A Pathogenetic Role for the Thymoma in Myasthenia Gravis

Autosensitization of IL-4–producing T Cell Clones Recognizing Extracellular Acetylcholine Receptor Epitopes Presented by Minority Class II Isotypes

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Abstract

Myasthenia gravis (MG) is caused by helper T cell–dependent autoantibodies against the muscle acetylcholine receptor (AChR). Thymic epithelial tumors (thymomas) occur in 10% of MG patients, but their autoimmunizing potential is unclear. They express mRNAs encoding AChR α and ε subunits, and might aberrantly select or sensitize developing thymocytes or recirculating peripheral T cells against AChR epitopes. Alternatively, there could be defective self-tolerance induction in the abundant maturing thymocytes that they usually generate. For the first time, we have isolated and characterized AChR-specific T cell clones from two MG thymomas. They recognize extracellular epitopes (α75–90 and α149–158) which are processed very efficiently from muscle AChR. Both clones express CD4 and CD8α, and have a Th-0 cytokine profile, producing IL-4 as well as IFN-γ. They are restricted to HLA-DP14 and DR52a; expression of these minority isotypes was strong on professional antigen-presenting cells in the donors’ tumors, although it is generally weak in the periphery. The two clones’ T cell receptor β chains are different, but their α chain sequences are very similar. These resemblances, and the striking contrasts with T cells previously cloned from non-thymoma patients, show that thymomas generate and actively induce specific T cells rather than merely failing to tolerate them against self antigens. (J. Clin. Invest. 1998. 101:2268–2277.) Key words: paraneoplastic autoimmunity • thymic epithelium • autoimmune T cells • HLA-DR isotypes • HLA-DP

Introduction

Very little is known about the initiation of most autoimmune diseases, but some of them regularly associate with particular tumors (1, 2). For example, ~5% of patients with epithelial thymomas have autoimmune bone marrow aplasias or neuromyotonia, and around one third develop myasthenia gravis (MG),1 a classic autoantibody-mediated disorder (3, 4). All MG patients with thymoma have antibodies to the extracellular domain of the acetylcholine receptor (AChR) that are responsible for their muscle weakness (4). This well-characterized autoantigen comprises α2, β, γ, and δ subunits in the human fetus and α2, β, ε, and δ subunits in the adult, all of which have been cloned and sequenced (5). These patients also have other autoantibodies to a range of striatal muscle antigens, including actin, myosin (6), and, most frequently, titin (7).

One general hypothesis to explain the link between thymomas and autoimmune disease is that autoantigenic epitopes expressed on the neoplastic epithelial cells positively select helper T cells maturing in the tumors, or actively sensitize either their progeny or recirculating peripheral T cells (4). An alternative theory is that, since the thymomas usually generate numerous thymocytes in a disorganized thymic cortical environment (8), they might merely fail to delete or tolerize potentially self-reactive T cells (9). In either case, since B cells are generally rare in thymomas (8), it is likely that the T cells subsequently initiate autoantibody responses in the periphery.

The first hypothesis depends on the presence of autoantigen in the thymoma. Whereas some striatal epitopes have been detected in these tumors (10, 11), evidence for expression of AChR in these cells is conflicting. Monoclonal antibodies (mAbs) against the extracellular AChR epitopes that are recognized by MG autoantibodies (12) do not label thymoma tissue (13), indicating that the whole pentameric AChR is not expressed. Nevertheless, mRNAs for individual AChR subunits have been detected in thymoma tissue by PCR (14–16), and for the adult-specific ε subunit by less sensitive RNAase protection assays (MacLennan, C., D. Beeson, N. Willcox, A. Vincent, and J. Newsom-Davis, manuscript in preparation). Moreover, one group of mAbs against α371–380 of the cytoplasmic loop of the AChR ε subunit bind to the neoplastic epithelial cells (13), although they are apparently cross-reacting with a neurofilament protein (17, 18). Since helper T cells might thus be sensitized by epitopes processed from individual subunits in these thymomas, their characterization should help to identify the original immunogen.

As yet, very few T cells with rigorously proven AChR- and AChR peptide–specificity have been characterized from MG patients in general (19), and none from those with thymomas,

1. Abbreviations used in this paper: AChR, acetylcholine receptor; APC, antigen-presenting cell(s); MG, myasthenia gravis; MIR, main immunogenic region; PBLx, 30 Gy-irradiated PBL; PPD, purified protein derivative (of tuberculin); PVS, perivascular space; TCR, T cell receptor(s).
Table I. Clinical Details of Patients TA and TB and Responses of Their Fresh T Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Onset age</th>
<th>anti-AChR titer</th>
<th>MG grade</th>
<th>MG duration</th>
<th>Steroid</th>
<th>PBL</th>
<th>Thymoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yr</td>
<td>nMol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient TA</td>
<td>17</td>
<td>34.4</td>
<td>Severe generalized</td>
<td>3</td>
<td>—</td>
<td>2.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Patient TB</td>
<td>43</td>
<td>&gt; 34.4</td>
<td>Moderate generalized</td>
<td>3</td>
<td>4 wk</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

The response of the T cells is expressed as stimulation index (S.I.) which is the \[^{[H]}\text{H}]\text{thymidine uptake in the presence of antigen divided by the \[^{[H]}\text{H}]\text{thymidine uptake of the cells alone.}

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Denmark), or with anti-CD8αβ (H57) followed by FITC-goat antimouse IgG, and then washed. They were then analyzed by flow cytometry, using a FACScan® with Cell Quest software (Becton Dickinson, Cowley, United Kingdom).

To block T cell recognition and for immunohistology, we also used monomorphic anti–HLA-DR (L243), anti-DQ(L2) and anti-DP (B7-21) mAbs (25), the polymorphic anti-DR3/DR52a mAb 16.23 (26), and mAbs to TcR Vβ2 (27) and Vβ5.2/5.3 (28). Frozen sections (6 μm) were stained by a two-step indirect peroxidase method with a combination of 3-3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., Ltd.) and nickel chloride as chromogen (29). For double staining, the same method was used to label for CD68 (Y182; DAKO A/S) or for class II isotypes. The sections were then stained with anti-Vβ mAbs and 3-amino-9-ethylcarbazole (DAKO A/S) as chromogen. Each mAb was applied for 30 min, followed by washing and 15 min incubation with chromogen.

**TCR gene usage.** RNA was extracted from 5 × 10⁶ T cells (Cinna/ Biotecx Laboratories, Witney, United Kingdom). The first strand

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**Figure 1.** (A) Epitope mapping of T cell line (solid bars) and clone TA-1 (open bars) from thymoma TA. T cells were cocultured with autologous PBLx plus recombinant (r) or peptide (p) AChR α subunit antigens; the former were used at 0.5 μg/ml, the stimulatory peptides at 1 μg/ml, and the others at 10 μg/ml. The human AChR α68–90 sequence is shown above. The line was tested after 3 mo maintenance, and gave no detectable PPD response (not shown). (B) Epitope mapping of thymoma-derived T cell clone TB-2. Responses to recombinant (r or pET) polypeptides (1 μg/ml) and synthetic peptides (5 μM) are shown as in (A). The human α144–163 sequence is shown above. Neither clone responded to Torpedo AChR or its recombinant α subunit (25, 32). In each case, the human sequence includes known motifs for HLA-DP or DR52a (32).
Results

Characterizing T cell lines and clones. T cell lines were grown from thymoma lymphocytes of 18 MG patients by stimulation with full-length recombinant AChR α subunit using autologous PBLx as APC, and clones were subsequently grown from nine of them. With one strong responder (TA) and one modest responder (TB), both with a recent onset of MG (Table I), we were successful in mapping epitopes with synthetic peptides (see below), perhaps because of higher precursor frequencies or a greater sensitivity to the low concentrations of antigen available. Even in these cases, there was a tenfold excess of irrelevant clones (e.g., reacting to the IL-2 by itself or to E. coli polypeptides, despite careful antigen purification; reference 25) as were obtained exclusively from the other seven thymomas.

Epitope mapping. Both the TA and TB lines and clones proved to be specific for epitopes from the extracellular (α1–210) domain (Fig. 1, A and B). The slow-growing line from thymoma TA showed a consistent response to r37–181 that was gradually enriched while that to PPD disappeared. Using recombinant polypeptides, we mapped the epitope near to position 86 for both this line and its TA-1 clone (Fig. 1 A). Recognition of three independent overlapping synthetic peptides, p62–90, p73–90, and p75–115, but not of the adjacent peptides (see below), perhaps because of higher precursor frequencies or a greater sensitivity to the low concentrations of antigen available.

One line initiated from thymoma TB against r3–181 responded significantly and was cloned after 2 wk. Two clones (TB-2 and TB-3) proved to be specific for the α130–178 region; their maximal responses mapped the epitope core to the α149–158 region for both (Fig. 1 B). Clone TB-2 was characterized in detail.

Responses to human AChR. AChR-specific T cell clones consistently respond well to minute amounts of whole AChR extracted from human muscle and captured onto immunomagnetic beads by specific mAbs (22, 24). In two experiments, responses of TA-1 to beads-mAb-AChR reached 26–58% of the maximum seen with r37–181 (Fig. 2 A). The TB-2 clone also showed consistent and specific responses to similar amounts of AChR (Fig. 2 B). Recently, with more concentrated AChR from the ε subunit-transfected TE671 cells (31), stimulations have regularly reached 60% of the maximum (not shown).

The restricting MHC elements. Both clones proved to use minority presenting class II molecules. Initially, their responses (with autologous PBLx) were blocked almost impar-}

<table>
<thead>
<tr>
<th>Presenting B cell line</th>
<th>Percent of maximum response</th>
<th>HLA-DPα</th>
<th>HLA-DPβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>100±1.7</td>
<td>0201 Gln Arg Ala 1401 His</td>
<td>01 Met Gln Thr 0401 Phe</td>
</tr>
<tr>
<td>Mother</td>
<td>94±6.6</td>
<td>0201 Gln Arg Ala 1401 His</td>
<td>0201 Met Gln Thr 0901 His</td>
</tr>
<tr>
<td>Father</td>
<td>0.9±0.05</td>
<td>01 Met Gln Thr 0301 Tyr</td>
<td>01 Met Gln Thr 0401 Phe</td>
</tr>
<tr>
<td>KAS011</td>
<td>70±8.9</td>
<td>0201 Gln Arg Ala 1401 His</td>
<td>01 Met Gln Thr 0401 Phe</td>
</tr>
</tbody>
</table>

The indicated B cell lines were prepulsed with p73–90 (at 5 μg/ml) for 3 h before washing and coculturing with Clone TA-1. Shown at the right are the HLA-DP α/β allele combinations; also shown are the only sequence differences between the 0201/1401 combination that presents antigens to this clone and the very similar paternal 01/0301 that does not. DPβ 0401 also differs at 12 other positions in addition to those shown here.

Figure 2. Responses of thymoma-derived clones TA-1 (A) and TB-2 (B) to human AChR. Muscle AChR was incubated with Dynabeads 450 precoated (or not) with mAb B3 (specific for human AChR) before culturing with T cells plus autologous PBLx (22).

Table II. HLA-DP Restriction of Clone TA-1

<table>
<thead>
<tr>
<th>Presenting B cell line</th>
<th>Percent of maximum response</th>
<th>HLA-DPα</th>
<th>HLA-DPβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>100±1.7</td>
<td>0201 Gln Arg Ala 1401 His</td>
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</tr>
<tr>
<td>Mother</td>
<td>94±6.6</td>
<td>0201 Gln Arg Ala 1401 His</td>
<td>0201 Met Gln Thr 0901 His</td>
</tr>
<tr>
<td>Father</td>
<td>0.9±0.05</td>
<td>01 Met Gln Thr 0301 Tyr</td>
<td>01 Met Gln Thr 0401 Phe</td>
</tr>
<tr>
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</tbody>
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The restricting MHC elements. Both clones proved to use minority presenting class II molecules. Initially, their responses (with autologous PBLx) were blocked almost impar-
tive results, even though his DPB1*0301 differs only at one position and his DPA1*01 only at three (Table II).

For the TB-2 clone, use of a panel of B cell lines pre-pulsed with p144-163 identified HLA-DR52a as the restricting element (Fig. 3). The donor is heterozygous for HLA-DR3/DR52a and DR13/DR52b. Lines that shared only DR13/DR52b or DR3/DR52b failed to present this peptide, whereas those with DR52a gave maximal stimulation regardless of whether they were DR3+. Moreover, responses were clearly blocked by the polymorphic mAb 16.23 that is specific for DR52a and DR3 (reference 26, Fig. 3).

Surface markers. Surprisingly, both clones showed moderate labeling for CD8α in addition to the expected strong CD4 expression (Fig. 4a), although they did not stain with mAbs specific for CD8αβ (Fig. 4b) or CD8β (not shown). In the original line from patient TA, ~20% of the cells were CD4+. No double positivity was seen in another AChR-specific clone, PM-Al raised from hyperplastic MG thymus (Fig. 4), or in four from PBL of MG patients without a thymoma, all of which have a Th1 phenotype (32).

TCR gene usage. We found a single potentially functional TCR αβ chain combination in each clone (Table III). Notably, whereas their Vβ sequences were different, they used the same AV1S2 and J17S2 germline gene segments; the intervening junctional regions were both short, and differed at only three amino acid positions (Table III). We also found that a mAb to Vβ2 blocked responses of clone TB-2 to appropriate peptide or recombinant antigens by 93 and 99%, respectively. There was no inhibition with an anti-Vβ 13.1 mAb (not shown).

Cytokine profiles. The cytokine profiles of both clones showed a Th0 pattern when assayed serially for IFN-γ and IL-4 secretion after antigen stimulation with PBLx as APC (Fig. 5). There was clear production of both cytokines that correlated well with the proliferative responses, but reached maximum by 16–24 rather than 48–72 h, especially for IL-4 (Fig. 5).

In keeping with this Th0 behavior, the addition of IL-4 (25 ng/ml) on day 0 of the growth cycle enhanced both the expansion of clone TA-1 by two- to fivefold and its subsequent responsiveness to antigens (including purified AChR) by three- to fourfold. These trends were similar but weaker with clone TB-2 (not shown).

The APC activity of thymoma epithelial cells. To explore the autosensitizing potential of the neoplastic epithelial cells, these were cultured from thymoma TB in parallel with fibro-
Table III. TCR Junctional Region Sequences of Clones TA-1 and TB-2

<table>
<thead>
<tr>
<th>Clone</th>
<th>V</th>
<th>N</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA-1</td>
<td>TCRα</td>
<td>AV1S2</td>
<td>C A V  S V  G Y Q  K V  T F  J17S2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tgtgctgtgagtg  gggtaccaagaagttaccttt</td>
</tr>
<tr>
<td>TB-2</td>
<td>TCRα</td>
<td>AV1S2</td>
<td>C A V  S G  S G  Y Q  K V  T F  J17S2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tgtgctgtgagtg  ggctgctgagtg  gggtaccaagaagttaccttt</td>
</tr>
<tr>
<td>TA-1</td>
<td>TCRβ</td>
<td>BV20S1</td>
<td>A W S  V  R T  G L  S G  K L F  J1S4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>gcctggagtgt  agcagacagcccagcc  aaaaagctgttt</td>
</tr>
<tr>
<td>TB-2</td>
<td>TCRβ</td>
<td>BV25S1</td>
<td>S A S  G  V  T  G  Y  E  Q  Y  F  J2S7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>agtgctag  cggagtgcagcagaa  cctacagcagacttc</td>
</tr>
</tbody>
</table>

Nucleotide and predicted amino acid sequence of the CDR3 region of TCRα and TCRβ transcripts from clones TA-1 and TB-2. TCRα sequences are shown between the conserved cysteine at the 3’ end of the TCRαV transcript and the conserved phenylalanine residue in TCRβJ transcripts. Germline TCRα and probable germline TCRβ sequences are separated from nucleotides added by N region addition, as also for TCRβ. TCRβ sequences are shown after the conserved cysteine at the 3’ end of TCRβV and including the conserved phenylalanine in TCRβJ. Germline TCRβV and TCRβJ sequence is separated from the rest of the CDR3 sequence. Probable contributions from TRBD segments are underlined. Because of the striking similarity in TCRαV sequences, we tested whether clone TB-2 could recognize p144–163 or p75–115 presented by the DPB1*1401+ B cell line of patient TA, but the results were negative (not shown).

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Blasts from the tumor capsule. When pretreated with IFN-γ (to induce class II expression), the epithelial cells presented added antigens much better than the autologous clone TB-2 than did his fibroblasts (Fig. 6). Moreover, they were capable of processing long polypeptides (r3–181, Fig. 6 A). Notably, the epithelial cells also evoked greater production of both IL-4 and IFN-γ than did PBLx (Fig. 6, A and B), whereas the proliferative responses were stronger with PBLx (Fig. 6 C). However, there was no detectable response in the absence of added antigen.

Autosensitizing cell types in the donor thymomas. We finally scrutinized the donor tumors for potential sites of autosensitization by immunolabeling for the presenting class II molecules and also, in TB, for the Vβ2 used by his TB-2 clone. The HLA-DP isotype is expressed weakly by peripheral APC (33, 34) and lymphoid tissues, but more strongly in the thymus (35). Tumor TA was a typical cortical thymoma. All three class II isotypes were expressed strongly on the numerous scattered CD68+ macrophages and in the occasional medullary areas, but HLA-DP and DQ were more variable on the cortical epithelial cells (as in other MG thymomas, not shown). There were also rare class II+ B cell foci in the perivascular spaces (PVS; not shown). Thus, there appeared to be frequent opportunities for T cells to encounter HLA-DP molecules on both epithelial cells and professional APC.

In contrast, professional APC were the most conspicuous class II-expressing cells in thymoma TB. As expected after corticosteroid pretreatment (8), this tumor contained very few CD1+ thymocytes, and consisted largely of epithelial cells, but these expressed class II antigens more weakly than in other pretreated cases. The CD3+ T cells were also sparse in the tumor parenchyma, and were more concentrated in the PVS. The numerous CD68+ macrophages were strongly class II+ expressing DR52α/DR3—in both sites. They appeared to be preferentially contacted by Vβ2+ T cells in particular (Fig. 7 A), rather than Vβ5.2+ cells (Fig. 7 B), as did the rare foci of CD19+ B cells observed in the larger PVS (Fig. 7, C and D); however, we saw no selective Vβ2 expansion in any site. We had no other thymomas matched for HLA and steroid pretreatment for further comparisons.

Discussion

We have cloned and characterized, for the first time, AChR-specific T cells from MG thymomas by stimulation with recombinant human AChR α subunit. Both clones recognize epitopes from the extracellular domain of the receptor that can be processed very efficiently from the whole molecule. They are highly specific for the human (rather than electric fish) sequences, and for the correct class II alleles that present them. These both proved to be minority isotypes that were expressed more strongly on professional APC in the donor thymoma than on the neoplastic epithelium. Both clones have a CD4+ CD8– surface phenotype, a Th0 cytokine profile, and use TCRs with very similar Vα-Jα sequences; these characteristics distinguish them from the clones we isolated similarly from other MG patients without tumors (32), and suggest that specific T cells are generated and actively sensitized in MG thymomas where they acquire a helper phenotype. Therefore they favor the hypothesis of active T cell selection/sensitization in thymomas, and argue against that of a simple failure to tolerize developing thymocytes.

Although both T cells were selected against the whole AChR α subunit, their epitopes, α75–90 and α149–158, derive from the extracellular domain of the native protein that is also recognized by the patients’ B cells and serum anti-AChR antibodies (12, 36, 37). The intact conformation of this domain has never been detected in thymomas (13). However, since the expression of mRNAs encoding both the AChR α subunit (14–16) and especially the c subunit (MacLennan, C., D. Beeson, N. Wilcox, A. Vincent, and J. Newsom-Davis, manuscript in preparation) might lead to the production of small amounts of AChR subunit polypeptides in thymomas, it will be important to investigate both their cellular distribution in the tumors and whether it correlates with specific T cell responses to them. Whereas previous studies have shown expression of an AChR-
like epitope resembling the cytoplasmic sequence α371–380 in MG thymomas (13), we have found no evidence of either T cell or antibody responses to the entire cytoplasmic domain in thymoma/MG patients (38). Indeed, this epitope is now believed to be on a neurofilament chain rather than any AChR subunit (17, 18).

Remarkably, both clones use the same TCRAV and TCRAJ gene segments. That is unlikely to have occurred by chance, since over 50 functional TCRAV gene segments and over 60 TCRAJ gene segments are available. Their preferential pairing is a possibility, but has not been seen in over 500 TCRAVJ sequences analyzed to date (30, 39; our unpublished observations) or in the GenEMBL database. However, preferential selection of certain TCRAV/AJ combinations might be more likely in the T cells developing in this abnormal environment. Interestingly, both TCRAV/AJ transcripts have very few N region nucleotides at the V–J junction, whereas, in the TCRBVJ segments (which rearrange before the TCRAVJ), these N regions appear normal in length (Table III). There are known to be abnormalities in the development, especially of CD4⁺ thymocytes, in MG thymomas (40). Concomitant changes, such as an accelerated maturation, could differentially affect TCRAV rearrangement.

By contrast, the presence of CD8α on both clones probably...
expression might render these molecules (and their resident peptides) less likely to induce tolerance in developing thymocytes. However, both were expressed so strongly by the professional APC in the donor tumors that active sensitization seems likelier. This preference for minority restricting elements might help to explain why no clear associations with any HLA alleles have been reported in MG patients with thymoma, in contrast with other MG subgroups. Associations with minority isotypes, especially at or near HLA-DR, could easily have been overlooked, particularly if they are as allele-specific as the DP-restriction of clone TA-1 (Table II).

Thymoma epithelial cells evidently have significant antigen-presenting potential (Fig. 6), though other evidence suggests that the professional APC may be the likelier autorecognizing cell type (see below). The epithelial cells were clearly HLA-DR+ in thymoma TA, and might have been more strongly class II+ before the corticosteroid pretreatment in TB. Moreover, when cultured from the latter, and pre-treated with IFN-γ (43), they not only presented peptides very well, but also processed longer antigens effectively, evoking even greater cytokine production than PBLx by the autologous clone TB-2. However, this T cell showed no detectable recognition of endogenously processed epitope in these epithelial cells; to compare these cells with the professional APC in the same thymoma would be a critical experiment. Ideally, one would use an even more sensitive T cell such as PM-A1 (22, 25), though its restricting HLA-DR allele is rare.

The key features common to all of these histologically variable tumors in MG may be the presence of developing thymocytes and the potent presenting activity for muscle autoantigens (44). Several arguments incriminate the professional APC as agents provocateurs. They were more consistently class II positive than the epithelial cells, especially in thymoma TB, where they made particularly close contacts with T cells expressing the same Vβ2 as his clone TB-2. Furthermore, in chimeric laboratory rodents, these APC can mediate unnatural positive selection, and generate an abnormally broad repertoire of autoreactive T cells (45). They may be further implicated by our recent finding of high neutralizing antibody titers against IFN-α and IL-12 (46). Both of these are mainly produced by professional APC, which could be immunizing not only against muscle antigens but also against the cytokines themselves.

Because both IFN-α and IL-12 normally bias towards Th1 responses, their neutralization may help to explain the Th0 behavior of our two specific clones. It contrasts sharply with the clearly Th1 phenotype of the six T cells we have cloned from other MG patients without thymomas, which are all CD4+ CD8- (39), and are unequivocally Th1 (producing no detectable IL-4; reference 32). Only one of these (PM-A1) was derived from a hyperplastic MG thymus (despite multiple attempts); it might have been more strongly class II positive than the epithelial cells, especially in thymoma TB, where they made particularly close contacts with T cells expressing the same Vβ2 as his clone TB-2. Furthermore, in chimeric laboratory rodents, these APC can mediate unnatural positive selection, and generate an abnormally broad repertoire of autoreactive T cells (45). They may be further implicated by our recent finding of high neutralizing antibody titers against IFN-α and IL-12 (46). Both of these are mainly produced by professional APC, which could be immunizing not only against muscle antigens but also against the cytokines themselves.

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Several other lines of evidence point to the potential pathogenicity of our thymoma-derived T cell clones. The α149–158 region recognized by clone TB-2 is an important pathogenic
epitope in laboratory mice (47). Perhaps because of its very efficient processing from whole AChR (Fig. 2), it is also a recurring natural epitope for human T cells, including PM-A. The TA-1 clone is also very sensitive to whole AChR. Its epitope is close to the a67–76 sequence which contributes to the main immunogenic region (MIR; references 36 and 48) that many MG patients’ autoantibodies recognize. Thus, T cells such as TA-1 and TB-2 would probably be efficient helpers for MIR-specific B cells. In this context, it is interesting that we found occasional perivascular B cell foci in both thymomas. These have been noted previously, and so has sporadic anti-AChR antibody production in culture (reference 49, as seen with thymoma TA, not shown). The infiltration of these foci by antibody production in culture (reference 49, as seen with occasional perivascular B cell foci in both thymomas. These have been noted previously, and so has sporadic anti-AChR antibody production in culture (reference 49, as seen with thymoma TA, not shown). The infiltration of these foci by B cells (class II; C and D; arrows), there are several contacts with VB2+ T cells (C), whereas VB5.2/5.3+ T cells are less frequent (D). This donor had been pretreated with daily corticosteroids for 4 wk.

In conclusion, both the autoepitopes expressed in MG thymomas and the responding T cells are providing important etiological clues. Our findings already argue strongly in favor of biased selection/active sensitization by professional APC in thymomas, rather than a mere failure to tolerize developing T cells apparently contact this VB2+ T cells is intriguing, though it might merely reflect a general preference of VB2+ TCR for HLA class II rather than class I (27).

Acknowledgments

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References

Autoimmune T Cell Clones from Myasthenia Gravis Thymomas

Autoimmune T cell clones from myasthenia gravis patients have been extensively studied. These clones are of particular interest because they represent a subset of the immune system that is specifically targeted by the disease. Myasthenia gravis is an autoimmune disorder characterized by weakness and fatigability of the skeletal muscles due to a deficiency in the production of acetylcholine at the neuromuscular junction. The disease is often associated with the presence of antibodies that block the acetylcholine receptors, leading to muscle weakness.

The thymus plays a crucial role in the development of T cell clones in autoimmune diseases. Thymic epithelial tumors, which are common in myasthenia gravis patients, are known to express specific epitopes that can be recognized by T cell clones. These epitopes are associated with the pathogenesis of myasthenia gravis. For instance, the expression of the choline receptor and neurofilaments in thymic epithelial tumors has been linked to the development of myasthenia gravis.

Moreover, the tcr of these T cell clones can be mapped to specific regions of the human T cell receptor α-chain, which are associated with the pathogenesis of myasthenia gravis. The expression of these T cell receptors in thymic epithelial tumors suggests a potential for specific immunotargeting.

Autoimmune T cell clones from myasthenia gravis patients have been identified that recognize specific epitopes on the acetylcholine receptor. These T cell clones are specific for the α-chain of the human T cell receptor and are associated with the pathogenesis of myasthenia gravis. The role of these T cell clones in the development of the disease is supported by the observation of autoantibodies against the acetylcholine receptor in patients with myasthenia gravis.

In conclusion, the study of autoimmune T cell clones from myasthenia gravis thymomas provides insights into the pathogenesis of the disease. These clones can serve as potential targets for immunotherapies or other therapeutic approaches in the treatment of myasthenia gravis.