Myasthenia Gravis

CD4$^+$ T Epitopes on the Embryonic γ Subunit of Human Muscle Acetylcholine Receptor

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Abstract

In myasthenia gravis (MG) an autoimmune response against muscle acetylcholine receptor (AChR) occurs. Embryonic muscle AChR contains a γ subunit, substituted in adult muscle by a homologous ϵ subunit. Antibodies and CD4$^+$ cells specific for embryonic AChR have been demonstrated in MG patients.

We identified sequence segments of the human γ subunit forming epitopes recognized by four embryonic AChR-specific CD4$^+$ T cell lines, propagated from MG patients' blood by stimulation with synthetic peptides corresponding to the human γ subunit sequence. Each line had an individual epitope repertoire, but two 20-residue sequence regions were recognized by three lines of different HLA haplotype. Most T epitope sequences were highly diverged between the γ and the other AChR subunits, confirming the specificity of the T cells for embryonic AChR. These T cells may have been sensitized against AChR expressed by a tissue other than innervated skeletal muscle, possibly the thymus, which expresses an embryonic muscle AChR-like protein, containing a γ subunit. Several sequence segments forming T epitopes are similar to regions of microbial and/or mammalian proteins unrelated to the AChR. These findings are consistent with the possibility that T cell cross-reactivity between unrelated proteins ("molecular mimicry"), proposed as a cause of autoimmune responses, is not a rare event. (*J. Clin. Invest.* 1992. 90:1558–1567.) Key words: embryonic acetylcholine receptor • myasthenia gravis • T epitopes

Introduction

In myasthenia gravis (MG)[1] an autoimmune response against muscle nicotinic acetylcholine receptor (AChR) occurs (1–4). Anti-AChR T helper (CD4$^+$, Th) cells can be propagated in vitro from the blood and thymus of MG patients (5–9). They stimulate the production of anti-AChR antibodies in vitro (10) and recognize the AChR in association with HLA-DR molecules (11). Because Th cells recognize denatured AChR (6), they can be propagated in vitro using synthetic sequences of the AChR to yield polyclonal CD4$^+$ T cell lines enriched in AChR-specific T cells (7, 12, 13).

The AChR is a complex protein, formed in nonneuronal tissue by four homologous subunits (14). Skeletal muscle AChR exists in two forms, whose expression is developmentally regulated (15–17). An embryonic AChR is expressed before innervation occurs, whereas a different AChR is expressed by adult innervated muscle (15–17). Both AChRs contain two α, one β, and one δ subunits (15). Embryonic AChR also contains a γ subunit, substituted in adult AChR by a homologous ϵ subunit (15–17). Adult mammalian thymus expresses a protein similar or identical to embryonic muscle AChR, containing a γ subunit in addition to α, β, and δ subunits (18).

We previously propagated Th cell lines specific for embryonic AChR from the blood of MG patients, using a pool of overlapping synthetic peptides corresponding to the complete sequence of the human γ subunit (19). This finding has ramifications for the pathogenesis of MG, because it has been proposed that the anti-AChR sensitization in MG may originate within the thymus (20): the existence of MG patients of Th cells specific for embryonic AChR strongly supports this hypothesis. Further, antibodies specific for embryonic muscle AChR are frequently present in MG patients (21, 22). A study that used junctional (i.e., adult) and extrajunctional (i.e., embryonic) rat muscle AChRs indicated that antibodies in MG patients recognized determinants either unique to embryonic AChR or common to both forms, never determinants unique to adult AChR (21). Finally, a case of MG has been described where the antibodies recognized *only* the embryonic form of rat muscle AChR (23). The thymus of MG patients is frequently hypertrophic or it contains a thymoma (24), and it contains anti-AChR Th and B cells (8, 25). Thymectomy is beneficial for the evolution of MG and is a staple in the treatment of MG (26).

In this study we sought to identify the sequence segments of the human AChR γ subunit that form epitopes recognized by autoimmune CD4$^+$ cell lines specific for embryonic AChR (19), propagated from four MG patients of different HLA-DR type, by challenging the lines with individual overlapping peptides, spanning the complete sequence of the human AChR γ subunit. For the sequence regions found to contain T epitopes, we searched for similarities with unrelated proteins to determine if the results reported here are compatible with the proposed origin of autoimmune responses from mechanisms of molecular mimicry between self components and microbial proteins (27).

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1. *Abbreviations used in this paper:* AChR, muscle nicotinic acetylcholine receptor; APC, antigen presenting cells; FBAChR, fetal (embryonic) bovine muscle AChR; γ Pool, a pool of synthetic peptides corresponding to the complete sequence of human muscle AChR γ subunit; MG, myasthenia gravis; TCM, tissue culture medium; Th, anti-AChR T helper cells.

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Table I. Characteristics of the Myasthenic Patients

Patient No.	Age	Sex	Disease class*	Ab titer	Thymectomy	Treatment	HLA haplotype‡
	yr			*nM*			
1	33	M	2	59.0	+	Prednisone	Aw24, A26, B8, Bw58 DRw15 (2)-Dw2, DRw11 (5), DRw52, Dw25 DQw6 (1), DQw7 (3)
2	73	M	2	3.55	−	Prednisone	A2, A29, B7, Bw44 DR2, DR7 DQw1, DQw2
3	33	F	2	0.0	+	Prednisone	A2, A3, Bw35, Bw22 DRw1 (1), DRw14 (6), Dw9, DRw52, Dw25 DQw5 (1)
4	46	F	2	2.31	+	Prednisone	A1, B7, Bw44 DRw15 (2)-Dw2, DR4 (Dw4), DRw53 DQw6 (w1), DQw7 (w3)
5	57	M	2	3.22	+	Prednisone	A1, A29, B8, Bw44 DR3, DR7

* Classified as described in reference 28. ‡ Determined by restriction fragment length polymorphism (69) and (for the DR4 subtypes) by oligonucleotide hybrydization for patients 1, 3, and 4 and by microcytotoxicity (70) for patients 2 and 5.

Methods

Patients and T cell lines. The clinical characteristics of the myasthenic patient donors of the anti-AChR γ subunit T cell lines, classified according to a modification of the Osserman classification (28), their serum anti-AChR antibody titer (measured as described in reference 29), and HLA haplotype are summarized in Table I. Propagation and characterization of the $CD4^+$ T lines are described in detail in (19). Briefly, the lines were propagated from PBMCs by cycles of stimulation with a pool of synthetic peptides ([γ Pool] 1 μg/ml of each peptide) corresponding to the complete sequence of human γ subunit (as reported in reference 30) for 2 d, followed by stimulation with IL-2 (10 U/ml) for 5 d, using as antigen presenting cells (APC) irradiated (4,000 rad) HLA-DR–matched PBMC for the lines from MG patients and irradiated autologous PBMC for the control lines. Autologous or DR-matched PBMC are equally good APC for propagation of anti-AChR Th lines (11, 31), which are DR restricted (11). The cell phenotype of the T lines, determined by FACS analysis (7), was uniformly $CD3^+$, $CD4^+$, $CD8^-$. They could be propagated for several months and specifically recognize embryonic AChR (19 and below) whereas they cross-react to a very small extent or not at all with adult muscle AChR (19). Propagation of T cell lines using the γ pool was also attempted from six healthy subjects, four of which controls (1–4) were those used in the study reported in reference 19. T lines could be propagated for a short time (30–40 d) from controls 3, 4, and 5.

$CD8^+$ T cells depletion of PBMC. For patients 3 and 4 we also tested the response to the γ subunit synthetic sequence of unselected $CD8^+$-depleted, $CD4^+$-enriched T cells from peripheral blood. $CD8^+$ T cell depletion of PBMC was done using mouse anti-CD8 antibody (OKT8; Ortho Pharmaceutical, Raritan, NJ) and paramagnetic beads coated

Table II. Amino Acid Composition of Synthetic Peptides Forming Epitopes Recognized by the T Cell Lines

	Hγ39–49		Hγ75–94		Hγ135–194		Hγ297–312		Hγ321–340		Hγ336–355		Hγ470–489	
	Expected	Found	Expected	Found	Expected	Found	Expected	Found	Expected	Found	Expected	Found	Expected	Found
D, N	3	3	2	2.2	3	3.1	2	2.5			2	2.2	2	2.3
E, Q	3	3	1	1.3	3	3.4			1	1.1	3	3.4		
S	2	2.1	1	1	3	3	2	2.1			1	1.0		
G											1	1.2	1	1
H									1	1	1	1	1	1
R	1	1.3	2	2.2			1	1.7	4	4.7	2	2.3	2	2.2
T	2	1.9	1	1.1	2	1.8					1	1.1		
A	1	1.2					1	1.1			3	2.9	2	2.1
P			2	2.1	1	0.8	1	1.2	2	2.2	2	2	5	5
Y					1	1.3							1	1
V	1	1.1	4	4.1			6	4.9	3	3	2	1.9	1	1.1
M			1	1					1	1			1	1
I	1	0.9	1	0.8	1	1	1	0.8						
L	5	4	3	2.5	1	1	2	1	6	5.2	2	1.4	2	1.9
F					3	2.4			1	1.1			2	2
K	1	1							1	1.1				

with goat anti-mouse Ig antibody (Advanced Magnetics Inc., Cambridge, MA) (7). The $CD8^+$-depleted, $CD4^+$-enriched cells thus obtained (referred to as $CD4^+$-enriched cells) were consistently 45–55% of the starting PBMC. The cellular composition of the $CD4^+$-enriched cells was determined in four pilot experiments by FACS analysis (FACStar; Becton Dickinson, Mountain View, CA), using phycoerythrin-conjugated Leu 2 (anti-CD8), Leu 3 (anti-CD4), and Leu 4 (anti-CD3) antibodies (Becton Dickinson) (7), and was (n = 4): T cells ($CD3^+$), 61.4±15%; $CD4^+$ cells, 55.5±14.3%; $CD8^+$ cells, 0.5±0.1%.

Peptide synthesis and characterization. 35 peptides, 11–20 residues long and corresponding to the complete sequence of the human AChR γ subunit (30), were synthesized (32). They overlapped each other by approximately five residues to reduce the chance of missing epitopes "split" between peptides. The codes of the peptides included the letters Hγ for human γ subunit and two numbers indicating the position on the γ subunit sequence of the first and the last residue of the peptide. They corresponded to the sequence segments of the γ subunit indicated along the abscissa of Fig. 2. HPLC analysis of the peptides (19) using a C18 column (Ultrasphere ODS) consistently showed one main peak of optical density. Amino acid composition analysis (33) yielded an excellent correspondence between experimental and expected values for all peptides. The amino acid composition of the peptides containing Th epitopes is reported in Table II. Sequence and purity of randomly selected peptides from different syntheses were verified by gas-phase sequencing (Applied Biosystems, Foster City, CA). Only the expected sequence was found. Contaminating sequences (shorter homologous peptides missing one or more residues because of incomplete coupling), when detectable, accounted for 5–15% of the total signal.

Purification of AChRs from bovine muscle. Fetal (embryonic) bovine muscle AChR (FBAChR) was purified from fetuses 8–12-in long (34).

Microproliferation assay. T line cells (blasts) (2×10^5/ml in tissue culture medium [TCM]: RPMI 1640 with 10% heat-inactivated human AB serum, 2 nM L-glutamine, 100 U/ml penicillin, and 50 μg/ml streptomycin) and autologous (for control lines) or HLA-DR-matched APC (2×10^6 in TCM) were plated in triplicate in 96-round-bottom-well plates (100 μl of blast cells and 100 μl of APC) and stimulated with one of the following antigens: γ pool (0.05, 0.1, 0.5, 1, and 5 μg of each peptide/ml), PHA (10 μg/ml; Wellcome Reagent Ltd., Beckenham, UK), FBAChR (5, 2.5, and 1.25 μg/ml), or each individual synthetic peptide (10 μg/ml). Triplicate wells with blasts alone were seeded as controls. Blanks were wells with blasts plus APC and/or wells with blasts and APC plus 10 μg/ml of a 19-residue synthetic peptide unrelated to the human γ subunit (peptide E73, corresponding to residues 1–19 of the major intrinsic protein of bovine lens [35]). After 24 h the cells were labeled for 16–20 h with [^{3}H]thymidine (1 μCi/well, specific activity 6.7 Ci/mmol; Amersham Corp., Arlington Heights, IL), harvested (Titertek multiple harvester; Skatron Inc., Sterling, VA) and the incorporation of [^{3}H]thymidine was measured by liquid scintillation. Similar microproliferation assays were carried out (for patients 3 and 4) with $CD4^+$-enriched cells, using 1×10^5 cells/well and labeling with [^{3}H]thymidine after 120 h.

Search for sequence similarities between γ subunit sequence segments containing T epitopes and unrelated proteins. The protein sequences contained in the databases PIR 29 and Swiss-Prot 19 were searched for sequence similarities with the segments of the γ subunit sequence found to contain T epitopes (γ30–49, γ60–79, γ75–94, γ135–154, γ165–184, γ297–312, γ321–340, γ336–355, γ411–430, γ470–489, and γ476–495, see Results), using the program FASTDB (version 5.4) (36) of the Intelligenetics suite (IG, Inc., Mountain View, CA).

Results

The $CD4^+$ lines propagated from MG patients with the synthetic γ pool are AChR specific. The specificity of the T cell lines for the AChR was tested by their ability to recognize FBAChR (Fig. 1). AChR from bovine muscle is a good substitute for the exceedingly scarce human muscle AChR because it can be purified in sufficient amounts (34) and is highly homologous to human AChR (14, 30, 37): the bovine γ subunit is 92% identical to the human γ subunit (30, 37). Lines 2, 3, and 4 responded to 5 μg/ml of FBAChR at least as strongly as to the highest concentration of γ pool used (5 μg/ml of each peptide) whereas the response to FBAChR of line 1, although significant, was consistently lower than the response to the γ pool.

Sequence segments of the human γ subunit recognized by $CD4^+$ lines from MG patients. The segments of the γ subunit sequence forming epitopes recognized by the $CD4^+$ cell lines were identified by challenging the lines with the individual synthetic peptides present in the γ pool. The lines were tested for reactivity to the individual synthetic peptides as soon as a satisfactory enrichment in reactivity to γ pool was achieved, i.e., when the response of the T cell lines to the γ pool in microproliferation assays was comparable to the response to PHA (Fig. 1), which occurred after 3–4 wk of culture, and every 1–2 wk during the first 5 mo of propagation of the lines, to verify the consistency of the recognition and detect any clonal enrichment/loss. The lines frequently had a high rate of basal cell proliferation, which frequently occurs when T blasts are used (e.g., see references 7, 38, 39). Each line had an individual pattern of recognition of the peptides, which was consistent during the propagation of the lines, although recognition of less dominant peptides was somewhat erratic, either because of clonal loss or because the high basal rate of cell proliferation caused an unfavorably high background (Fig. 2).

Line 1 had the most complex pattern of peptide recognition. Peptides Hγ75–94 and Hγ321–340 were strongly recognized in all experiments. Two other peptides, Hγ30–49 and

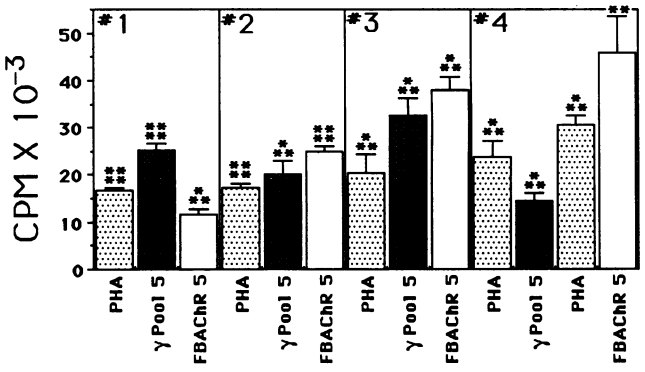


Figure 1. Responses of anti-γ subunit $CD4^+$ T cell lines propagated from four MG patients to PHA (10 μg/ml), γ pool (5 μg/ml), and purified FBAChR (5 μg/ml) measured in 2-d microproliferation assays. Values are incorporation of [^{3}H]thymidine by the T cells (average of triplicate determinations±SD). The responses to γ pool and FBAChR of line 4 were assessed in two different experiments and the corresponding values of [^{3}H]thymidine incorporation induced by PHA are reported as positive controls. All the lines responded vigorously both to γ pool and FBAChR. Basal [^{3}H]thymidine incorporation of the blasts in the presence of APC only was subtracted and was as follows: line *1:* 1,970±1,063; line *2:* 8,745±186; line *3:* 15,032±2,707; line *4*, first experiment: 9,540±506; second experiment: 28,873±1,800. Asterisks indicate responses significantly higher than the unstimulated proliferative rate of the blasts (****$P < 0.001$, ***$P < 0.005$, **$P < 0.01$). See text for experimental details.

Hγ297–312, were consistently recognized to a lesser extent, and recognition of Hγ297–312 decreased during propagation of the line. Peptides Hγ135–154 and Hγ470–489 elicited a small but significant increase of T cell proliferation in some experiments. Line 2 strongly recognized peptide Hγ75–94 in all experiments and peptide Hγ470–489 in one experiment. Line 3 recognized two sequence segments, peptide Hγ75–94 and the overlapping peptides Hγ321–340 and Hγ336–355 in all experiments, and two other peptides (Hγ135–154 and Hγ411–430) in two experiments. Line 4 consistently, strongly recognized one peptide, Hγ321–340.

Lack of detectable response to γ subunit synthetic sequences of unselected blood CD4⁺ cells. We attempted assessment of the faithfulness of the epitope repertoire of the long-term cell lines compared with that of the unselected blood CD4⁺ cells by directly testing in microproliferation assay the CD4⁺-enriched population from the blood of patients 3 and 4. As expected from patients suffering from mild symptoms (40, 41), the CD4⁺-enriched cells did not respond detectably to any γ subunit peptide.

Response to γ subunit synthetic sequences of CD4⁺ lines from healthy controls. We investigated the response to the individual peptides of T cell lines that could be propagated for a few weeks from three normal subjects. Line from control 5 did not recognize any peptide detectably (data not shown) whereas lines from controls 3 and 4 responded to several peptides (see Fig. 4). Control line 3 was tested three times: it clearly recognized several peptides (Hγ75–94, Hγ297–312, Hγ321–340 and the overlapping peptides Hγ470–489 and Hγ476–495) only during the first experiment (Fig. 3 *A*). Control line 4 had a slow rate of growth and could be tested only once (Fig. 3 *B*). Peptides Hγ165–184 and Hγ476–495 were clearly recognized. The overlapping peptides, Hγ120–139 and Hγ135–154, elicited a low but significant response.

Similarities of the γ subunit epitope-forming sequences with sequences of unrelated proteins. Several peptides recognized by the CD4⁺ lines had strong similarities with sequence regions from unrelated proteins, as summarized in Table III, where the γ subunit peptide epitopes are aligned on the unrelated, similar sequences. Residues that are identical or conser-

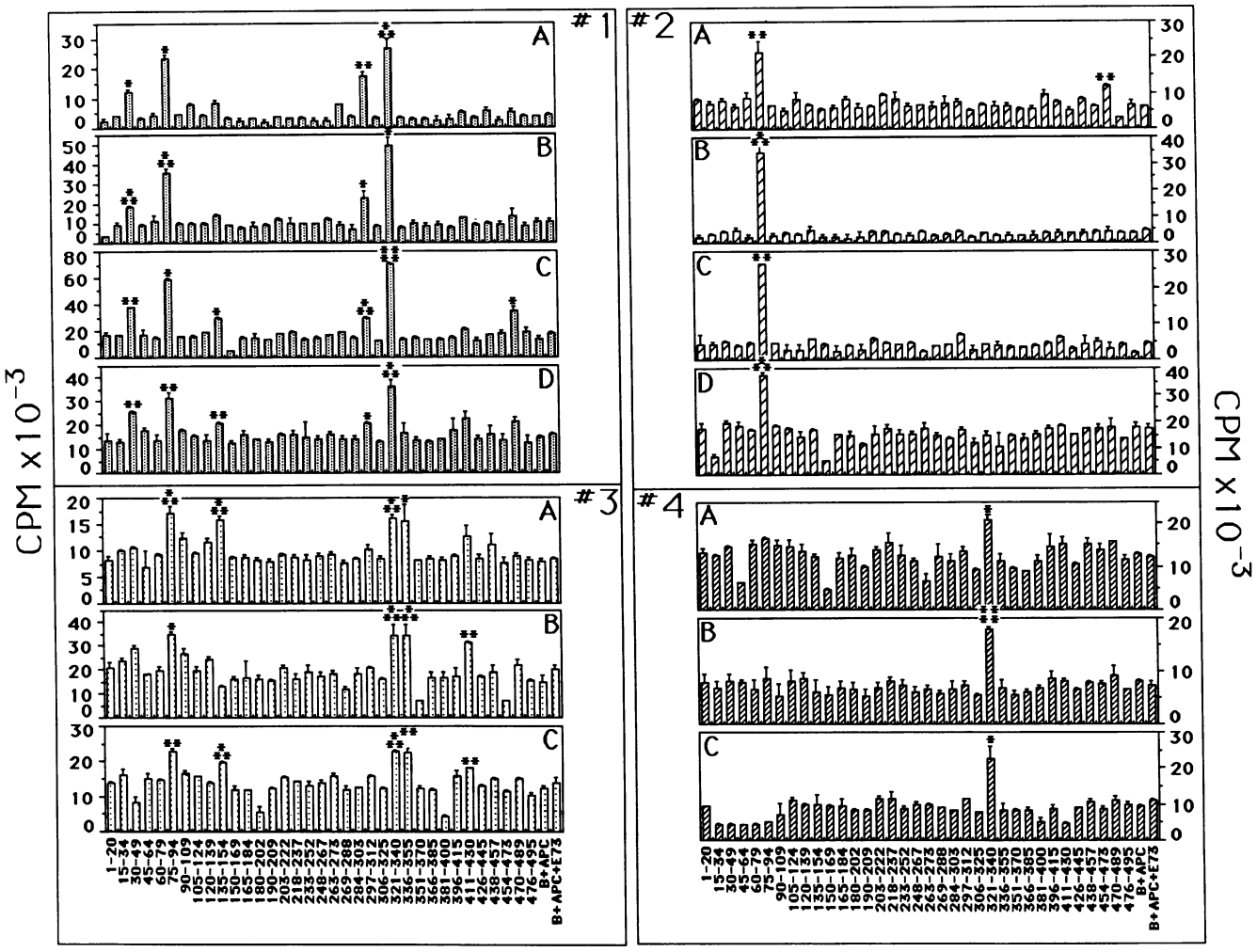


Figure 2. Response of anti-γ subunit CD4⁺ T cell lines from MG patients to the individual synthetic peptides forming the γ Pool (10 μg/ml) measured in 2-d microproliferation assays. The first test was carried out after a satisfactory enrichment in γ pool-specific T cell was reached, indicated by reactivity to the γ pool as high or higher than the response to PHA (after two or three cycles of stimulation with the antigen). A, B, C, and D refer to successive tests, carried out at intervals of ≥ 1–2 wk. The asterisks indicate responses significantly higher than the unstimulated proliferative rate of blasts in the presence of APC only, with or without 10 μg/ml of the unrelated peptide E73 (****$P < 0.001$, ***$P < 0.005$, **$P < 0.01$, *$P < 0.025$). See text for experimental details.

vatively substituted are indicated with boldfaced characters. Of the sequence pairs containing substantial similarities, we selected those containing sequence segments 10–16 residues long, with ≥ 75% amino acids being identical or conservatively substituted. Insertions/deletions introduced to optimize the alignment were counted as extra positions. A length of 10–16 residues was chosen as a conservative compromise between the minimal length found in functional assays for class I–restricted T epitopes (5–7 residues [42, 43]), and the length of the sequences found to bind to MHC molecules, 8–9 residues for class I–presented peptides (44–47), and somewhat longer sequence segments for class II–presented peptides (48, 49).

Discussion

We have identified here sequence segments of the human γ subunit that form epitopes recognized by autoimmune Th cell lines specific for embryonic AChR, obtained from MG patients of different HLA-DR type. Other epitopes may have gone undetected because of the small size of the patient population, because of poor representation by our peptide panel, or because of selection of clones recognizing epitopes within peptides better processed into the relevant epitope and/or better able to bind the DR-restricting elements. Within these limitations, our approach permitted unambiguous identification of several epitopes.

Fig. 4 summarizes the sequence regions of the γ subunit-containing epitopes recognized by the MG lines and the HLA-DR haplotype of the patients. The specificity of the lines for the epitope peptides was stable and was not a reflection of the duration of the culture because the pattern of peptide recognition remained consistent during propagation of the lines. Although some peptide sequences were recognized only by one line, as might be expected for Th cells of different HLA-DR haplotype, a substantial overlap existed in the epitope repertoires of the lines, and the sequences Hγ75–94 and Hγ321–340 were recognized by three lines. Immunodominant regions were previously identified along the AChR α subunit sequence, containing epitopes recognized by most MG patients irrespective of their HLA-DR haplotype (13, 41). In multiple sclerosis a peptide corresponding to aminoacids 87–106 of the myelin basic protein is frequently recognized by T cells of these patients: the

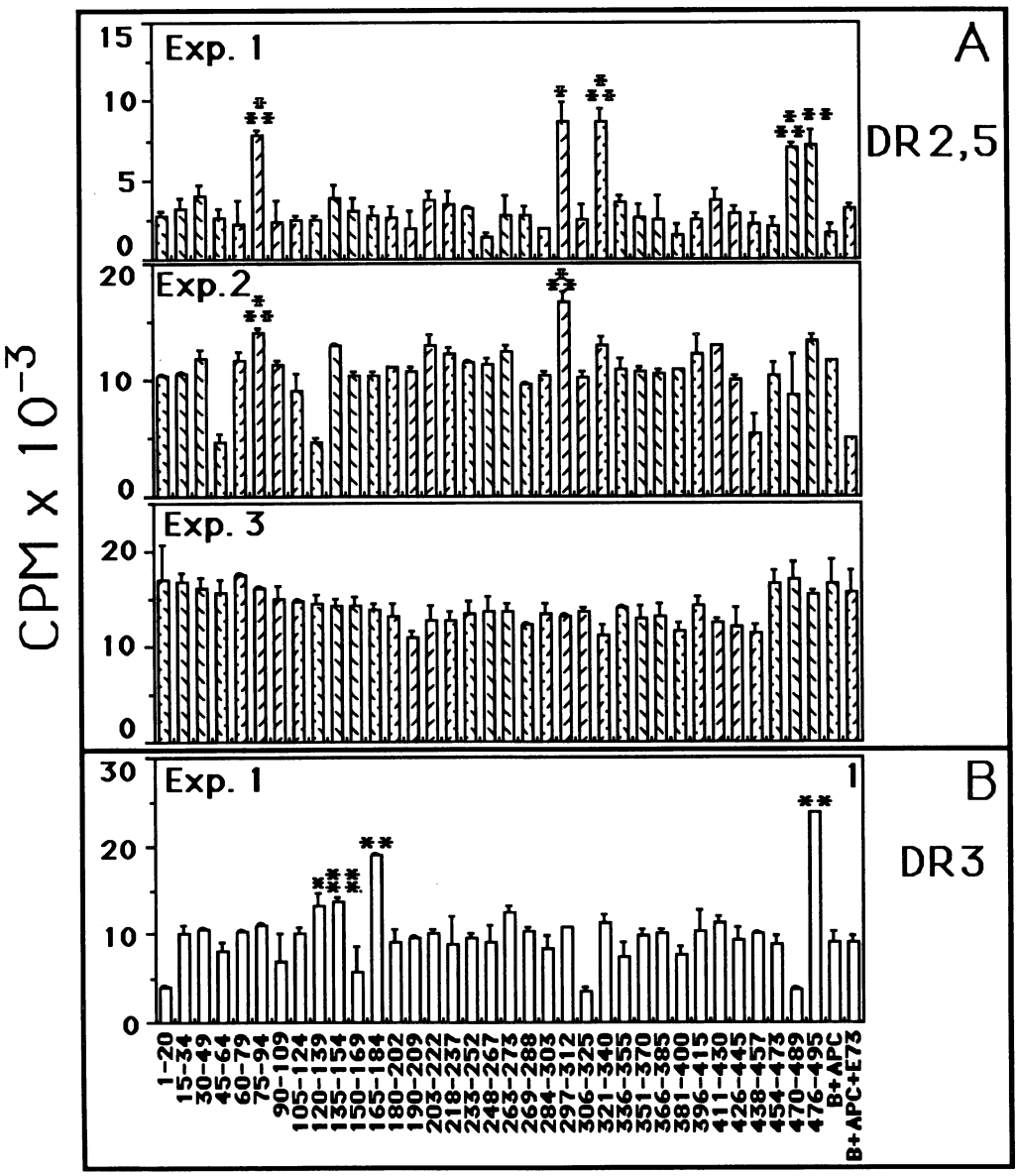


Figure 3. Response of short-term T cell lines obtained from two healthy controls to the individual synthetic peptides forming the γ pool (10 μg/ml). Asterisks indicate responses significantly higher than the unstimulated proliferative rate of blasts in the presence of APC only, with or without 10 μg/ml of the unrelated peptide E73 (****$P < 0.001$, ***$P < 0.005$, **$P < 0.01$, *$P < 0.025$). See text for experimental details.

*Table III. Sequence Regions of the Human γ Subunit Forming T Epitopes: Similarities with Sequences of Unrelated Proteins**

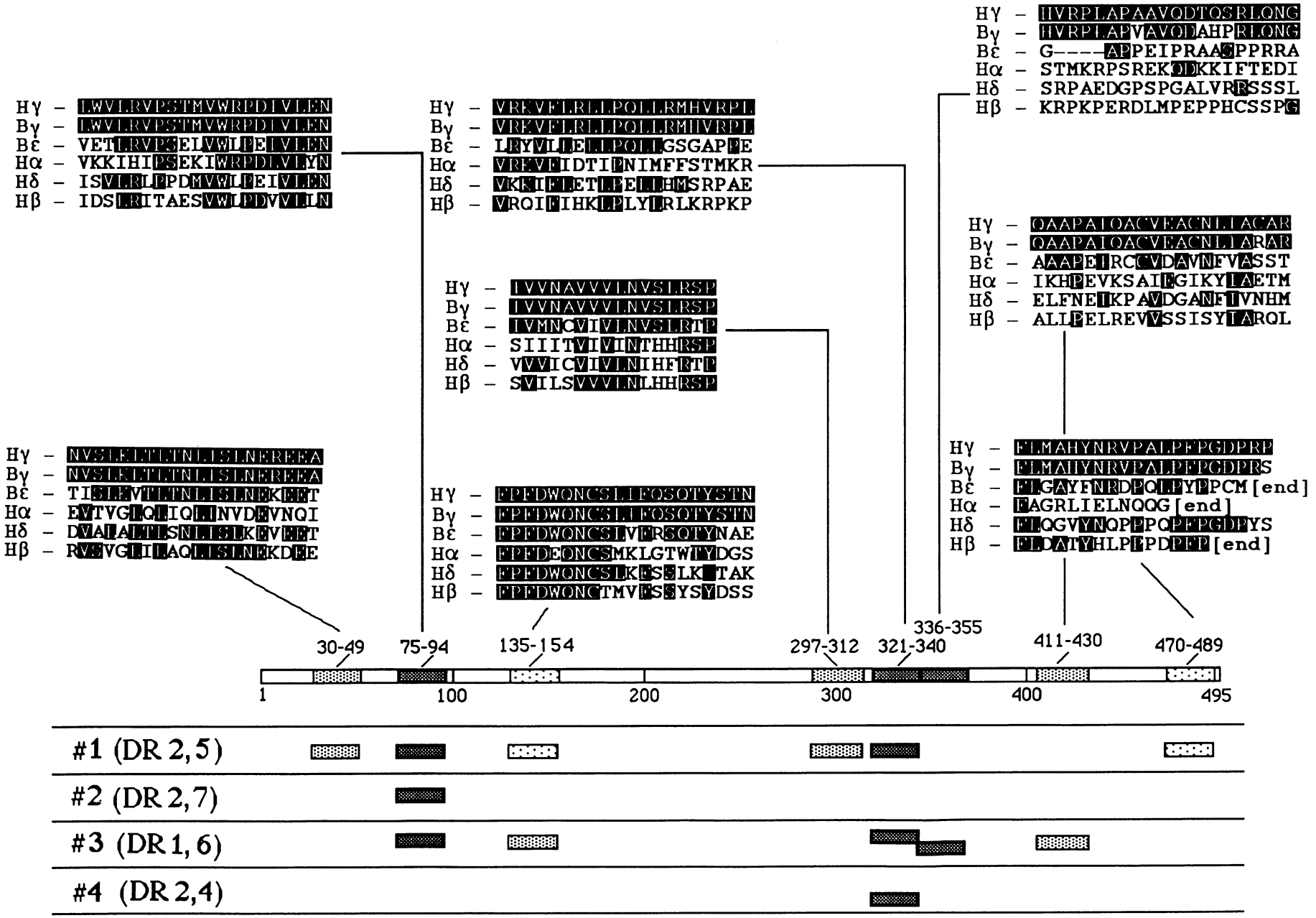
T epitope	Sequences	Protein containing a similar sequence (database accession #)
γ30–49	NVSLKLTLTNLISLNEREEA NVDKKLLKKALEQLNEREKQ	RNA polymerase sigma-E factor precursor. *Bacillus subtilis* (RPSE$BACSU‡)
	NVSLKLTLTNLISLNEREEA SVSLPLTLKELI-----EEL	T cell activation protein P600. Mouse (p600$MOUSE‡).
	NVSLKLTLTNLISLNEREEA IVSLLTFLNVLITLNNKYKH	Early E3 22.2 kD protein. Adenovirus type 1 (E322$ADEC1‡).
	NVSLKLTLTNLISLNEREEA NVLQKLYISMLISLQILLIM	NADH-Ubiquinone Oxidoreductase Chain 4. Mouse (NU4M$MOUSE)‡.
γ135–154	FPFDWQNCSLIFQSQTYSTN TWFDWNNQSLPYHSQKLRLE	B cell receptor CD22 precursor. Human (CD22$HUMAN‡).
γ336–355	EVAL-CLPRSELLFQQWQRQG RVAVNHLPR-ELIFQVWQRSW	VPX protein. Human immunodeficiency virus type 2 (VPX$HIV2D‡).
	EVALCLPRSELLFQQWQRQG EVGLKGERSELLLSEKVVDA	Erythroid differentiation factor precursor. Human (A30884)§.
γ411–430	QAAPAIQACVE--ACNLIACAR QAAPAIQNDVEMAAVNLSVFVD	Ribonucleoside-diphosphate reductase. Human cytomegalovirus (RIR1$HCMVA‡).
	QAAPAIQACVEACNLIACAR QAAPDLQENVHATVLIEETM	Probable RNA-directed RNA polymerase. Tobacco rattle virus (194K$TRVSY‡).
γ476–495	NRVPALPF-PGDPRPYLPSP-D PN-LLAL-FAPRDPIPYLP-PLE	70K U1 small nuclear ribonucleoprotein. Human (RU17$HUMAN‡).
	NRVPA-LPFPGDPRPYL---PSP TQ-PPAPVG-PGDPDVYLKGVPSA	DNA-binding protein. Herpes simplex virus (type 1, strain 17) (DNB$HSV11‡).

* For all sequence segments of the human AChR γ subunit identified here as forming T epitopes (γ30–49, γ60–79, γ75–94, γ120–139, γ135–154, γ165–184, γ297–312, γ321–340, γ336–355, γ411–430, γ470–489, and γ476–495) we searched for sequence similarities with the proteins in the databases PIR 29 and Swiss-Prot 19. The table reports only the T epitope sequence regions for which similarities with unrelated proteins were found. See text for further details. ‡ From Swiss-Prot 19. § From PIR 29.

use of long-term myelin basic protein-specific T cell lines allowed identification of a core sequence (residues 89–99) recognized in the context of four different HLA-DR types (50). Because T epitopes can be as small as 5–7 residues (42, 43), the sequence regions identified here may contain several nested T epitopes, as it occurs in sequence regions of similar or even shorter length from other protein antigens (51–54). Alternatively, one or more of these sequence segments may contain a single immunodominant T epitope, recognized within several or any MHC-class II haplotype as described for T epitopes on other proteins (39, 55). The Th immunodominance of the regions identified here may be due to easier cleavage and processing, since they are probably located on the AChR surface (see below), and to the ability of human DR molecules to interact with unrelated peptides (56, 57).

A potential caveat to the use of long-term T cell lines is that selective clonal loss/enrichment may bias their repertoire. Comparison of the repertoire of the lines with that of unselected blood $CD4^+$ cells could not be done because, due to the paucity of AChR-specific $CD4^+$ cells in the blood of MG patients (7), testing of blood $CD4^+$ cells with AChR sequences is successful only in acutely, severely affected patients, never in the moderately affected patients from which the repetitive bleedings necessary for establishment of the lines can be obtained (7, 13). Accordingly, unselected $CD4^+$ cells from patients 3 and 4 did not detectably recognize any γ subunit sequence. In favor of good concordance of the epitope repertoires of unselected $CD4^+$ cells and of long-term Th lines is the finding that the sequence regions of the AChR α subunit recognized by unselected $CD4^+$ cells of severely affected MG patients are very similar to those recognized by long-term AChR-specific lines propagated from moderately affected patients by the use of a peptide pool corresponding to the α subunit sequence (41).

Patients 2 and 4 were donors of cells used to establish anti-α subunit $CD4^+$ T cell lines (13). Comparison of the four T immunodominant regions of the α subunit sequence (Hα32–67, Hα 101–120, Hα304–337, Hα419–437: the latter segment corresponds to the carboxyl terminus of the human α subunit) (13, 41) with those identified here on the γ subunit sequence, reveals a similar distribution of the T epitope segments along the α and the γ subunit sequence. Four γ subunit epitope peptides (Hγ30–49, Hγ75–94, Hγ321–355, and Hγ470–489) have a sequence location similar to that of the immunodominant α subunit, although these segments are highly divergent in the α and the γ subunit (Fig. 4). In both α and γ subunits the immunodominant segments are within regions predicted to form non-transmembrane parts of the AChR molecule, and


Hγ - LWVLRVPSTMVWRPDIVLEN
Bγ - LWVLRVPSTMVWRPDIVLEN
Bε - VETLRVPSELVWLPEIVLEN
Hα - VKKIHIPSEKIWRPDLVLYN
Hδ - ISVLRLPPDMVWLPEIVLEN
Hβ - IDSLRITAESVWLPDVVLLN
Hγ - VREVFLRLLPQLLRMHVRPL
Bγ - VREVFLRLLPQLLRMHVRPL
Bε - LRYVLLELLPQLLGSGAPPE
Hα - VREVFIDTIPNIMFFSTMKR
Hδ - VKKIFLETLPELLEMSRPAE
Hβ - VRQIFIHKLPLYLRLKRPKP
Hγ - IVVNAVVVLNVSLRSP
Bγ - IVVNAVVVLNVSLRSP
Bε - IVMNCVIVLNVSLRTP
Hα - SIIITVIVIINTHHRSP
Hδ - VVVICVIVLNIHFRTP
Hβ - SVILSVVVLNLHHRSP
Hγ - HVRPLAPAAVQDTQSRLQNG
Bγ - HVRPLAPVAVQDAHPRLQNG
Bε - G----APPEIPRAASPPRRA
Hα - STMKRPSREKQDKKIFTEDI
Hδ - SRPAEDGPSPGALVRRSSSL
Hβ - KRPKPERDLMPEPPHCSSPG
Hγ - QAAPAIQACVEACNLIACAR
Bγ - QAAPAIQACVEACNLIARAR
Bε - AAAPELRCGVDAVNFVASST
Hα - IKHPEVKSAIEGIKYLAETM
Hδ - ELFNEIKPAVDGANFTVNHM
Hβ - ALLPELREVVSSISYIARQL
Hγ - NVSLEITLTNLISLNEREEA
Bγ - NVSLEITLTNLISLNEREEA
Bε - TISLEVTLTNLISLNEKEET
Hα - EVTVGLQLIQLINVDEVNQI
Hδ - DVALALTLSNLISLKEVEET
Hβ - RVSVGLILAQLISLNEKDEE
Hγ - FPFDWQNCSLIFQSQTYSTN
Bγ - FPFDWQNCSLIFQSQTYSTN
Bε - FPFDWQNCSIVERSQTYNAE
Hα - FPFDEQNCSMKLGTWTYDGS
Hδ - FPFDWQNCSLKESSSLKITAK
Hβ - FPFDWQNCTMVESSSYSYDSS
Hγ - FLMAHYNRVPALPFPGDPRP
Bγ - FLMAHYNRVPALPFPGDPRS
Bε - FLGAYFNRDPQLPYPPCM[end]
Hα - DAGRLIELNQQG[end]
Hδ - FLQGVYNQPPPQPFPGDPYS
Hβ - FLDAIYHLPLPDPFP[end]
30-49
75-94
135-154
297-312
321-340
336-355
411-430
470-489
1
100
200
300
400
495
#1 (DR 2,5)
#2 (DR 2,7)
#3 (DR 1,6)
#4 (DR 2,4)

because of the presence of several charged and hydrophilic residues they may be exposed on the AChR surface. All but one T epitope peptides on the γ subunit identified here are within sequence segments proposed to form surface domains of the AChR, either extracellular (sequences 30–49, 75–94, 135–154, and possibly 470–489) or cytoplasmic (sequences 321–355 and 411–430) (14), suggesting that T epitopes might easily originate from surface regions of a protein.

Th cells against autoantigens, including the AChR, exist in normal subjects (e.g., references 9, 58). A $CD4^+$ T line specific for the δ subunit of human muscle AChR was propagated, for a short time only, from a normal subject (12), and AChR-specific T cell clones could be obtained from healthy humans by stimulation with a biosynthetic fragment of mouse AChR α subunit, but less frequently than from MG patients (59), suggesting that potentially autoreactive T cells exist in the normal population, but in lesser amounts or binding the antigen less efficiently than bona fide autoimmune T cells. In agreement with those results, γ pool–specific $CD4^+$ cell lines could be propagated from three controls, but only for a short time. Control line 5 responded detectably to the γ pool, but not to any individual peptide (not shown), suggesting that $CD4^+$ T cells specific for AChR γ subunit sequences existed in this subject, but they interacted with several γ epitopes with too low affinity for any one peptide epitope to be clearly recognized in vitro. Control line 3 had a pattern of peptide recognition similar to that of MG line 1, which had the same DR haplotype: both lines recognized the "immunodominant" peptides Hγ75–94 and Hγ321–340 and peptides Hγ297–312 and Hγ470–489 (Fig. 3). The latter peptides were not recognized by any other line and may therefore be restricted by the DR5 molecule, which was only expressed by MG line 1 and control line 3. Of the peptide recognized by control line 5, Hγ 135–154 was also recognized by MG lines 1 and 3 (Fig. 4). Autoreactive T cells have been demonstrated in many experimental systems where the host does not normally develop an autoimmune disease (60), including experimental autoimmune myasthenia gravis (61, 62). The presence of $CD4^+$ cells reactive to self antigens does not indicate a failure of tolerance because the autoreactive cells clonally deleted during maturation within the thymus are probably those with optimal (highest) affinity for the self antigen/MHC complex (for review see reference 60). Autoreactive T cells with low affinity antigen receptor may escape clonal deletion and never be activated at the periphery because of the low affinity of their T cell receptors (60, 63).

Immunological cross-reactivity between viral or bacterial antigens and normal protein constituents of the host may be involved in development of autoimmune diseases, including MG, because microbial proteins and human autoantigens contain identical or similar sequence segments, which may form cross-reacting epitopes (27). Some T epitope sequences of the AChR γ subunit resembled sequence regions of other proteins, frequently of microbial origin (Table III). This may indicate that molecular mimicry with microbial proteins is indeed involved in autoimmune responses or that some of our peptides were recognized as a result of cross-reactivity, facilitated by the higher concentration of peptide in vitro compared with the in vivo conditions, of T cells sensitized against common viruses and bacteria. In support of the latter possibility the sequence Hγ476–495, recognized by two control lines (Figs. 3 and 4), has strong similarities with a protein component of the *Herpes simplex* virus, which is very common in humans. The relative high frequency of sequence similarities of AChR sequence regions with unrelated proteins is not surprising because certain short amino acid sequences (3–5 residues), presumably corresponding to specific tridimensional structural motifs, occur in the sequence of known proteins with a much higher frequency than statistically expected, and they have a propensity to cluster together, making it likely and indeed frequent that fragments of alien proteins up to 7–18 residues in length (i.e., enough for T epitope formation [43–49]) share substantial sequence similarities with fragments of self proteins (64). Further "filters" must exist in the immune system to avoid a widespread occurrence of autoimmune phenomena. Among them, the necessity for an alien sequence resembling a self protein to be processed and presented, which may not occur if it is not on the protein surface, or flanked by residues incompatible with processing (65) and the availability of a potentially reactive T clone, which may have been deleted during the thymic education of the T cells. The two immunodominant regions Hγ75–94 and Hγ321–340 did not yield any sequence similarities with unrelated known proteins.

Fig. 4 depicts alignments of the T epitope sequence segments of the γ subunit with the homologous segments of the other known subunits of human muscle AChR and of the γ and ϵ subunits of bovine muscle AChR. The bovine ϵ subunit substitutes for the still unknown sequence of the human ϵ subunit (14). Some T epitope peptide sequences are highly diverged (e.g., Hγ321–355 and Hγ 411–430) and it is unlikely that the corresponding T cells cross-react with homologous segments of other AChR subunits. Others contain stretches of residues conserved or conservatively substituted in other AChR subunits (e.g., the sequences TLTNLISLN, FPFDWQNCSL, SLKLTLLNILSNE, FPFDWQNCSLVF, and VVVLNVSLRSP, identical or almost identical in the γ and ϵ subunits) and may have stimulated and propagated T cells non-γ subunit specific. This may explain the reported cross-reactivity of line 1 with both embryonic and adult AChRs (19).

The preferential or unique response of the $CD4^+$ cell lines to embryonic AChR (19) and the finding that several epitopes they recognize are within sequence regions highly diverged between the γ and the other AChR subunits prove that these T cells are specific for embryonic AChR. Because embryonic AChR is not expressed by adult innervated muscle, this finding lends credence to the proposed origin of the anti-AChR sensitization in MG within the thymus (20), which expresses an AChR similar or identical to embryonic muscle AChR, containing a γ subunit (18). Because the thymus AChR also contains α, β, and δ subunits (18), T cells sensitized against epitopes formed by subunit present also in the more abundant muscle AChR may be preferentially expanded. Anti-γ Th cells, as the disease progresses, may become a small fraction of the anti-AChR Th population and have a marginal role in the pro-

Figure 4. Sequence segments recognized by the four T cell lines and their HLA-DR haplotypes. Peptides that elicited a strong response are indicated as densely hatched segments, peptides that elicited a lower response are indicated as increasingly lighter hatched segments. The sequence segments of the γ subunit containing Th epitopes are reported and are aligned with the corresponding segments of the other known human AChR subunits and with the bovine AChR γ and ϵ subunit, which substitutes for the still unknown sequence of the human ϵ subunit.

duction of pathogenic anti-AChR antibodies. Still, their existence suggests a nonmuscle origin of the anti-AChR response.

Although the autoimmune Th response against AChR in MG is polyclonal, a limited sequence of at least two of AChR subunits, the α (13, 49) and the γ subunits, seems to be involved in Th cell sensitization. This may allow development of specific immunosuppressive procedures, based on the use of synthetic peptides analogues, able to tightly bind to the class II molecules but unable to bind the T cell receptor of the autoimmune T cell and competing with the pathogenetic T epitope for their presentation, as it was successfully done in experimental autoimmune encephalomyelitis (66–68). Availability of human autoimmune anti-AChR $CD4^+$ T cell lines and knowledge of the epitopes recognized are necessary steps towards this goal.

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References

1. Lindstrom, J. D., D. Shelton, and Y. Fujii. 1988. Myasthenia gravis. *Adv. Immunol.* 42:233–284.

2. Drachman, D. 1983. Myasthenia gravis: immunobiology of a receptor disorder. *Trends Neurosci.* 6:446–448.

3. Engel, A. G. 1984. Myasthenia gravis and myasthenic syndromes. *Ann. Neurol.* 16:519–534.

4. Schönbeck, S., S. Chrestel, and R. Hohlfeld. 1990. Myasthenia gravis: prototype of the antireceptor autoimmune diseases. *Int. Rev. Neurobiol.* 32:175–201.

5. Hohlfeld, R., K. V. Toyka, K. Heininger, H. Grosse-Wilde, and I. Kalies. 1984. Autoimmune human T-lymphocytes specific for acetylcholine receptor. *Nature (Lond.).* 310:244–246.

6. Hohlfeld, R., K. V. Toyka, S. J. Tzartos, W. Carson, and B. M. Conti-Tronconi. 1987. Human T helper lymphocytes in myasthenia gravis recognize the nicotinic receptor α subunit. *Proc. Natl. Acad. Sci. USA.* 84:5379–5383.

7. Protti, M. P., A. A. Manfredi, C. Straub, X. Wu, J. F. Howard, and B. M. Conti-Tronconi. 1990. Use of synthetic peptides to establish anti-human acetylcholine receptor $CD4^+$ cell lines from myasthenia gravis patients. *J. Immunol.* 144:1711–1720.

8. Melms, A., S. Chrestel, B. C. G. Shalke, H. Wekerle, A. Mauron, M. Ballivet, and T. Barkas. 1989. Autoimmune T lymphocytes in myasthenia gravis. Determination of target epitopes using T lines and recombinant products of the mouse nicotinic acetylcholine receptor gene. *J. Clin. Invest.* 83:285–289.

9. Harcourt, G. C., N. Sommer, J. Rothbard, H. N. A. Willcox, and J. Newsom-Davis. 1988. A juxta-membrane epitope on the human acetylcholine receptor recognized by T cells in myasthenia gravis. *J. Clin. Invest.* 82:1295–1330.

10. Hohlfeld, R., I. Kalies, B. Kohleisen, K. Heininger, B. M. Conti-Tronconi, and K. V. Toyka. 1986. Myasthenia gravis: stimulation of antireceptor autoantibodies by autoreactive T-cell lines. *Neurology.* 36:618–621.

11. Hohlfeld, R., B. M. Conti-Tronconi, I. Kalies, J. Bertrams, and K. V. Toyka. 1985. Genetic restriction of autoreactive acetylcholine receptor-specific T lymphocytes in myasthenia gravis. *J. Immunol.* 135:2393–2399.

12. Protti, M. P., A. A. Manfredi, X. D. Wu, L. Moiola, J. F. Howard, Jr., and B. M. Conti-Tronconi. 1991. Myasthenia gravis: T epitopes of the δ subunit of human muscle acetylcholine receptor. *J. Immunol.* 146:2253–2261.

13. Protti, M. P., A. A. Manfredi, C. Straub, J. F. Howard, Jr., and B. M. Conti-Tronconi. 1990. Immunodominant regions for T helper sensitization on the human nicotinic receptor α subunit in myasthenia gravis. *Proc. Natl. Acad. Sci. USA* 87:7792–7796.

14. McCarthy M. P., J. P. Earnest, E. F. Young, S. Choe, and R. M. Stroud. 1986. The molecular neurobiology of the acetylcholine receptor. *Annu. Rev. Neurosci.* 9:383–414.

15. Mishina, M., T. Takai, K. Imoto, M. Noda, T. Takahashi, S. Numa, C. Methfessel, and B. Sakmann. 1986. Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature (Lond.).* 321:406–411.

16. Witzemann, V., B. Barg, Y. Nishikawa, B. Sakmann, and S. Numa. 1987. Differential regulation of muscle acetylcholine receptor γ- and ϵ-subunit mRNAs. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 223:104–112.

17. Gu, Y., and Z. W. Hall. 1988. Immunological evidence for a change in subunits of the acetylcholine receptor in developing and denervated rat muscle. *Neuron.* 1:117–125.

18. Nelson, S., and B. M. Conti-Tronconi. 1990. Adult thymus expresses an embryonic nicotinic acetylcholine receptor-like protein. *J. Neuroimmunol.* 29:81–92.

19. Protti, M. P., A. A. Manfredi, J. F. Howard, Jr., and B. M. Conti-Tronconi. 1991. T cells in myasthenia gravis specific for embryonic acetylcholine receptor. *Neurology.* 41:1809–1814.

20. Wekerle H., R. Hohlfeld, U.-P. Ketelsen, J. Kalden, and I. Kalies. 1981. Thymic myogenesis, T lymphocytes and the pathogenesis of myasthenia gravis. *Ann. NY Acad. Sci.* 377:455–476.

21. Weinberg, C. B., and Z. W. Hall. 1979. Antibodies from patients with myasthenia gravis recognize determinants unique to extrajunctional acetylcholine receptors. *Proc. Natl. Acad. Sci. USA.* 76:504–508.

22. Vincent, A., P. J. Whiting, M. Schluep, F. Heidenreich, B. Lang, A. Roberts, and N. Wilcox. 1987. Antibody heterogeneity and specificity in myasthenia gravis. *Ann. NY Acad. Sci.* 505:106–120.

23. Schuetze, S. M., S. Vicini, and Z. W. Hall. 1985. Myasthenic serum selectively blocks acetylcholine receptors with long channel open times at developing rat endplates. *Proc. Natl. Acad. Sci. USA.* 782:2533–2537.

24. Li, A., A. Papatestas, A. Kark, R. S. Osserman, and K. Osserman. 1971. An histologic reappraisal of the thymus in myasthenia gravis. A correlative study thymic pathology and response to thymectomy. *Arch. Pathol.* 91:55–61.

25. Lisak, R. P., A. I. Levinson, B. Zweiman, and M. R. Kornstein. 1986. Antibodies to acetylcholine receptor and tetanus toxoid: in vitro synthesis by thymic lymphocytes. *J. Immunol.* 136:1221–1225.

26. Olanow, C. W., A. S. Wechsler, M. Sirotkin-Roses, J. Stajich, and A. D. Roses. 1987. Thymectomy as primary therapy in myasthenia gravis. *Ann. NY Acad. Sci.* 505:595–606.

27. Oldstone, M. B. A. 1987. Molecular mimicry and autoimmune disease. *Cell.* 50:819–820.

28. Sanders, D. B., J. F. Howard, Jr., T. R. Johns, and J. F. Campa. 1979. High-dose daily prednisone therapy in myasthenia gravis. *In* Plasmapheresis and Immunobiology of Myasthenia Gravis. Vol. 27. P. C. Dau, editor. Houghton-Mifflin Professional Publishers, Boston. 289–306.

29. Lindstrom, J. 1977. An assay for antibodies to human acetylcholine receptor in serum from patients with myasthenia gravis. *Clin. Immunol. Immunopathol.* 7:36–42.

30. Shibahara, S., T. Kubo, H. J. Perski, H. Takahashi, M. Noda, and S. Numa. 1985. Cloning and sequence analysis of genomic DNA encoding γ subunit precursor of muscle acetylcholine receptor. *Eur. J. Biochem.* 146:15–21.

31. Hohlfeld, R., M. Michels, H. Tesch, A. Fahsbender, K. Heininger, B. M. Conti-Tronconi, and K. M. Toyka. 1986. Epstein-Barr virus-transformed B cells can present acetylcholine receptor to autologous autoreactive T cells. *Immunol. Lett.* 12:171–174.

32. Houghten, R. A. 1985. General method for the rapid solid phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA.* 82:5131–5135.

33. Heinrickson, R. L., and S. C. Meredith. 1984. Amino acid analysis by reverse phase high performance liquid chromatography; precolumn derivatization with phenylisothiocyanate. *Anal. Biochem.* 136:65–74.

34. Gotti, C., B. M. Conti-Tronconi, and M. A. Raftery. 1982. Mammalian muscle acetylcholine receptor purification and characterization. *Biochemistry.* 21:3148–3154.

35. Gorin, M. B., S. Yancey, J. Cline, J.-P. Revel, and J. Horwitz. 1984. The major intrinsic protein (MIP) of the bovine lens fiber membrane: characterization and structure based on cDNA cloning. *Cell.* 39:49–55.

36. Brutlag, D. L., J. P. Dautricort, S. Manlik, and J. Relph. 1990. Sensitive similarity searches of biological sequence databases. *Comput. Appl. Biosci.* 6:237–245.

37. Takai, T., M. Noda, Y. Furutani, H. Takahashi, M. Notake, S. Shimizu, T. Kayano, T. Tanabe, K. Tanaka, T. Hirose, et al. 1984. Primary structure of γ subunit precursor of calf muscle acetylcholine receptor deduced from the cDNA sequence. *Eur. J. Biochem.* 143:109–115.

38. Ellerman, K. E., J. M. Powers, and S. W. Brostoff. 1988. A suppressor T-lymphocyte cell line for autoimmune encephalomyelitis. *Nature. (Lond.).* 331:265–267.

39. Sinigaglia, F., M. Guttinger, J. Kilgus, D. M. Doran, H. Matile, H. Etlinger, A. Trzeciak, A. Gillessen, and J. R. L. Pink. 1988. A malaria T-cell epitope recognized in association with most mouse and human MHC Class II molecules. *Nature (Lond.).* 336:778–780.

40. Protti, M. P., A. A. Manfredi, C. Straub, J. Howard, and B. M. Conti-Tronconi. 1990. $CD4^+$ T cell response to human acetylcholine receptor α subunit correlates with myasthenia gravis severity. A study with synthetic peptides. *J. Immunol.* 144:1276–1281.

41. Manfredi, A. A., M. P. Protti, X. D. Wu, J. F. Howard, Jr., and B. M.

Conti-Tronconi. 1992. CD4+ T epitope repertoire on the human acetylcholine receptor α subunit in severe myasthenia gravis. A study with synthetic peptides. *Neurology.* 42:1092–1100.

42. Livingston, A. M., and C. Garrison-Fathman. 1987. The structure of T-cell epitopes. *Annu. Rev. Immunol.* 5:477–501.

43. Reddehase, M. J., J. B. Rothbard, and U. H. Koszinowski 1989. A pentapeptide as minimal antigenic determinant for MHC class I-restricted T lymphocytes. *Nature (Lond.).* 337:651–652.

44. Van Bleek, G. M., and S. G. Nathenson. 1990. Isolation of an endogenously processed immunodominant viral peptide from the class I H-2K^b molecule. *Nature (Lond.).* 348:213–218.

45. Falk, K., O. Rotzshke, K. Deres, J. Metzger, G. Jung, and H.-G. Rammensee. 1991. Identification of naturally processed viral nonapeptides allows their quantification in infected cells and suggests an allele-specific T cell epitope forecast. *J. Exp. Med.* 174:425–433.

46. Madden, D. R., J. C. Gorga, J. L. Strominger, and D. C. Wiley. 1991. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature (Lond.).* 353:321–326.

47. Jardetzky, T. S., W. S. Lane, R. A. Robinson, D. R. Madden, and D. C. Wiley. 1991. Identification of self peptides bound to purified HLA-B27. *Nature (Lond.).* 353:326–329.

48. Demotz, S., H. M. Grey, E. Appella, and A. Sette. 1989. Characterization of a naturally processed MHC class II-restricted T-cell determinant of hen egg lysozyme. *Nature (Lond.).* 342:682–684.

49. Rudensky, A. Y., P. Preston-Hurlbut, S.-C. Hong, A. Barlow, and C. A. Janeway, Jr. 1991. Sequence analysis of peptides bound to MHC class II molecules. *Nature (Lond.).* 353:622–624.

50. Martin, R., M. D. Howell, D. Jaraquemada, M. Flerlage, J. Richert, S. Brostoff, E. O. Long, D. E. MacFarlin, and H. F. McFarland. 1991. A myelin basic protein peptide is recognized by cytotoxic T cells in the context of four HLA-DR types associated with multiple sclerosis. *J. Exp. Med.* 173:19–24.

51. Lambert, L. E., and E. R. Unanue. 1989. Analysis of the interaction of peptide hen egg white lysozyme (34–45) with the I-A^k molecule. *J. Immunol.* 143:802–807.

52. Nicholas, J. A., M. E. Levely, M. A. Mitchell, and C. W. Smith. 1989. A 16-amino acid peptide of respiratory syncytial virus 1A protein contains two overlapping T cell-stimulating sites distinguishable by class II MHC restriction elements. *J. Immunol.* 143:2790–2796.

53. Brett, S. J., K. B. Cease, C.-A. S. Ouyang, and J. A. Berzofsky. 1989. Influences of antigen processing on the expression of the T cell repertoire evidence for MHC-specific hindering structures on the products of processing. *J. Immunol.* 143:771–779.

54. Sakai, K., A. A. Sinha, D. J. Mitchell, S. S. Zamvil, J. B. Rothbard, H. O. McDevitt, and L. Steinman. 1988. Involvement of distinct murine T-cell receptors in the autoimmune encephalitogenic response to inbred epitopes of myelin basic protein. *Proc. Natl. Acad. Sci. USA.* 85:8608–8612.

55. Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswel, D. C. Berustein, D. L. Mann, G. M. Sheares, and J. A. Berzofsky. 1989. Interleukin-2 production used to detect antigenic peptide recognition by T-helper lymphocytes from asymptomatic HIV-seropositive individuals. *Nature (Lond.).* 339:383–385.

56. Sette, A., S. Buus, S. Colon, C. Miles, and H. M. Grey. 1989. Structural analysis of peptides capable of binding to more than one Ia antigen. *J. Immunol.* 142:35–40.

57. Panina-Bordignon, P., A. Tan, A. Termijtelen, S. Demotz, G. Corradin, and A. Lanzavecchia. 1989. University immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. *Eur. J. Immunol.* 19:2237–2242.

58. Pette, M., K. Fujita, B. Kitze, J. N. Whitaker, E. Albert, L. Kappos, and H. Wekerle. 1990. Myelin myelin basic protein-specific T lymphocyte lines from multiple sclerosis patients and healthy individuals. *Neurology.* 40:1770–1776.

59. Zhang, Y., M. Schluep, S. Frutiger, G. J. Hughes, M. Jaenner, A. Steek, and T. Barkas. 1990. Immunological heterogeneity of autoreactive T lymphocytes against the nicotinic acetylcholine receptor in myasthenic patients. *Eur. J. Immunol.* 20:2577–2583.

60. Gammon, G., and E. E. Sercarz. 1990. Does the presence of self-reactive T cells indicate the breakdown of tolerance? *Clin. Immunol. Immunopathol.* 56:287–297.

61. Bellone, M., N. Ostlie, S. Lei, and B. M. Conti-Tronconi. 1991. Experimental myasthenia gravis in congenic mice. Sequence mapping and H-2 restriction of T helper epitopes on the α subunits of *Torpedo californica* and murine acetylcholine receptors. *Eur. J. Immunol.* 21:2302–2310.

62. Granato, D. A., B. W. Fulpius, and J. F. Moody. 1986. Experimental myasthenia in Balb/c mice immunized with rat acetylcholine receptor from rat denervated muscle. *Proc. Natl. Acad. Sci. USA.* 73:2872–2876.

63. Gammon, G., and E. E. Sercarz. 1989. How some T-cells escape tolerance induction. *Nature (Lond.).* 342:183–185.

64. Ohno, S. 1991. Many peptide fragments of alien antigens are homologous with host proteins, thus canalizing T-cell responses. *Proc. Natl. Acad. Sci. USA.* 88:3065–3068.

65. Del Val, M., H.-J. Schlicht, T. Ruppert, M. J. Reddehase, and U. H. Koszinowski. 1991. Efficient processing of an antigenic sequence for presentation by MHC class I molecules depends on its neighboring residues in the protein. *Cell.* 66:1145–1153.

66. Sakai, K., S. S. Zamvill, D. J. Mitchell, S. Hodgkinson, J. B. Rothbard, and L. Steinman. 1989. Prevention of experimental encephalomyelitis with peptides that block interaction of T cells with major histocompatibility complex proteins. *Proc. Natl. Acad. Sci. USA.* 86:9470–9474.

67. Wraith, D. C., D. E. Smilek, D. J. Mitchell, L. Steinman, and H. O. McDevitt. 1989. Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. *Cell.* 59:247–255.

68. Gauam, A. M., and P. Glynn. 1990. Competition between foreign and self proteins in antigen presentation. Ovalbumin can inhibit activation of myelin basic protein-specific T cells. *J. Immunol.* 144:1177–1180.

69. Noreen, H. J., M. L. Davidson, J. McCullough, F. H. Bach, and M. Segall. 1989. HLA Class Ii typing by restriction fragment length polymorphism (RFLP) in unrelated bone marrow transplant patients. *Transplant. Proc.* 21:2968–2970.

70. National Institute of Health Transplantation and Immunology Branch Staff. 1979. NIA lymphocyte microcytotoxicity technique T_{40}39. *In* Manual of Tissue Typing Techniques. NIH Publication No. 80-545. T. Ray, Editor. U.S. Dept. of Health, Education, and Welfare, Washington, DC. 39–40.