Both $CD4^+$ and $CD8^+$ T Cells Are Essential to Induce Experimental Autoimmune Myasthenia Gravis

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Summary

$CD4^+$ T cells have been shown to be crucial in the development of experimental autoimmune myasthenia gravis (EAMG). The role of $CD8^+$ T cells in EAMG is less well established. We previously showed that antibody depletion of $CD8^+$ T cells in rats effectively suppresses EAMG. To further study the role and relationship of $CD4^+$ versus $CD8^+$ T cells in induction of EAMG, $CD4^{-/-}$, $CD8^{-/-}$, and $CD4^-8^-$ mutant C57BL/6 mice and the parent $CD4^+8^+$ wild-type mice were immunized with Torpedo acetylcholine receptor (AChR) plus complete Freund's adjuvant. Clinical EAMG was nearly completely prevented in $CD4^-8^-$, $CD4^{-/-}$, and $CD8^{-/-}$ mice. This was associated with strongly reduced AChR-specific T and B cell responses, and with reduced levels of AChR-reactive interferon γ (IFN-γ) and interleukin 4 (IL-4) mRNA-expressing cells in lymphoid organs when compared with $CD4^+8^+$ wild-type mice. We conclude that (*a*) both $CD4^+$ and $CD8^+$ T cells are essential for development of EAMG, and a collaboration between these cell types may be necessary; (*b*) $CD4^+$ as well as $CD8^+$ T cells secrete IFN-γ and IL-4, and both cytokines are involved in the development of EAMG; and (*c*), besides T cells, other immune cells might also be responsible for help of anti-AChR antibody production.

Experimental autoimmune myasthenia gravis (EAMG)[1] can be induced in susceptible mouse strains by repeated injections of acetylcholine receptor (AChR) emulsified in CFA, and serves as a useful animal model for exploring the pathogenesis of human myasthenia gravis (MG) (1). The muscle weakness and fatigue that are the hallmarks of myasthenia gravis and EAMG are due to an antibody-mediated autoimmune attack directed against AChR of the neuromuscular junctions (2). The production of anti-AChR antibodies depends on cytokines produced by T cells (3). T cell responses to AChR, its subunits, and the α-subunit peptides have been described in myasthenia gravis (4, 5) and EAMG (6–8). AChR subunit and peptide-specific rat $CD4^+$ T cells secrete both Th1 (IL-2, IFN-γ) and Th2 (IL-4) cytokines after stimulation with AChR, and provide help for anti-AChR antibody production (3). EAMG is suppressed in mice treated with mAb to murine $CD4^+$ T cells and in mice lacking $CD4^+$ T cells, further supporting a role for these T cells in the pathogenesis of EAMG (9, 10).

We have shown that depletion of $CD8^+$ T cells in Lewis rats also suppresses EAMG and the levels of anti-AChR antibodies (11), indicating that $CD8^+$ T cells are also involved as helper or effector cells in the pathogenesis of this disease. Another report, however, showed that $\beta_2m^{-/-}$ mice, which are $CD8^-$ and MHC class I deficient, have a higher incidence of EAMG than control mice (12). To further analyze the relative contribution of CD4 and CD8 T cells in the induction of EAMG, we induced the disease in $CD4^{-/-}$, $CD8^{-/-}$, and $CD4^-8^-$ mutant C57BL/6J mice and the same strain of $CD4^+8^+$ wild-type mice.

Materials and Methods

Animals and Antigen Preparation. Female C57BL/6J ($H\text{-}2^b$) $CD8^{-/-}$, $CD4^{-/-}$, and $CD4^-8^-$ mutant mice were obtained from Dr. Tak W. Mak using homologous recombination in pluripotent embryonic stem cells, and were bred at the animal house, Division of Immunology, Karolinska Institute. The same strain of female $CD4^+8^+$ mice was obtained from the same animal house. All mice used were 8–12 wk of age, weighing 20–30 g.

[1]*Abbreviations used in this paper:* α-BGT, α-bungarotoxin; AChR, acetylcholine receptor; EAMG, experimental autoimmune myasthenia gravis; M-AChR, muscle AChR extract; MBP, myelin basic protein; MNC, mononuclear cell; p.i., postprimary immunization; MG, myasthenia gravis; PILN, popliteal and inguinal lymph node; SI, stimulation index.

Torpedo AChR was purified from the electric organs of *Torpedo Californica* (Pacific Biomarine, Venice, CA) by affinity chromatography on an α-cobrotoxin-agarose resin (Sigma Chemical Co., St. Louis, MO) as described previously (13). The isolated product was pure as judged by SDS-PAGE. Muscle AChR extract from normal C57BL mice (M-AChR) was prepared as described (13). Myelin basic protein (MBP) used as control antigen was purified from normal mouse brains as described (14).

Induction of EAMG. Mice were immunized with 20 μg *Torpedo* AChR emulsified in CFA in a total volume of 100 μl, injected into five intradermal sites along the back, the hind footpads, and the base of the tail (1), and were boosted on days 30 and 60 after the primary sensitization. The mice were observed every other day in a blinded fashion for signs of clinical muscle weakness. The disease symptoms were graded as follows (9): 0, no definite muscle weakness; 1+, normal strength at rest but weak with chin on the floor and inability to raise the head after exercise consisting of 20 consecutive paw grips; 2+, as grade 1+ weakness at rest; and 3+, moribund, dehydrated, and paralyzed. Clinical EAMG was confirmed by i.p. administration of neostigmine bromide and atropine sulphate. Mice were killed 100 d postprimary immunization (p.i.).

RIA for M-AChR Content. The concentration of AChR of mouse muscle carcass was determined by RIA (10). Briefly, triplicate 2-pmol aliquots of ^{125}I–α-bungarotoxin (α-BGT; Amersham Corp., Arlington Heights, IL)–labeled Triton X–100 solubilized mouse muscle extract were mixed with a standard pooled mouse anti-AChR antiserum. After overnight incubation, rabbit anti–mouse Ig (Dakopatts, Copenhagen, Denmark) was added. The resulting precipitates were pelleted, washed, and pelleted again. Radioactivity of the pellet was counted in a gamma counter (Packard Instrument Co., Inc., Meriden, CT). AChR content is expressed as moles of ^{125}I–α-BGT binding sites per milligram protein of carcass, and the percentage loss of M-AChR in test mouse carcass was calculated (13).

RIA for Serum Anti-AChR Antibodies. Blood specimens were collected from the tail vein just before and then at weekly intervals, and serum antibody concentrations to M-AChR were measured by RIA (13). Briefly, 1 nmol mouse AChR was incubated with 2 nmol ^{125}I–α-BGT. To 1 ml of labeled M-AChR, 1 μl serum was added, followed by rabbit anti–mouse Ig. The samples were centrifuged, washed, and counted in a gamma counter. The AChR precipitated minus the background value permits calculation of the titer in moles of toxin-binding sites bound per liter of serum. Serum samples from CFA-injected CD4$^+$8$^+$ mice were analyzed in parallel.

Mononuclear Cell Suspensions. Suspensions of mononuclear cells (MNC) from the popliteal and inguinal lymph nodes (PILN), spleen, and thymus were prepared as described (11), and cells were suspended in DME supplemented with 1% (vol/vol) MEM (both from Gibco, Paisley, UK), 2 mM glutamine (Flow Laboratories, Irvine, UK), 50 IU/ml penicillin, 50 μg/ml streptomycin, and 10% (vol/vol) FCS (Gibco). The cells were washed three times and then rediluted to a cell concentration of 2×10^6/ml.

Enumeration of AChR-specific IgG Antibody-secreting Cells. A solid-phase enzyme-linked immunospot assay (ELISPOT) was used with some modifications (11). Briefly, wells were coated with 100 μl AChR or MBP (10 μg/ml in PBS). Aliquots of 100-μl cell suspensions containing 2×10^5 MNC were added in triplicate to individual wells. After incubation for 24 h, the wells were emptied, followed by addition of rabbit anti–mouse IgG (Sigma Chemical Co.), biotinylated swine anti–rabbit IgG (Dakopatts), and avidin–biotin peroxidase complex (ABC; Dakopatts). After peroxidase staining, the red-brown immunospots that corresponded to cells having secreted anti-AChR IgG were counted and standardized to number per 10^5 MNC.

AChR-reactive IFN-γ–secreting Cells. Nitrocellulose-bottomed microtiter plates were coated with 100 μl rat IFN-γ capture antibody (DB1; 15) at 15 μg/ml. Aliquots of 200-μl suspensions containing 4×10^5 MNC were added to individual wells in triplicate, followed by antigen (AChR or MBP) or the mitogen Con A (Sigma Chemical Co.) in 10-μl aliquots at a final concentration of 10 μg/ml AChR or MBP, or 5 μg/ml Con A. After 48 h of culture, the wells were emptied. Secreted and bound IFN-γ was visualized by sequential application of polyclonal rabbit anti–rat IFN-γ (15), biotinylated anti–rabbit IgG (Dakopatts), and ABC (Dakopatts). After peroxidase staining, the red-brown immunospots that corresponded to the cells that had secreted IFN-γ were enumerated in a dissection microscope. To calculate the numbers of T cells responding to a particular antigen or mitogen, numbers of spots in culture without antigen added were subtracted from the values obtained after antigen or mitogen exposure. The data were expressed as numbers per 10^5 MNC.

Lymphocyte Proliferation Responses. Triplicate aliquots (200 μl) of MNC suspensions were applied to 96-well round-bottomed microtiter plates (Nunc, Copenhagen, Denmark) at a cell density of 2×10^6/ml. 10-μl aliquots of either AChR, MBP, or Con A were added to appropriate wells at a final concentration of 10 μg/ml (AChR or MBP) or 5 μg/ml (Con A). After 60 h of incubation, the cells were pulsed for 12 h with 10-μl aliquots containing 1 μCi of [^{3}H]methylthymidine (specific activity, 42 Ci/mmol; Amersham Corp.). Cells were harvested onto glass fiber filters and thymidine incorporation was measured. The results were expressed as stimulation index (SI), which was calculated by dividing the counts per minute from culture in the presence of antigen or mitogen by the counts per minute from culture without antigen.

Detection of IFN-γ, IL-4, and TGF-β mRNA Expression by In Situ Hybridization. In situ hybridization was performed as described (16). Briefly, 200-μl aliquots of suspensions from PILN containing 4×10^5 MNC were plated into round-bottomed microtiter plates (Nunc, Roskilde, Denmark) in triplicate. 10-μl aliquots of either AChR, MBP, or Con A were added to appropriate wells at a final concentration of 10 μg/ml (AChR or MBP) or 5 μg/ml (Con A). After culture for 24 h, the cells were washed, counted, and dried onto restricted areas of glass slides (ProbeOn slides; Fisher Scientific, Pittsburgh, PA). Synthetic oligonucleotide probes (Scandinavian Gene Synthesis AB, Köping, Sweden) were labeled using [^{35}S]deoxyadenosine-5′-α-(thio)-triphosphate with terminal deoxynucleotidyl transferase (Amersham Corp.). To increase the sensivity of the method, a mixture of four different oligonucleotide probes was employed for each cytokine. The oligonucleotide sequences were obtained from EMBL/GenBank/DDBJ using the MacVector software (IBI). The rat IFN-γ probes (17) (EMBL/GenBank/DDBJ accession numbers M29315, M29316, and M29317) were complementary to bases 298-345 (exon 1), 80-125 (exon 2), 303-350 (exon 3), and 180-227 (exon 4). The rat IL-4 probes (18) (EMBL/GenBank/DDBJ accession number X16058) corresponded to bases 83-130, 209-256, 270-317, and 331-378. The TGF-β probes (19) (EMBL/GenBank/DDBJ accession number X02812) were complementary to bases 1766-1813 and 1953-2000. The oligonucleotide probes were approximately 48 bases long and checked for the absence of palindromes or long sequence of homology within the species against available EMBL/GenBank/DDBJ data. Cells were hybridized with 10^6 cpm of labeled probe per 100 μl of hybridization mixture. After emulsion autoradiography, development, and fixation,

the coded slides were examined by dark field microscopy for positive cells containing >15 grains per cell in a starlike distribution. The intracellular distribution of the grains was always checked by light microscopy, and labeled cells were expressed as numbers per 10^5 MNC.

Statistical Analysis. Overall differences between the four groups were evaluated by the Kruskal-Wallis one-way analysis of variance (ANOVA). Student's *t* test was used for the comparison of serum anti-AChR antibodies, and the Mann-Whitney U test was used for the other variables analyzed. All significance tests were two-sided.

Results

Muscle Weakness in CD4- and/or CD8-knockout Mice. One out of 15 wild-type ($CD4^+8^+$) mice developed muscle weakness 3 wk after primary sensitization without boosting. Seven more mice developed paralysis after boosting. These animals deteriorated progressively until two died and two were humanely killed (Fig. 1). After the first boosting, two $CD8^{-/-}$ mice exhibited mild and moderate weakness, respectively, lasting to the end. In the $CD4^{-/-}$ group, one mouse got mild EAMG after the first boosting and recovered about 1 mo later; another mouse developed moderate weakness after the second boosting and improved 20 d later. Among the $CD4^-8^-$ mutant mice, only one developed mild and short-term muscle weakness 12 d after the first boosting (Fig. 1).

Loss of M-AChR. The mean values for losses of M-AChR in the $CD4^{-/-}$, $CD8^{-/-}$, and $CD4^-8^-$ mice after immunization with AChR and CFA were 21 ± 18%, 29 ± 15%, and 9 ± 3%, respectively. In contrast, the mean M-AChR loss in the $CD4^+8^+$ wild mice was 54 ± 11%, being significantly higher than in the other three groups ($P < 0.05$, 0.05, and 0.002, respectively). No differences were found among $CD4^{-/-}$, $CD8^{-/-}$, and $CD4^-8^-$ mice.

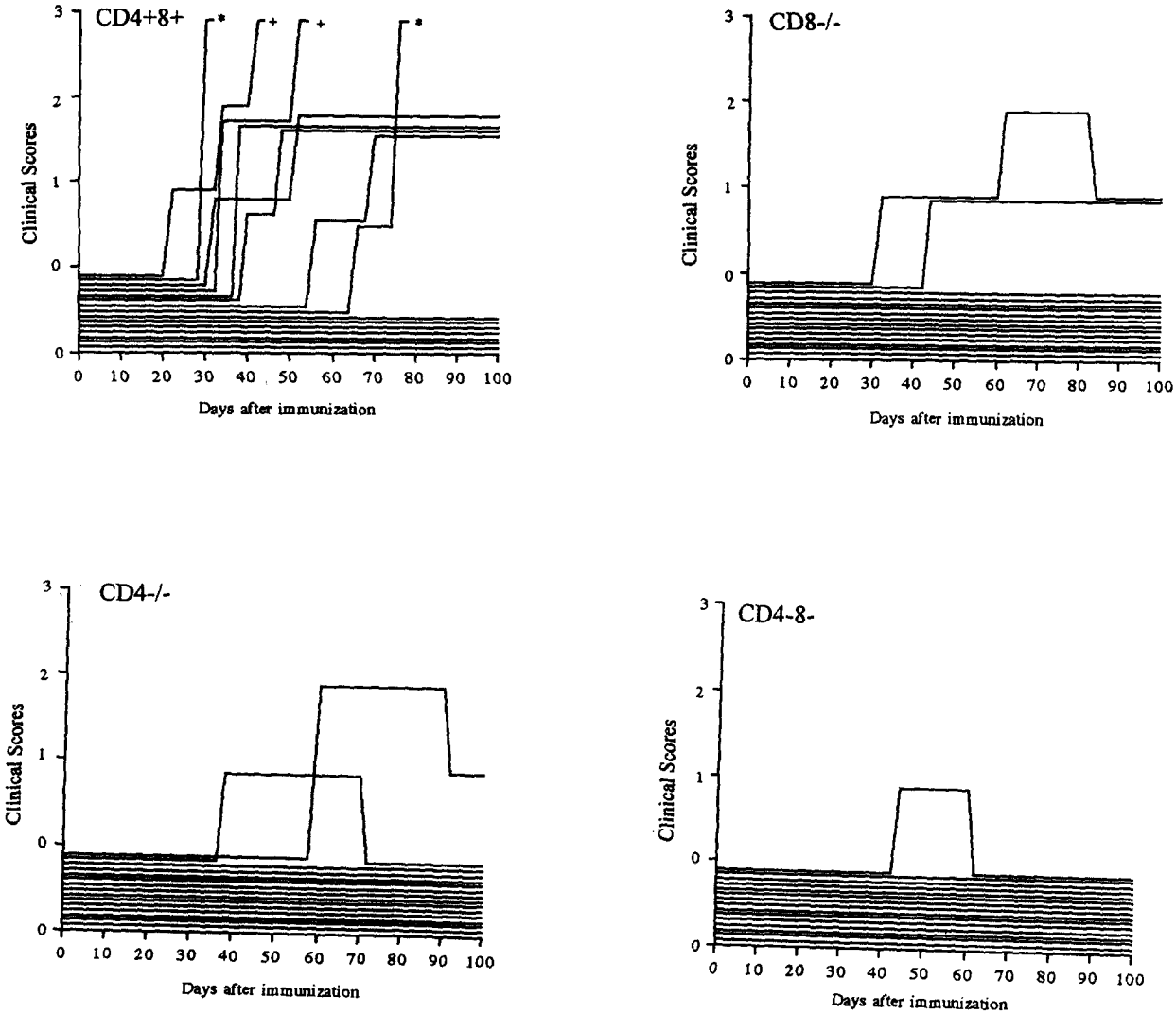

Figure 1. Clinical course of EAMG in $CD4^+8^+$, $CD8^{-/-}$, $CD4^{-/-}$, and $CD4^-8^-$ C57BL/6J mice (n = 15 in each group). Mice were immunized with AChR plus CFA and boosted twice monthly. (*) Died; (+) humanely killed.

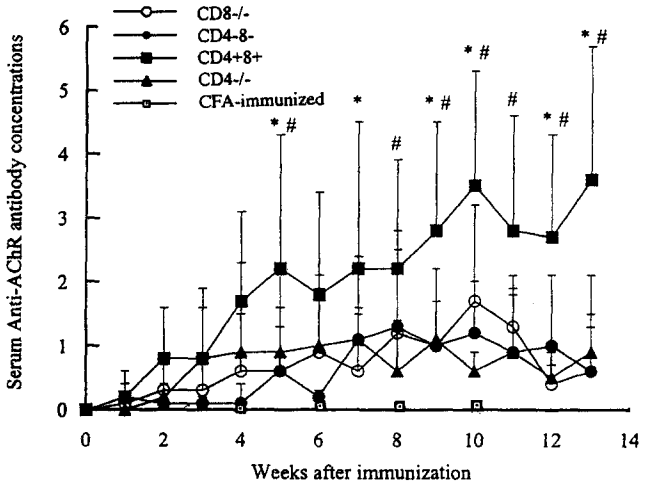

Figure 2. Serum anti-M-AChR antibody levels in the different groups. The antibody concentrations were measured weekly by RIA and expressed as moles of α-BGT–binding sites bound per liter of serum. Symbols refer to mean values, and bars to SD. The differences between $CD4^-8^-$ and $CD4^+8^+$ mice were significant from week 2 p.i. up to end (significant P values were omitted). (*) $P < 0.05$ between $CD4^+8^+$ and $CD8^{-/-}$ mice; (#) $P < 0.05$ between $CD4^+8^+$ and $CD4^{-/-}$ mice.

Anti-AChR Antibodies in Serum and AChR-specific IgG Antibody-secreting Cells. Serum anti-M-AChR antibodies were detected in the $CD4^+8^+$ wild-type mice from week 2 p.i. The levels increased gradually over the observation period (Fig. 2). In contrast, the levels of antibodies in the $CD4^-8^-$ mice were significantly lower from week 2 p.i. up to the end. The levels in the $CD4^{-/-}$ and $CD8^{-/-}$ mice were also significantly lower than in the $CD4^+8^+$ wild-type mice from week 5 p.i. onwards, except at a few time points. There were no differences in antibody levels between $CD4^{-/-}$, $CD8^{-/-}$, and $CD4^-8^-$ mice. No anti-AChR antibodies were found in sera from the CFA-immunized control mice.

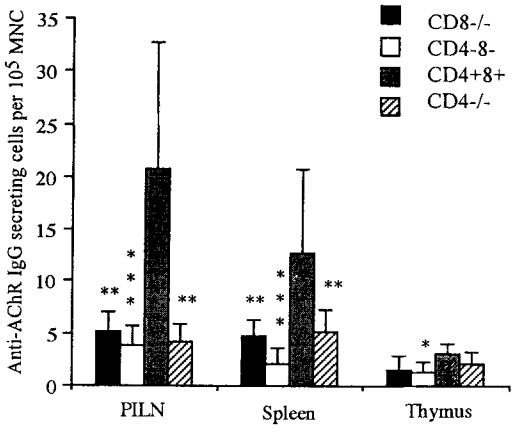

Figure 3. Numbers of anti-AChR IgG antibody-secreting cells per 10^5 MNC isolated from PILN, spleen, and thymus of the different groups. Symbols refer to mean values, and bars to SD. P values refer to comparisons between the $CD4^+8^+$ mice and the other three groups. (*) $P < 0.05$; (**) $P < 0.01$; and (***) $P < 0.001$.

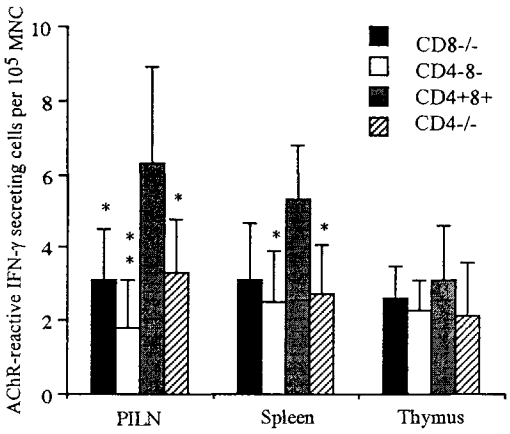

Figure 4. Numbers of AChR-reactive IFN-γ–secreting T cells per 10^5 MNC isolated from PILN, spleen, and thymus of the different groups. Symbols refer to mean values, and bars to SD. P values refer to comparisons between the $CD4^+8^+$ mice and the other three groups. (*) $P < 0.05$; (**) $P < 0.01$.

The highest numbers of anti-AChR IgG antibody-secreting cells were found in lymph nodes and the lowest in thymus (Fig. 3). Lymph nodes as well as spleens from $CD4^+8^+$ mice contained higher numbers of these cells than the other groups. In thymus, numbers of anti-AChR IgG-secreting cells were low throughout, but a significant difference was noticed between $CD4^+8^+$ and $CD4^-8^-$ mice.

AChR-reactive IFN-γ–secreting Cells. As shown in Fig. 4, the lymph nodes from $CD4^+8^+$ EAMG mice contained higher numbers of AChR-reactive IFN-γ–secreting T cells compared with $CD4^-8^-$, $CD4^{-/-}$, or $CD8^{-/-}$ mice. In spleen, $CD4^+8^+$ mice also had higher numbers of these cells than $CD4^-8^-$ and $CD4^{-/-}$ mice. There were no differences between the four groups in thymus. No differences were found when cells from lymphoid organs were evaluated in control experiments after culture with MBP

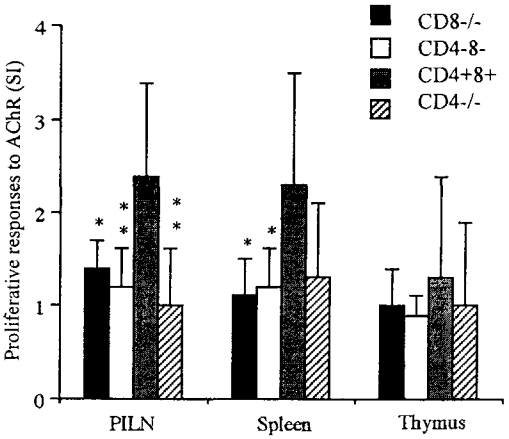

Figure 5. Proliferative responses to AChR by MNC from PILN, spleen, and thymus of the different groups. Symbols refer to mean values, and bars to SD. P values refer to comparisons between the $CD4^+8^+$ mice and the other groups. (*) $P < 0.05$; (**) $P < 0.01$.

(0.4–0.8/10^5 MNC) or without antigen or mitogen (1.0–2.1/10^5 MNC), respectively. After culture with Con A for MNC from lymph nodes, the lowest numbers of IFN-γ–secreting cells were found in $CD4^-8^-$ mice (6.5 ± 2.4/10^5 MNC) and the highest in $CD4^+8^+$ mice (13.6 ± 6.1/10^5 MNC) ($P < 0.05$). No differences were found between other groups.

AChR-induced Lymphocyte Proliferation. Proliferative responses to AChR were higher in lymph node cells of $CD4^+8^+$ mice compared with the other groups. Significant differences were also found in spleens between $CD4^+8^+$ and $CD4^-8^-$ or $CD8^{-/-}$ mice (Fig. 5). All groups showed similar high SI levels after stimulation by Con A (5.4–9.0), and similar low SI levels after culture with the control antigen MBP (0.7–1.3).

AChR-reactive IFN-γ, IL-4, and TGF-β mRNA-expressing Cells. Lymph nodes from $CD4^+8^+$ mice contained higher numbers of AChR-reactive IFN-γ and IL-4 mRNA-expressing cells when compared with $CD4^{-/-}$, $CD8^{-/-}$, and $CD4^-8^-$ mice. The numbers of IFN-γ–mRNA-expressing cells in $CD4^{-/-}$ mice were also higher than in $CD4^-8^-$ mice. There were no differences among the three groups of mutant mice for IL-4 mRNA-expressing cells. Numbers of AChR-reactive TGF-β mRNA-expressing cells were lower in $CD4^-8^-$ mice compared with $CD4^+8^+$ wild-type mice, but not among the other groups (Fig. 6). No differences were detected between the four groups for MBP-induced cytokine mRNA-expressing cells (2.0–3.5/10^5 MNC for IFN-γ, 1.3–2.5 for IL-4, and 2.6–3.3 for TGF-β) or after culture of MNC without antigen or mitogen (2.2–3.8 for IFN-γ, 1.3–2.5 for IL-4, and 2.2–3 for TGF-β). After being stimulated by Con A, the numbers of mRNA-expressing cells for IFN-γ were 43.6 ± 9.7 in $CD4^-8^-$ mice and 62.3 ± 10.2 in $CD4^+8^+$ mice; for IL-4 they were 5.3 ± 1.9 in $CD4^-8^-$ mice and 10.2 ± 4.0 in $CD4^+8^+$ mice ($P < 0.05$, respectively). There were no differences between the other groups for IFN-γ and IL-4, or between all four groups for TGF-β (20.6 ± 4.7–35.3 ± 13.2/10^5 MNC).

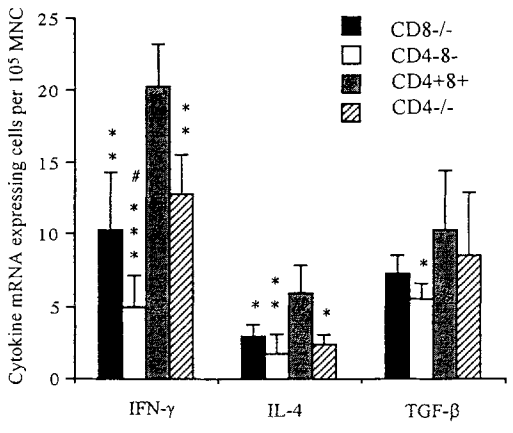

Figure 6. Numbers of AChR-reactive IFN-γ, IL-4, and TGF-β mRNA-expressing cells per 10^5 MNC isolated from PILN of the different groups. Symbols refer to mean values, and bars to SD. (*) $P < 0.05$; (**) $P < 0.01$ between the $CD4^+8^+$ mice and the other three groups; and (#) $P < 0.05$ between $CD4^{-/-}$ and $CD4^-8^-$ mice.

Discussion

This study provides evidence that both CD4 and CD8 T cells are involved in the induction of EAMG as helper or effector cells. The importance of $CD4^+$ T cells in the pathogenesis of EAMG is in line with previous data on EAMG and human MG (6, 7, 10, 20). However, the role of $CD8^+$ T cells in EAMG is unclear. Shenoy et al. (12) reported that $\beta_2m^{-/-}$ mice with deficient MHC class I expression and low CD8 cell counts showed more severe EAMG compared with controls, and postulated that the increased disease severity was a result of a lack of regulator/suppressor $CD8^+$ T cells (12). By using $CD8^{-/-}$ and $CD4^-8^-$ mice, we now present evidence that $CD8^+$ T cells also function as effector/helper cells in EAMG. There are several reasons why results between $CD8^{-/-}$ and $\beta_2m^{-/-}$ mice differ. (*a*) $\beta_2m^{-/-}$ mice indeed process functional $CD8^+$ T cells (21, 22). After in vivo priming and in vitro restimulation with $H\text{-}2^d$ spleen cells, $\beta_2m^{-/-}$ ($H\text{-}2^b$) responder mice developed the same strong cytotoxic responses against the allospecific $H\text{-}2^d$–expressing targets as $\beta_2m^{+/-}$ control mice (21). The specific ability of residual $CD8^+$ T cells in β_2m-deficient mice to react with class I $H\text{-}2^b$ molecules on normal $H\text{-}2^b$–expressing cells indicates that these T cells have not undergone negative selection (23), and $CD8^+$ T cells specific for self-peptides consequently may escape elimination. (*b*) Besides their restriction by MHC class I, $CD8^+$ T cells are also restricted by MHC class II (24). These class II–restricted $CD8^+$ T cell subsets, which can secrete IFN-γ, IL-4, and IL-10 (23), will survive in β_2m-deficient mice. (*c*) Rats depleted of $CD8^+$ cells with mAb had less severe EAMG accompanied by decreased B and T cell responses to AChR (11). (*d*) An unexpected function of MHC class I was observed in experimental SLE, an autoantibody-mediated disease, since SLE-susceptible mice lacking MHC class I did not develop any clinical SLE manifestations, whereas mice depleted of $CD8^+$ T cells remained susceptible (25). These data indicate that experimental manipulation of $CD8^+$ T cells will not parallel manipulation of MHC class I.

T cells have for a long time been functionally classified according to their CD phenotype, i.e., $CD4^+$ T cells are helper/inducer and $CD8^+$ T cells are suppressor/cytotoxic cells. Recent studies suggest that the functions of T cells are more complicated (26). There are suppressor $CD4^+$ T cells that can produce TGF-β and are important in inducing tolerance (27). $CD8^+$ T helper cells can also be classified into Th1 and Th2 subsets according to their phenotypes and production of cytokines (28, 29). Based on the production of IL-4 and IFN-γ, the $CDw60^+CD8^+$ cells are similar to the $CD4^+$ Th2 subset, whereas the $CDw60^-CD8^+$ cells resemble the Th1 subset (28). $CD8^+$ cells were also found to help B cells in antibody production through the expression of CD40 ligand (30). Elimination of either $CD4^+$

or $CD8^+$ T cells suppresses some T cell– or antibody-mediated experimental autoimmune diseases (31–34). The present study suggests that both $CD4^+$ and $CD8^+$ cells are essential for the development of EAMG. Interestingly, in human MG, there is a striking association with the MHC class I allele HLA B8, which is stronger (higher relative risk), than with the MHC class II allele DR3 (35). In view of the fact that MHC class I is the main restriction element for $CD8^+$ T cells, the present observation is in agreement with a MHC class I association in MG.

Mice lacking $CD4^+$ T cells have normal development and function of $CD8^+$ T cells and some TCR-$\alpha/\beta^+CD4^-8^-$ cells (36). Mice lacking $CD8^+$ T cells have normal development and function of $CD4^+$ cells (37). In $CD4^-8^-$ mutant mice, B cells and TCR-$\alpha/\beta^+CD4^-8^-$ cells have expanded to occupy the compartment that would otherwise have been occupied by $CD4^+8^-$ and $CD4^-8^+$ cells (38). These results indicate that a reduced communication/adhesion between antigen-specific T cells and APC, as a consequence of the absence of marker molecules (i.e., CD4/CD8 surface antigens) in remaining T cells, might also play a role in the amelioration of EAMG in our animal model. On the other hand, CD4 or CD8 cells separately are not sufficient to induce EAMG, suggesting that a collaboration between CD4 and CD8 T cells may be necessary. In EAE, it was speculated that $CD4^+$ effector cells initially home to the central nervous system, and $CD8^+$ T cells are subsequently recruited and may subserve an effector function that augments the damage of myelin (31). In nonobese diabetic mice, $CD4^+$ cells home to the pancreas and promote the influx of $CD8^+$ cells, which finally leads to the full development of insulitis (39). Whether or not this is the case in antibody-mediated EAMG is currently under investigation.

The Th1-associated proinflammatory IFN-γ may play a role in both human MG and EAMG (5, 8). In transgenic mice expressing IFN-γ in neuromuscular junctions, autoantibody deposits at motor end plates imply that IFN-γ in the milieu of the muscle tissue induces humoral autoimmunity (40). After tolerance induction by oral or nasal administration of AChR, numbers of AChR-reactive IFN-γ–secreting cells are reduced in association with decreased muscular weakness (8, 41). The present study suggests that both $CD4^+$ and $CD8^+$ antigen-specific T cells produce IFN-γ and, thereby, provide help to induce EAMG. In EAE, both $CD4^+$ and $CD8^+$ MBP-specific T cells can secrete large amounts of IFN-γ. Furthermore, oral tolerance to EAE could be established after depletion of either $CD4^+$ or $CD8^+$ T cells (42). Taken together, IFN-γ seems to be an important promotor in both B– and T cell–mediated autoimmune diseases.

The decrease of anti-AChR antibodies in CD4 and CD8 mutant mice might be a result of reduced levels of IL-4 mRNA-expressing cells. IL-4 is involved in the differentiation of resting B cells to Ig-secreting cells (43) and is the major anti-AChR antibody-promoting factor (3). It has been observed that human MG and EAMG are associated with elevated numbers of IL-4 mRNA-expressing cells (16, 44). Besides $CD4^+$ T cells, type 2 $CD8^+$ T cells can produce similar amounts or even two or three times more IL-4 mRNA (24). Our results are in line with these observations. In the present study, lack of either $CD4^+$ or $CD8^+$ T cells does not substantially decrease the amounts of TGF-β, whereas lack of both subsets does, indicating that both $CD4^+$ and $CD8^+$ T cells are to some extent sources of TGF-β. Though TGF-β functions as an immunosuppressive cytokine, the increased TGF-β amounts in wild-type $CD4^+8^+$ mice are not high enough to counteract disease development. It can be concluded that the suppression of EAMG in CD4- and/or CD8-knockout mice is mainly caused by a decrease in the production of effectors like IFN-γ and IL-4, but not mediated by TGF-β.

Notably, $CD4^-8^-$ mice developed some amounts of anti-AChR antibodies in serum after immunization with AChR plus CFA. This would suggest that B cells can be activated to produce anti-AChR antibodies to some degree without the help of costimulatory cell surface antigens on $CD4^+$ and $CD8^+$ cells. Another possibility is that, besides T cells, additional types of cells such as macrophages (45) can provide B cell help. Further studies defining the roles of immune cells beyond T cells are thus crucial to understand the development of MG and EAMG.

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