of each Jβ gene segment as previously described (23).

Statistical Analysis

The nonparametric Wilcoxon-Mann-Whitney two-tailed test was used.

TABLE 1.   Median and range (min–max) values for relative expression of individual TCR Vβ gene segments in CD4+ and CD8+ T cells from healthy blood donors (n = 7)

	Relative Vβ Gene Expression in T Cell Subsets	
Vβs	CD4+	CD8+
1	1.5 (0.7–9.8)	2.1 (0.8–7.6)
2	6.0 (2.0–6.6)	5.2 (0.3–7.1)
3	4.6 (2.8–8.9)	6.1 (1.6–12.0)
4	4.1 (2.6–4.7)	4.5 (3.1–12.5)
5.1	4.5 (3.1–8.5)	7.5 (1.1–8.1)
5.2–3	4.9 (3.0–6.6)	6.0 (0.9–7.4)
6.1–3	4.0 (2.5–9.0)	5.2 (1.5–7.9)
7	7.2 (6.0–9.2)	7.2 (6.5–10.0)
8	4.7 (0.2–7.6)	5.0 (2.2–8.5)
9	5.1 (0.5–7.9)	3.7 (2.0–6.5)
10	2.0 (0.0–6.0)	1.9 (0.8–6.1)
11	3.0 (1.6–6.2)	3.3 (0.5–8.3)
12	2.1 (0.0–7.0)	2.0 (0.7–4.9)
13.1	6.1 (2.4–8.1)	4.5 (2.6–9.2)
13.2	4.0 (1.0–5.5)	3.2 (1.1–5.7)
14	4.1 (2.0–8.3)	5.0 (3.5–6.2)
15	3.0 (0.6–5.1)	5.0 (1.5–7.9)
16	3.2 (0.4–7.4)	3.0 (0.6–11.0)
17	1.8 (0.6–7.5)	1.7 (1.2–4.5)
18	5.6 (3.1–8.7)	5.1 (2.7–7.2)
19	4.1 (3.3–8.1)	4.8 (1.7–7.0)
20	3.5 (2.5–4.4)	3.2 (1.6–5.7)

RESULTS

Analysis of Relative Usage of TCR Vβ Gene Segments

By analyzing the binding of distinct TCR V gene product–specific monoclonal antibodies, many MG patients displayed individual V gene–specific expansions among their T lymphocytes. Importantly, no given Vα or Vβ gene segment product was, however, consistently overexpressed (data not shown). To accomplish a more thorough analysis of the distribution patterns of Vβ gene family usage, we established, by combining RT-PCR strategy and Southern blot technique, the relative frequency of utilization of 22 Vβ exons in CD4+ and CD8+ T cell subsets from eight MG patients and seven healthy controls.

Median and range values of expression for each of the 22 individual Vβ gene segments in CD4+ and CD8+ T cell subpopulations from 7 healthy controls were calculated. Results are indicated in Table 1. Next, when the relative usage of the 22 Vβs were determined in MG patients, an overrepresentation of Vβ1, Vβ13.2, Vβ17, and Vβ20 gene families was observed—importantly, in both CD4+ and CD8+ T lymphocytes. Table 2 presents the relative levels of overexpressed Vβ gene families in MG-derived, separated T cell subsets. These observations are graphically illustrated in Fig. 1, depicting the distribution of relative usage of overrepresented Vβ1, Vβ13.2, Vβ17, and Vβ20 gene segments in CD4+ and CD8+ T cell subsets derived from MG patients and controls. All MG patients had been subjected to typing of their MHC class I and II

TABLE 2.   Median values for expression of overrepresented TCR Vβ gene segments in CD4+ and CD8+ T cells from Myasthenia gravis patients (n = 8)

	Relative Vβ Gene Segment Expression in T Cell Subsets			
Vβs	CD4+	p Value[a]	CD8+	p Value[a]
1	5.2 (2.2–5.8)[b]	<0.05	6.7 (6.0–14.7)[b]	<0.005
13.2	5.0 (4.4–8.2)	<0.05	5.5 (3.8–7.9)	<0.05
17	4.8 (3.1–6.4)	<0.05	4.2 (1.9–5.9)	<0.05
20	4.6 (3.0–6.6)	<0.05	4.9 (3.9–8.0)	<0.05

[a]The nonparametric Wilcoxon-Mann-Whitney two–tailed test was used to determine p values for comparison of MG patients and healthy controls regarding relative expression of the Vβs in CD4+ and CD8+ T cells.
[b]Numbers in parentheses indicate the range (min–max) of values.

Frequency of Vβ gene expression in CD4 T cells (%)
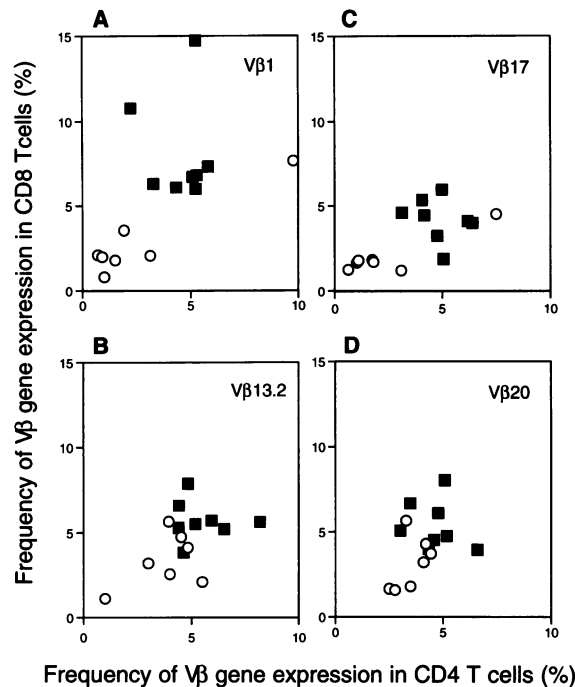
FIG. 1. Distribution of relative expression of TCR Vβ gene segments among CD4⁺ and CD8⁺ peripheral T cells from myasthenia gravis patients (■) and healthy controls (○).

Analyses of frequency of usage of individual Vβ gene families were performed by a comparative PCR-based approach. Four out of 22 Vβs were determined to be overrepresented—importantly, in both CD4⁺ and CD8⁺ T cell subsets derived from MG patients: Vβ1 (A), Vβ13.2 (B), Vβ17 (C) and Vβ20 (D).

TABLE 3. Median relative level of utilization of individual TCR Jβ gene segments recombined to Vβ1 in CD4⁺ T cells from Myasthenia gravis patients (MG) (n = 8) and healthy controls (HC) (n = 7)

	Relative Jβ Gene Expression in Vβ1⁺/CD4⁺ T Cells	
Jβs	MG	HC
1.1	5.8 (3.6–15.0)[a]	5.8 (2.6–9.4)
1.2	5.5 (3.8–7.2)	7.6 (2.4–21.6)
1.3	1.0 (0–2.2)	1.6 (0–6.0)
1.4	0 (0–1.6)	0.4 (0–2.0)
1.5	0.3 (0–1.6)	0.7 (0–1.6)
1.6	1.7 (0.6–6.9)	2.1 (0.4–4.1)
2.1	17.8 (15.4–28)	17.7 (9.5–29.7)
2.2	9.8 (5.2–32.7)	7.0 (3.1–9.4)
2.3	5.2 (0.9–9.7)	6.2 (1.0–15.7)
2.4	0 (0–0.02)	0.2 (0–3.3)
2.5	11.3 (3.6–14.7)	13.2 (5.0–20.0)
2.6	0 (0–2.3)	0.5 (0–7.5)
2.7	33.1 (20.1–36.8)	31.5 (10.4–61.7)

p values, as determined by the nonparametric Wilcoxon-Mann-Whitney two-tailed test, indicate that the patterns of Jβ gene segment expression in MG patient-derived Vβ1⁺/CD4⁺ T cells are not significantly different from those observed in the corresponding subset from healthy controls.
[a]Numbers in parentheses represent the range (min–max) of values.

alleles, which displayed a broad scatter of HLA haplotypes (data not shown).

Analysis of Relative Usage of TCR Jβ Gene Segments

Considering the relevance of Jβ-encoded sequences of the complementarity-determining region 3-β loop of the variable domain for nominal antigen recognition, we next attempted, using Vβ1 as a representative of overexpressed Vβ gene segments, to determine distribution patterns of all 13 of the Jβ multigene family members expressed by Vβ1⁺/CD4⁺ and Vβ1⁺/CD8⁺ T cells from MG patients in comparison to healthy individuals. Analyses of frequency of usage of individual Jβ gene segments in CD4⁺ and CD8⁺ Vβ1⁺ T cell subpopulations are indicated in Tables 3 and 4, respectively. Median values for the relative utilizations of individual Jβ gene segments in Vβ1⁺/CD4⁺ and Vβ1⁺/CD8⁺ T cells are

graphically represented in Fig. 2 A and B, respectively. Upon comparison, strikingly similar nonrandom distribution patterns of expression of Jβs were observed in Vβ1⁺/CD4⁺ and Vβ1⁺/CD8⁺ T cells from MG patients and healthy controls.

DISCUSSION

Although the actual cause of MG is unknown, the disorder does carry all the conventional hallmarks of being an autoimmune disease with at least one identified major autoantigen as target, the muscle nicotinic acetylcholine receptor (AChR). Occurrence of high-affinity IgG autoantibodies reactive with AChR (1) and/or non-AChR muscle or synaptic proteins (28,29) is a common observation in MG patients and activities exerted by certain members of this antibody pool are the ultimate cause of the myasthenic

TABLE 4. Median relative level of utilization of individual TCR Jβ gene segments recombined to Vβ1 in CD8$^+$ T cells from Myasthenia gravis patients (MG) ($n = 8$) and healthy controls (HC) ($n = 7$)
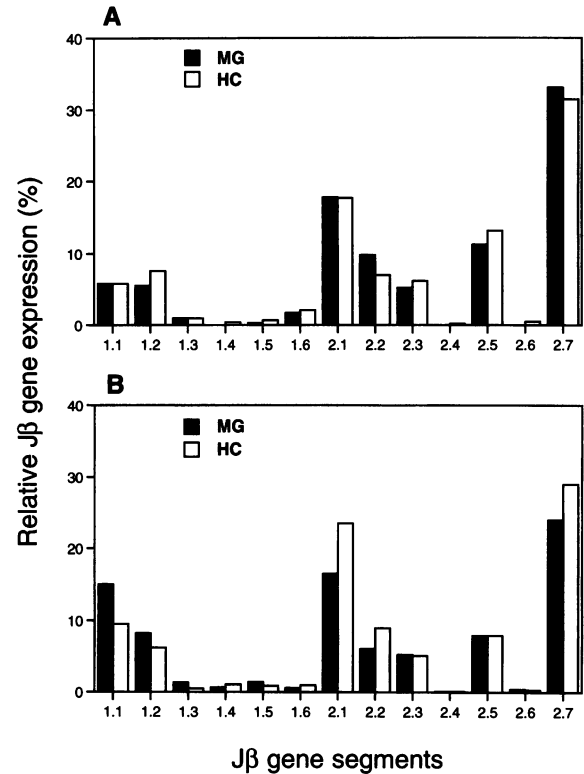
	Relative Jβ Gene Expression in Vβ1$^+$/CD8$^+$ T Cells	
Jβs	MG	HC
1.1	15.3 (8.7–20.0)[a]	8.9 (2.9–23.3)
1.2	8.2 (1.8–11.8)	6.1 (0.9–7.4)
1.3	1.3 (0.9–2.9)	0.5 (0–4.4)
1.4	0.7 (0–3.1)	1.1 (0–5.2)
1.5	1.4 (0–2.2)	0.8 (0–2.8)
1.6	0.6 (0.1–1.5)	1.1 (0–37.3)
2.1	16.2 (7.3–35.4)	24.0 (2.7–33.4)
2.2	6.0 (2.3–45.6)	9.3 (2.3–9.9)
2.3	5.2 (0.6–14.7)	5.0 (2.9–12.6)
2.4	0.1 (0–4.7)	0.1 (0–0.1)
2.5	7.9 (4.3–43.6)	7.5 (5–11.4)
2.6	0.4 (0–4.8)	0.3 (0–1.2)
2.7	23.5 (12.8–35.4)	29.7 (25.9–43.2)

p values, as determined by the nonparametric Wilcoxon-Mann-Whitney two-tailed test, indicate that the pattern of Jβ gene segment expression in MG patient-derived Vβ1$^+$/CD8$^+$ T cells are not significantly different from those observed in the corresponding subset from healthy controls.
[a]Numbers in parentheses represent the range (min–max) of values.

FIG. 2. Relative frequencies of individual TCR Jβ gene segments, as measured in TCR Vβ1-Cβ PCR-amplified material

Median values for TCR Jβ gene segment usage by Vβ1$^+$/CD4$^+$ (A) and Vβ1$^+$/CD8$^+$ (B) T cells derived from eight myasthenia gravis patients (MG) and seven controls (HC) are illustrated. Individual values were obtained from blotting and hybridization of PCR products with a complete panel of ^{32}P-5'-end-labeled Jβ-specific probes. Resulting autoradiographs were then scanned and the relative expression of individual Jβ exons calculated.

symptoms. Basic and clinical observations suggest, however, the existence of additional parameters as key elements involved in the pathogenic mechanisms of the autoimmune response. Among these parameters, T lymphocytes are highly interesting. As already discussed in the introduction, several strong indications suggest a critical role for T cells in the pathogenesis of MG. Herein, we observe that MG patients regularly overexpress usage of the Vβ1, Vβ13.2, Vβ17, and Vβ20 gene families in both CD4$^+$ and CD8$^+$ PBL T cells. Whether this overexpression is a consequence of a numerical expansion of such Vβ gene-expressing normal T cells or of the presence of selectively activated, nonproliferating T cells, or of a combination of both factors, is the focus of an ongoing study.

CD4$^+$ T lymphocytes specific for a large number of various AChR epitopes have been frequently identified in healthy individuals (30–32). Thus, the mere presence of self-reactive

T cells cannot be responsible for the mechanisms underlying the development of MG. Rather, induction and maintenance of the pathogenic process of MG may be explained by immunoregulatory imbalance and activation of a particular set of anti-AChR-specific T cell clones (30). It can be envisaged that cross-reactivity (molecular mimicry) between a single AChR determinant and a microbial structure or even certain autologous proteins may initially trigger the autoimmune response. Then, during the course of development of manifest MG—through antigen determinant spreading—presentation of numerous distinct sequences of endogenous AChR will result in stimulation of specific CD4$^+$ T cells expressing pathology-related characteristics.

Alternatively, although not mutually exclusive with the molecular mimicry model, a recently described group of toxic proteins, designated superantigens (SA), may constitute a panel of candidate molecules by which certain infectious agents can mediate activation of autoreactive T cells, potentially leading to termination of the state of tolerance to self antigens and, eventually, inducing manifest autoimmune illness (reviewed in Ref. 14). The overall potency of SA stimulation is dependent upon the level of avidity of the dual recognition of the relevant $V\beta$ gene product and the MHC class II molecule. Most exogenous SAs induce activation of both $CD4^+$ and $CD8^+$ T cells expressing the specific $V\beta$ gene family products. Activation of α/β T cells by exogenous bacterial toxins, in the context of MHC class II, is mainly controlled by a high-avidity binding between the SA and the MHC class II molecule and the targeted $V\beta$ product. Conversely, SA preferentially activating $CD4^+$ T cells can compensate for low avidity binding via a co-binding of the CD4 molecule to the MHC class II (33).

Superantigen-activated T cell subsets expressing the relevant $V\beta(s)$ have as one additional characteristic distinguishing them from nominal antigen-driven oligoclonal expansions: a broad, normal relative expression of $J\beta$ multigene family members. We analyzed the $V\beta1$-overexpressing T lymphocytes in MG patients and determined that they behaved in concordance with superantigen activation in that their usage of $J\beta$ gene segments was equal in distribution pattern to normal control $V\beta1^+$ T cells. Hence, we reach the tentative conclusion that MG encompasses, as one reproducible element of the disease, a biased overusage of $V\beta1$, $V\beta13.2$, $V\beta17$, and $V\beta20$ exons within both $CD4^+$ and $CD8^+$ T cell subsets. We are unaware of any presently defined SA that selectively targets $V\beta1$-, $V\beta13.2$-, $V\beta17$-, and $V\beta20$-expressing T cells. The driving molecule is thus likely to be a novel SA, which is yet to be defined, and a search for this hypothetical molecule has now been initiated.

A problem associated with data indicating positive correlation between two parameters (in this case, induction/maintenance of the MG disease and overrepresentation of TCR $V\beta$s), relates to the question of whether the actual connection represents a causal or a consequential relationship. Nevertheless, our identification of a positively disease-associated overrepresentation of select $V\beta$s in the T cell pool within MG patients may provide new therapeutic strategies of intervention for myasthenia gravis.

ACKNOWLEDGMENTS

REFERENCES

1. Lindstrom J, Shelton D, Fujii Y. (1988) Myasthenia gravis. *Adv. Immunol.* **42:** 233–284.
2. Vincent A, Newsom-Davis J. (1980) Anti-acetylcholine receptor antibodies. *J. Neurol. Neurosurg. Psychiatry* **43:** 590–600.
3. Melms A, Schake B, Kirchner TH, Hermelink H, Albert E, Wekerle H. (1988) Thymus in myasthenia gravis. *J. Clin. Invest.* **81:** 902–908.
4. Åhlberg R, Pirskanen R, Lefvert AK. (1991) Defective T lymphocyte function in non-thymectomized patients with myasthenia gravis. *Clin. Immunol. Immunopathol.* **60:** 93–105.
5. Hohlfeld R, Toyka KV, Heininger K, Grosse-Wilde H, Kalies L. (1984) Autoimmune T-lymphocytes specific for acetylcholine receptor. *Nature* **310:** 244–246.
6. Hohlfeld R, Toyka KV, Tzartos SJ, Carson W, Conti-Tronconi BM. (1987) Human T-helper lymphocytes in myasthenia gravis recognize the nicotin receptor alpha subunit. *Proc. Natl. Acad. Sci. U.S.A.* **84:** 5379–5383.
7. Åhlberg R, Yi Q, Pirskanen R, et al. (1993) Clinical improvement of myasthenia gravis by treatment with a chimeric anti-CD4 monoclonal antibody. *Ann. N. Y. Acad. Sci.* **681:** 552–555.
8. Davis M, Bjorkman P. (1988) T cell antigen receptor genes and T cell recognition. *Nature* **334:** 395–402.
9. Drake C, Kotzin B. (1992) Superantigens: Biology, immunology and potential role in disease. *J. Clin. Immunol.* **12:** 149–161.
10. Woodland D, Blackman M. (1993) How do T cell receptors, MHC molecules and superantigens get together? *Immunol. Today* **14:** 208–212.
11. Marrack P, Kappler J. (1990) The staphylococcal enterotoxins and their relatives. *Science* **248:** 705–711.

12. Kappler J, Kotzin B, Herron L, et al. (1989) Vβ-specific stimulation of human T cells by staphylococcal toxins. *Science* **244**: 811–813.

13. Dellabona P, Peccoud J, Kappler J, Marrack P, Benoist C, Mathis D. (1990) Superantigens interact with MHC class II molecules outside of the antigen groove. *Cell* **62**: 1115–1121.

14. Friedman S, Posnett D, Tumang J, Cole B, Crow MA. (1991) Potential role for microbial superantigens in the pathogenesis of systemic autoimmune disease. *Arthritis Rheum.* **34**: 468–480.

15. Howell MD, Diveley JP, Lundeen KA. (1991) Limited T cell receptor β chain heterogeneity among interleukin-2 receptor positive synovial T cells suggests a role for superantigen in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U.S.A.* **88**: 10921–10925.

16. Davies TF, Martin A, Concepcion ES, Graves P, Cohen L, Ben-Nun A. (1991) Evidence of limited variability of antigen receptors on intrathyroidal T cells in autoimmune thyroid disease. *N. Engl. J. Med.* **325**: 238–244.

17. Davies TF, Martin A, Concepcion ES, et al. (1992) Evidence for selective accumulation of intrathyroidal T lymphocytes in human autoimmune thyroid disease based on T cell receptor V gene usage. *J. Clin. Invest.* **89**: 157–162.

18. Oksenberg JR, Stuart S, Begovich AB, et al. (1990) Limited heterogeneity of rearranged T cell receptor Vα transcripts in brain of multiple sclerosis patients. *Nature* **345**: 344–346.

19. Zouali M, Kalsi J, Isenberg D. (1993) Autoimmune diseases at the molecular level. *Immunol. Today* **14**: 473–476.

20. Grunewald J, Åhlberg R, Lefvert AK, Dersimonian H, Wigzell H, Janson CH. (1991) Abnormal T cell expansion and V-gene usage in myasthenia gravis patients. *Scand. J. Immunol.* **34**: 161–168.

21. Oosterhuis HJGH. (1964) Studies in myasthenia gravis: A clinical study of 180 patients. *J. Neurol. Sci.* **1**: 512–546.

22. Chomczynski P, Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156–159.

23. Grunewald J, Tehrani MJ, Pisa E, Janson CH, Andersson R, Wigzell H. (1992) Analysis of Jβ gene segment usage by CD4+ and CD8+ human peripheral blood T lymphocytes. *Int. Immunol.* **4**: 643–650.

24. Choi Y, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J. (1989) Interaction of *Staphylococcus aureus* toxin superantigens with human T cells. *Proc. Natl. Acad. Sci. U.S.A.* **86**: 8941–8945.

25. Doherty PJ, Roifman CM, Pan S, et al. (1991) Expression of the human T-cell receptor Vβ repertoire. *Mol. Immunol.* **28**: 607–612.

26. Sambrook J, Fritsch EF, Maniatis T (ed.) (1989) *Molecular Cloning. A Laboratory Manual.* 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

27. Tehrani MJ, Grunewald J, Hodara V, Andersson R, Wigzell H. (1994) Non-random TCR Jβ usage pattern in human CD4+ and CD8+ T cells. *Hum. Immunol.* **40**: 93–100.

28. Lu CZ, Link H, Mo XA, et al. (1991) Antipresynaptic membrane receptor antibodies in myasthenia gravis. *J. Neurol. Sci.* **102**: 39–45.

29. Mygland A, Tysnes O-B, Aarli JA, Flood PR, Gilhus NE. (1992) Myasthenia gravis patients with a thymoma have antibodies against a high molecular weight protein in sarcoplasmic reticulum. *J. Neuroimmunol.* **37**: 1–7.

30. Zhang Y, Schleup M, Frutiger S, et al. (1990) Immunological heterogeneity of autoreactive T-lymphocytes against the nicotinic acetylcholine receptor in Myasthenia patients. *Eur. J. Immunol.* **20**: 2577–2583.

31. Protti MP, Manfredi AA, Wu XD, et al. (1991) T epitopes on the δ subunit of human muscle acetylcholine receptor. *J. Immunol.* **146**: 2253–2261.

32. Protti M, Manfredi A, Wu X, et al. (1992) CD4+ T epitopes on the embryonic γ subunit of the human muscle acetylcholine receptor. *J. Clin. Invest.* **90**: 1558–1567.

33. Kotzin BL, Leung DYM, Kappler J, Marrack P. (1993) Superantigens and their potential role in human disease. *Adv. Immunol.* **54**: 99–166.