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Because myasthenia gravis (MG) is an autoimmune disease mediated by Abs specific for the acetylcholine receptor, helper T cells play a role in Ab production. In this study, we have performed large-scale cross-sectional and longitudinal TCR studies by CDR3 spectratyping using PBL and thymus tissues from MG patients. We found that there was no preferential usage of any particular TCR β-chains that was identical among MG patients. However, the longitudinal study clearly demonstrated that one or more TCR Vβ expansions persisted frequently in MG patients. Importantly, persistent TCR expansions correlated with clinical severity and high anti-acetylcholine receptor Ab titer. Finally, examinations of T cells expressing CXCR5, i.e., follicular B-helper T cells, revealed that spectratype expansions in MG patients were detected mainly in the CD4+ CXCR5+ T cell populations, whereas CD8+ T cells were the major source of clonal expansion in healthy subjects. These findings suggest that persistent clonal expansions of T cells in MG patients are associated with the development and maintenance of MG. Close examination of pathogenic T cells in MG provides useful information to elucidate the pathogenesis and to estimate the disease status. The Journal of Immunology, 2006, 176: 5100–5107.

Myasthenia gravis (MG) is an autoimmune disorder characterized by weakness and fatigability of skeletal muscles caused by Abs against the acetylcholine receptor (AChR). The Abs bind to AChR at the postsynaptic membrane, leading to receptor destruction and disturbance in neuromuscular transmission (1). Although it is clear that Abs against AChR in most patients are final effectors, T cells play an important role in helping the Ab production in MG. Another characteristic feature of MG is abnormalities in the thymus. Lymphofollicular hyperplasia of the thymic medulla occurs in 65% and thymoma is found in 15% of MG patients (2). Furthermore, it was demonstrated that AChR, a key molecule for the development of MG, is expressed in the thymus (3) and that thymus showing hyperplasia or with thymoma generate more T cells, including AChR-specific T cells (4–6). On the basis of these findings, it is generally believed that anti-AChR Abs are generated with the help of T cells in the thymus of MG patients. Thus, characterization of the AChR-specific T cells, especially their TCR usage, is essential for the elucidation of the pathomechanisms of MG and for the development of specific immunotherapy for the disease.

Materials and Methods

Patients and healthy subjects

Thirty-eight MG patients, 8 males and 30 females (mean age, 57.9 ± 15.5 years; range, 25–80 years), attending the Departments of Neurology, Nagasaki Medical Center of Neurology, or Nagasaki University Hospital were included in the present study. All subjects’ consent was obtained, and the study was approved by the Institute Review Board. The diagnosis of MG was made on the basis of the clinical presentation and confirmed by laboratory examinations, such as repetitive nerve stimulation tests.

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3 Abbreviations used in this paper: MG, myasthenia gravis; AChR, acetylcholine receptor; MS, multiple sclerosis.
anti-AChR, and computerized tomography images of mediastinum. For clinical assessment of the severity of the disease, the Myasthenia Gravis Foundation of America clinical classification (from class I: purely ocular disease to class V: defined by intubation) (14) and MG-activities of daily living (15) were used. Muscle-specific tyrosine kinase-seropositive MG patients were not included in this study. Forty-four healthy volunteers, 21 males and 23 females (mean age, 40.1 ± 17.6 years; range, 22–88 years) who had no episodes of common cold or influenza infection within the previous month were examined as controls. Ten milliliters of heparinized blood was drawn from patients and control subjects, and PBL was isolated using the density gradient method. Thymus tissues (n = 9) of MG patients were obtained by thymectomy. Six of them showed hyperplasia and three were uninvolved thymus adjacent to a thymoma.

Cross-sectional and longitudinal studies

The cross-sectional study was performed as described above. When the same person was examined more than once, the results of the first examination were included in the cross-sectional study. The longitudinal study was performed on 22 MG patients and 17 healthy subjects, and the results obtained from patients and subjects with sufficient follow-up periods were selected for analysis. The spectratype expansion pattern obtained in the longitudinal study was classified into the three categories: 1) heterogeneous, where no particular spectratype expansion was observed in multiple examinations in which the normal pattern was included frequently; 2) persistent, where spectratype expansion of particular Vβs was constantly observed during the observation period; and 3) intermediate, where a particular spectratype expansion was occasionally observed.

cDNA synthesis and PCR amplification

RNA was extracted from PBL using RNAzol B (Biotecx Laboratories). cDNA was then synthesized by reverse transcription using ReverTra Ace (Toyobo) and amplified in a thermal cycler (PerkinElmer) using primer pairs for TCR. Primers for Vβ1-24 were the same as those used in a previous study (16). Cα primer was labeled with Cy-5 or rhodamine or was left unlabeled.

CDR3 spectratyping

CDR3 spectratyping was performed as described previously (17). cDNA was amplified with Vβ-specific and rhodamine-labeled Cβ primers, and undiluted or diluted PCR products were added to an equal volume of formamide/dye loading buffer and heated at 94°C for 2 min. Two microliters of the samples was applied to a 6% acrylamide sequencing gel. Gels were run at 30 W for 3 h 30 min at 50°C. Then, the fluorescence-labeled DNA profile on the gel was directly recorded using an FMBio fluorescence image analyzer (Hitachi). Spectratype expansion was evaluated by visual inspection and density analysis of the image using software attached to the fluorescence image analyzer as shown in Fig. 1. C and D, and graded into two categories: 1)”Oligoclonal pattern,” appearing as an increase of the density and thickness of one band within a normal spectratype profile (distortion of the Gaussian distribution) (18); and 2)”Monoclonal” one, with a marked increase of the density and thickness of one band with faint or no additional spectratypes (18). We tested for contaminations by the reagents used in PCR every 10 PCR analyses by doing PCR without templates; if present, all reagents used and results obtained during the period were discarded.

Sequencing of PCR products

cDNA isolated from spectratypes of interest on the acrylamide gel was reamplified with Vβ and unlabeled Cβ primers to remove the fluorescence attached to the primer used for CDR3 spectratyping. This process was essential for cloning. To avoid biased amplification of a particular TCR clone, PCR was performed for only five cycles. Then, PCR products were ligated into pT-Adv vector and cloned using the Advantages PCR Cloning Kit (Clontech Laboratories) according to the manufacturer’s instructions. The plasmid DNA was then sequenced using Cβ5-labeled Cβ primer and Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing Kit on an ALFexpress DNA sequencer (Pharmacia Biotech). CDR3 length is defined as a region starting from an amino acid residue after the CASS sequence of most Vβ segments and ending before the GXG box in the Jβ region, as described previously (19).

Isolation and TCR analysis of CXCR5-positive T cells

Because the majority of CXCR5-expressing cells were CD4+ T and B cells (20), we decided to examine CD4+, CD8+, and CD4+CD8+ T cells. The number of CD8+CXCR5+ T cells was too small for the analysis. CD4+CXCR5+ T cells were isolated using an AutoMACS (Miltenyi Biotec) following the manufacturer’s instructions. In brief, we removed adherent cells and some B cells from MG and control PBL by negative selection with IF5 (anti-CD20) plus LM2/1.6.11 (anti-Mac-1) followed by anti-mouse IgG-microbeads (Miltenyi Biotec). The cells were then positively (the CD8+ T cell population) or negatively (the CD4+ T cell population) selected with OKT8. Finally, CD4+ T cells were further separated into CD4+CXCR5+ and CD4+CXCR5- T cells with anti-CXCR5 mAb.

**FIGURE 1.** CDR3 spectratyping profiles of PBL showing the normal spectratype pattern (A) in a healthy subject and spectratype expansion (B) in a MG patient. In the normal pattern, each Vβ shows a Gaussian distribution without any spectratype expansion (A). In contrast, this MG patient exhibits expansion of several spectratypes indicated by arrows (B). The profiles of Vβ4, Vβ8, and Vβ20 spectratypes of a healthy subject (A) and Vβ3, Vβ8, and Vβ14 spectratypes of a MG patient (B) revealed by the density analysis are shown in C and D, respectively.
(DakoCytomation) and anti-mouse IgG-microbeads. Three populations, CD4⁺, CD8⁺, and CD4⁺CD8⁻, were subjected to the TCR analysis. We determined the purity of isolated cells by flow cytometry after incubation of the cells with FITC-labeled anti-mouse IgG and confirmed that each population was >90% pure.

Results

Cross-sectional study of MG patients and healthy individuals by CDR3 spectratyping

We first performed a cross-sectional study of 38 MG patients and 44 healthy subjects to characterize the TCR repertoire in MG. Of 38 patients, six cases of the ocular type, 15 cases of MG with thymoma, 13 cases of the early onset, and two cases of the late onset were included. Our previous studies on neuroimmunological disorders revealed that a particular type of VB TCR, i.e., VB5.2, showed clonal expansion more frequently in MS (13), whereas there was no preferential usage of TCR in Guillain-Barre syndrome (21). When multiple examinations were performed on the same individuals, the results obtained in the first examination were included in the cross-sectional study. Representative spectratyping profiles are shown in Fig. 1 and the results summarized in Table I. Nearly half of controls (46.5%) showed a normal spectratype pattern (Fig. 1A). The density analysis revealed unskewed Gaussian distributions (Fig. 1C). In contrast, only a quarter of the MG patients showed the normal pattern (p < 0.05) (Table I). Remaining control and MG samples contained one or more expanded spectratypes (arrows in Fig. 1B). The distorted Gaussian distributions (VB3 and VB8 in Fig. 1D) and the monoclonal pattern (VB14 in Fig. 1D) were detected in the density analysis. In healthy subjects, Vβ9 spectratype expansions seemed to be more frequent compared with other Vβs, but there was no significant difference. In MG patients, 29 cases (77.3%) showed the expansion of one or more spectratypes (Table I). Although these were more frequent in the MG patients than in healthy subjects, they did not show preferential usage of a common Vβ, unlike MS.

We also examined nine thymus samples from MG patients. Histological examination demonstrated hyperplasia in six cases and normal looking tissues taken adjacent to thymoma in three cases. Interestingly, they all showed the normal spectratype pattern without any spectratype expansion (Fig. 2).

Longitudinal study of selected MG patients

We have currently performed longitudinal studies on 22 MG patients and 17 healthy subjects. Among them, we selected 16 MG and 11 control cases; we were able to examine MG patients for more than 2 years and healthy subjects for 1 year (Table II and Table IV). Representative profiles obtained from MG patients are illustrated in Fig. 3, and all of the results are summarized in Table II. Longitudinal studies of oligoclonal expansions of TCR revealed three behavior patterns. 1) In persistent type, expansions were detected in all samples, as illustrated in Fig. 3 and Table II. In Fig. 3, Vβ9 and Vβ11 expansion persisted over 1 year and 5 mo. Nine of 16 patients showed this type of spectratype expansion during the observation periods (patients 4, 5, 7, 8, 10, 11, 12, 13, and 15 in Table II). 2) In intermediate type, a particular spectratype expansion was intermittently observed (patients 6 and 9 in Table II).

We also examined HLA-DR haplotypes of MG patients subjected for the longitudinal study as shown in Table II (fourth column from the left). As far as we examined, there was no correlation between the HLA haplotype and TCR Vβ usage.

CDR3 sequences of TCR clones derived from expanded spectratypes in MG

In a series of previous studies in MS (13) and its animal models (17, 22), we demonstrated that persistently expanded spectratypes...
represent expansions of particular TCR clones. To test whether that also applies in MG, we did a similar analysis using PBL from patient 7 (Table II), which showed Vβ11 expansion over 2 years in 4 of 5 examinations. The expanded spectratypes were cut from the gel, and cDNA was extracted, cloned, and sequenced. As clearly demonstrated in Table III, we found a TCR clone with the CASS-ATPNEQFF-GPG sequence in the CDR3 throughout the 2 years. Evidently, it represents an expansion of a single T cell clone.

Table II. Clinical characteristics and spectratyping analysis of MG patients

| Patient | Age | Sex | N.E. | Thy-as | Invasive | Vβ | 11 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 1       | 68  | F   |       | Ocular  | NA       | -  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2       | 77  | F   | N.E.  | Ocular  | NA       | -  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3       | 67  | M   | 4/6/51| Ocular  | NA       | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4       | 68  | F   | N.E.  | Ocular  | NA       | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5       | 44  | F   |       | Early-onset | Hyperplasia | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6       | 61  | M   | 15/2  | Early-onset | Hyperplasia | -  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 7       | 58  | F   | 8/11  | Early-onset | Hyperplasia | -  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8       | 47  | M   | 4/16  | Early-onset | Hyperplasia | -  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9       | 52  | F   | 9/13  | Early-onset | Hyperplasia | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10      | 80  | F   |       | Late-onset | Hyperplasia | -  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 11      | 53  | F   | N.E.  | Thy-as  | Thymoma  | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 12      | 61  | F   | 9/13  | Thy-as  | Thymoma  | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13      | 77  | F   |       | Thy-as  | Thymoma  | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14      | 72  | F   | 9/8   | Thy-as  | Thymoma  | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15      | 46  | M   | 15/8  | Thy-as  | Thymoma  | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16      | 71  | F   | 9/10  | Thy-as  | Thymoma  | +  | -  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

*a* N.E., Not examined; N, the normal spectratype pattern; 1–24, the spectratype expansion of each Vβ.
Spectratype expansion found in healthy subjects was unstable and did not last for a long period. We performed similar longitudinal studies in elderly healthy subjects who occasionally show clonal T cell expansion (23–25) to look for differences from those in our MG patients. As can be seen in Table IV, persistent spectratype expansions were found in subjects in their seventies and transient ones in their fifties and sixties. These TCR expansions are thought to occur due to exogenous Ag stimulation/infections and to reduce the T cell diversity (26). Interestingly, one volunteer (37 years old) residing in the community had a persistent cough when first tested (not included in Table IV). As shown in Fig. 4A, there were Vβ2, 3, 6, 14, 20, and 24 expansions at that time point. Three months later when the cough had disappeared, the number of expanded spectratypes had reduced markedly (Fig. 4B). This change strongly suggests that spectratype expansions in middle-aged to elderly subjects can be elicited by exogenous Ag stimulation and may be short-lived.

We also tested for correlations between spectratype pattern and MG severity (Table V) or anti-AChR Ab titer. The persistent pattern was observed more frequently in the patients with grade III-V than in patients with grade I-II and controls (p < 0.02) (Table V). With regard to anti-AChR Abs, the persistent pattern seemed to be found more frequently in patients with the titers above 10 nM rather than below 10 nM (data not shown). Taken together, they suggest that clonal expansions of particular Vβs correlate with disease severity and are thus involved in pathogenesis of MG.

Analysis of CXCR5-positive T cells provides useful information for identification of pathogenic T cells in MG

These longitudinal studies on total T cells may indicate persistent TCR clonal expansions in autoimmune responses in MG patients. However, some persistent clonal expansions in healthy subjects were indistinguishable from those found in MG patients. To pursue that further, we spectratyped CD4+CXCR5+ T cells isolated from healthy subjects and MG patients (Table VI). Because these T cells reportedly act as helpers for follicular B cells (20), they might play an essential role in anti-AChR Ab production and in thymic hyperplasia, a classic sample of lymphoid neogenesis in autoimmune disorders. As shown in Table VI, the spectratype expansions found in three healthy subjects were mainly restricted to CD8+ T cells, as reported previously (26, 27), whereas in 2 of 3 cases CD4+ and CD4+CXCR5+ T cells showed normal spectratype patterns. Characteristically, in most MG patients, spectratype expansions found in the CD4+ T cell population were also detected in CD4+CXCR5+ T cells. These findings clearly demonstrate that

Table III. Amino acid and nucleotide sequences of the CDR3 of Vβ11 TCRs extracted from patient 7a

<table>
<thead>
<tr>
<th>Sample</th>
<th>Follow-up</th>
<th>Vβ</th>
<th>(N)D(N)</th>
<th>Jβ</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4129</td>
<td>0 mo5</td>
<td>CASS</td>
<td>A T P N E Q</td>
<td>F F GPG</td>
<td>11/13 (85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CASS</td>
<td>L S V Y A V</td>
<td>F F GDG</td>
<td>1/13 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CACS</td>
<td>A T H N E Q</td>
<td>F F GPG</td>
<td>1/13 (8)</td>
</tr>
<tr>
<td>4148</td>
<td>6 mo6</td>
<td>CASS</td>
<td>A T P N E Q</td>
<td>F F GPG</td>
<td>2/3 (66)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CASS</td>
<td>A T P N E R</td>
<td>F F GPG</td>
<td>1/3 (33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4609</td>
<td>A T H N E Q</td>
<td>F F GPG</td>
<td>9/10 (90)</td>
</tr>
<tr>
<td>4846</td>
<td>2 years</td>
<td>CASS</td>
<td>A T P N E Q</td>
<td>F F GPG</td>
<td>8/8 (100)</td>
</tr>
</tbody>
</table>

5 PCR products were extracted from Vβ11 spectratype showing expansion, and reamplified and the CDR3 were sequenced after cloning.

6 The first examination in the longitudinal examination.

7 Six months after the first examination.
the clonal expansions of T cells in MG patients are different from those in healthy subjects.

Discussion
A large body of evidence indicates that the final effectors in MG are autoantibodies directed against targets at the neuromuscular junction (28, 29). Moreover, Ag-specific Th cells are essential for the generation of myasthenogenic autoantibodies and for the development of experimental autoimmune MG (30, 31). Hence, there have been several attempts to characterize MG-associated T cells. However, the TCR usage of pathogenic T cells is very controversial.

Most studies used either FACS analysis with anti-TCR mAbs or CDR3 spectratyping. By FACS analysis using PBL or thymic cells, Vβ 4.1- (7), Vβ12- (9), and Vβ13- (11) positive T cells were reported to be significantly increased in MG patients. In our experience, however, this method is not sufficiently sensitive for TCR analysis of pathogenic T cells in autoimmune diseases. In EAE induced in Lewis rats by immunization with myelin basic protein, several lines of evidence indicate that encephalitogenic T cells mainly use Vβ8.2. Clonal expansion of this cell type was detectable by CDR3 spectratyping in the lymphoid organ at the very early stages of the disease (32). In sharp contrast, by flow cytometry, this expansion could not be detected in the lymphoid organ throughout EAE (33). Vβ8.2 expansion was only detectable at the peak of EAE in the CNS, where Vβ8.2-positive cells accounted for >20% (33). This is mainly because the numbers of circulating encephalitogenic T cells are too small for detection by FACS analysis. So far, two CDR3 spectratyping studies have focused on the TCR repertoire in MG. Navneetham et al. reported that Vβ4 and Vβ6 were used significantly more in MG patients than in controls (10). In contrast, Infante et al. (12) found no significant difference in the TCR repertoire between MG patients and control subjects. The inconsistencies between these two reports and between these reports and our results may be largely attributable to the number of patients and controls and to the presence or absence of clinical symptoms.
absence of the longitudinal examination. In previous studies, the number of cases for the analysis was too small, and each case was examined only once.

We now summarize intriguing findings from the large-scale cross-sectional and longitudinal analyses of PBL and thymus taken from MG patients and healthy subjects by CDR3 spectratyping. First, TCR spectratype expansions were more frequently found in MG patients than healthy subjects (\( p < 0.05 \)), though without preferential usage of any particular TCR \( \beta \)-chains. Second, the longitudinal study clearly demonstrated persistent TCR \( \beta \)-chains more frequently in the patients with severe MG. Although persistent spectratype expansions varied from patient to patient, they correlated with severe clinical status and high anti-AChR Ab titers. Finally, examination of CXCR5\(^+\) T cells may help to focus on pathogenic Th cells in MG. In MG patients, spectratype expansions were found in CD4\(^+\) and CD4\(^+\)CD8\(^-\)CXCR5\(^+\) T cells, whereas spectratype expansion seen in healthy subjects was observed in CD8\(^+\) T cells. We have tried to exclude the possibility that preceding infection occurred in MG patients may induce spectratype expansion unrelated to the MG pathogenesis as follows. For this purpose, we have checked all of the patients subjected for the longitudinal study whether they have clinical signs and laboratory data suggesting infection. All of the patients did not have fever and increased white blood cells at the time of spectratype examinations. Only patient 12 at the second examination exhibited slightly increased C-reactive protein. However, it is unlikely that this would modulate the results of CDR3 spectratyping, because the results at the second examination were almost the same as those obtained in other examinations. Taken together, the present study strongly suggests that T cells showing persistent clonal expansion are involved in the development and maintenance of MG.

It should be noted that all nine thymus tissues, including six hyperplastic thymi, exhibited normal spectratype patterns. Although the thymus has previously been implicated as a possible site of the disease origin (~75% of patients have thymic abnormalities) (1), its role in MG is still a mystery (29). Our findings from CDR3 spectratyping suggest either that the thymus includes no clonally expanded pathogenic T cells or that their frequency is too low to detect even using this method. Importantly, >30% of

### Table V. Classification of the spectratyping pattern in the follow-up study of MG patients and healthy subjects

<table>
<thead>
<tr>
<th>Classification</th>
<th>Total</th>
<th>Grade III–V(^a)</th>
<th>Healthy Subjects(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent</td>
<td>9 (56%)</td>
<td>7 (78%)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2 (13%)</td>
<td>1 (11%)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Heterogeneous</td>
<td>5 (31%)</td>
<td>1 (11%)</td>
<td>7 (63.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^a\) The numbers of Grade III–V MG patients showing the persistent pattern and healthy subjects showing the heterogeneous pattern are significantly higher than that of their counterparts (\( p < 0.02 \) by \( \chi^2 \) test).

### Table VI. CDR3 spectratyping analysis of CD4\(^+\), CD8\(^+\), and CD4\(^+\)CXCR5\(^+\) T cells isolated from healthy subjects and MG patients

<table>
<thead>
<tr>
<th></th>
<th>CD4(^+) T Cells</th>
<th>CD8(^+) T Cells</th>
<th>CD4(^+)CXCR5(^+) T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>N(^a)</td>
<td>1+, 6+, 16+</td>
<td>N(^a)</td>
</tr>
<tr>
<td>2</td>
<td>8+, 24+</td>
<td>8+, 24+</td>
<td>8+</td>
</tr>
<tr>
<td>3</td>
<td>N(^a)</td>
<td>11+, 16+</td>
<td>N(^a)</td>
</tr>
<tr>
<td>MG patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2+, 5.1+,</td>
<td>9+, 11+, 12+</td>
<td>2+, 11+</td>
</tr>
<tr>
<td>2</td>
<td>6+, 11+, 16+</td>
<td>11+, 13+, 16+, 20+, 22+, 23+</td>
<td>11+, 16+</td>
</tr>
<tr>
<td>3</td>
<td>1+, 3+, 5.2+, 9+</td>
<td>6+, 13+, 14+, 20+, 21+</td>
<td>3+, 5.2+, 6+, 9+</td>
</tr>
<tr>
<td>4</td>
<td>11+</td>
<td>3+, 6+, 8+, 11+, 15+</td>
<td>11+</td>
</tr>
</tbody>
</table>

\(^a\) N, Normal spectratype pattern.
MG patients do not show clinical improvement after thymectomy (34). Collectively, it is possible that the thymus plays a role in exacerbation of the disease in some, but not all, MG patients. In this regard, recent progress in elucidating the mechanism of lymphoid neogenesis provides useful information for the understanding of the relationship between thymic abnormalities and MG. As reviewed by Weyand et al. (35), ectopic germinal centers are formed in several organs in the presence of autoantigens, CXCL13-producing tissue cells, follicular dendritic cells, and B and T cells. Although it remains to be elucidated whether all of these factors are present in the thymus of MG patients, it is possible because AChRs are found in the thymic myoid cells (36).

In a series of reports, Berrih-Aknin and colleagues (7, 37) have insisted that Vβ5.1-positive T cells are pathogenic in MG, and that circulating anti-Vβ5.1 Abs may regulate the excess of pathogenic Vβ5.1-positive T cells (8). This conclusion was drawn on the basis that by FACS analysis, Vβ5.1-positive T cells were slightly but significantly increased in number in the thymus of MG patients. However, this finding was not confirmed by other groups using flow cytometry and CDR3 spectratyping. Similarly, we have not observed significant Vβ5.1 clonal expansion in this study. Taking all these findings into consideration, it can be said that the conclusion by Berrih-Aknin et al. (7, 37) remains unestablished.

In summary, we were able to identify disease-associated and clonally expanded T cells in patients with MG by CDR3 spectratyping. Close examination of pathogenic T cells in MG provides useful information to elucidate the pathogenesis and to estimate the disease status.

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Disclosures

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