Interferon γ (IFN-γ) Is Necessary for the Genesis of Acetylcholine Receptor–induced Clinical Experimental Autoimmune *Myasthenia gravis* in Mice

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Summary

Experimental autoimmune myasthenia gravis (EAMG) is an animal model of human myasthenia gravis (MG). In mice, EAMG is induced by immunization with *Torpedo californica* acetylcholine receptor (AChR) in complete Freund's adjuvant (CFA). However, the role of cytokines in the pathogenesis of EAMG is not clear. Because EAMG is an antibody-mediated disease, it is of the prevailing notion that Th2 but not Th1 cytokines play a role in the pathogenesis of this disease. To test the hypothesis that the Th1 cytokine, interferon (IFN)-γ, plays a role in the development of EAMG, we immunized IFN-γ knockout (IFN-gko) (−/−) mice and wild-type (WT) (+/+) mice of H-2^b haplotype with AChR in CFA. We observed that AChR-primed lymph node cells from IFN-gko mice proliferated normally to AChR and to its dominant pathogenic α146–162 sequence when compared with these cells from the WT mice. However, the IFN-gko mice had no signs of muscle weakness and remained resistant to clinical EAMG at a time when the WT mice exhibited severe muscle weakness and some died. The resistance of IFN-gko mice was associated with greatly reduced levels of circulating anti-AChR antibody levels compared with those in the WT mice. Comparatively, immune sera from IFN-gko mice showed a dramatic reduction in mouse AChR-specific IgG1 and IgG2a antibodies. However, keyhole limpet hemocyanin (KLH)–priming of IFN-gko mice readily elicited both T cell and antibody responses, suggesting that IFN-γ regulates the humoral immune response distinctly to self (AChR) versus foreign (KLH) antigens. We conclude that IFN-γ is required for the generation of a pathogenic anti-AChR humoral immune response and for conferring susceptibility of mice to clinical EAMG.

Myasthenia gravis (MG)[1] is a T cell–dependent antibody-mediated disease whose hallmark is an autoimmune neuromuscular disorder (1). The cause is a loss of functional acetylcholine receptors (AChR) at the postsynaptic membrane, mediated by autoantibodies (AAbs) and complement (2). Experimental autoimmune MG (EAMG) is a well-established animal model for exploring the pathogenesis of this disease in humans. In EAMG, the autoimmune destruction of AChR produces a defect in neuromuscular transmission causing the characteristic muscle weakness and fatigue of the disease. EAMG can be induced in mice of the H-2^b haplotype by repeated immunizations with AChR emulsified in CFA (3).

The role of cytokines in the immunopathogenesis of AChR-induced EAMG is not clear. Because EAMG is an antibody-mediated disease, it has been thought Th2 cytokines play a major role in the pathogenesis of this disease. The prevailing notion in autoimmunity is that Th1 cytokines (IFN-γ) are associated with cell-mediated rather than antibody-mediated diseases. However, in previous studies from our laboratory, the ectopic expression of proinflammatory Th1 cytokine IFN-γ in the neuromuscular junction elicited a humoral IgG response to an unidentified antigen within the motor end plate, yielding a MG-like syndrome in mice (4). Therefore, we tested here the requirement of IFN-γ in the development of AChR-induced EAMG in mice. For this purpose, we used IFN-γ knockout (IFN-gko) mice in which IFN-γ gene activity was disrupted and wild-type (WT) mice whose IFN-γ gene was intact.

[1]*Abbreviations used in this paper:* AAbs, autoantibodies; AChR, acetylcholine receptor; EAMG, experimental autoimmune myasthenia gravis; HRPO, horseradish peroxidase; IFN-gko, IFN-γ knockout; LNC, lymph node cells; M-AChR, mouse AChR; MG, myasthenia gravis; NMS, normal mouse serum.

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Materials and Methods

Mice. IFN-gko mice of the H-2^b haplotype (5) were provided by Dr. D. Dalton (Trudeau Institute, NY). Heterozygous IFN-gko (+/−) (129/SvEv × C57BL/6)F1 mice were intercrossed in our animal facility to generate homozygous (−/−) gko (129/SvEv × C57BL/6) F2 mice. WT (129/SvJ × C57BL/6)F2 mice (+/+) were used as positive control mice and were purchased from The Jackson Laboratory (Bar Harbor, ME). In addition, C57BL/6 mice were used as additional controls (The Jackson Laboratory). Both 129/SvJ and 129/SvEV are derived from the same parental strain. The difference between the two substrains is that the SvEv was crossed once with C3H, then the F1 were backcrossed again 14 times back to Sv parental strain but SvEv substrain is very much (99.99%) similar to Svj (6). Mice were 8- to 10-wk-old when used in the experiments in compliance with institutional guidelines.

Isolation of Genomic DNA and PCR. IFN-gko (−/−) mice were screened by PCR of tail DNA. An aliquot of the genomic DNA was amplified in a PCR using primers binding to the *neo* gene (*Neo*490V, 5′-CGGTTCTTTTTGTCAAGAC-3′; *NB3*, 5′-ATCCTCGCCGTCGGGC ATGC-3′) (391-bp product) and exon 2 and 3 of the IFN gene (*A1*, 5′-AAGTGGCATAGATGTGGAAG-3′; *B1*, 5′-GAATGCATCCTTTTTCGCCT-3′) (223-bp product). PCR conditions were as follows: one step at 94°C for 1 min, 30 s; then 33 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The final step was at 72°C for 5 min.

Antigens. AChR was purified from Triton X-100 extracts of electric organ membranes from *Torpedo californica* by affinity chromatography on a conjugate of α-bungarotoxin coupled to agarose (7). AChR–α146–162 peptide LGIWTYDGTKVSISPES (8) was synthesized at >70% purity. KLH (Cal Biochem, San Diego, CA), OVA (Sigma Chemical Co., St. Louis, MO), purified protein derivative (PPD; Statens Seruminstitute, Copenhagen, Denmark), and Con A (Pharmacia Biotech, Piscataway, NJ) were purchased as designated.

Lymphocyte Proliferation Assay. Mice were immunized at the base of the tail with 20 μg of AChR or 100 μg of KLH in 100 μl of CFA emulsion. After 5 d of immunization, the mice were killed, and their draining para aortic and inguinal lymph node cells (LNC) were cultured in 0.2 ml of RPMI-10 at 4×10^5/well in 96-well, flat-bottomed microtiter plates (Becton Dickinson, Franklin Lakes, NJ) with and without AChR (10 μg/ml) or α146–162 peptide (20 μg/ml). KLH and OVA were used at 50 μg/ml and 20 μg/ml, respectively. RPMI-10 consisted of RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 20 mM Hepes, 3×10^{-5} M 2-ME, 2×10^{-3} M L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cultures were incubated for 4 d at 37°C in humidified 5% CO_2-enriched air and were pulsed with 1 μCi [^{3}H]TdR/well during the last 18 h of incubation. [^{3}H]TdR uptake was measured in a Beckman β scintillation counter. The results were expressed as a stimulation index, i.e., (mean cpm with antigen) / (mean cpm without antigen).

Cytokine ELISA. Single cell suspensions of draining LNC of AChR-primed mice were cultured at 10^6/ml in RPMI-10 and 2.5 μg/ml AChR in 24-well, flat-bottomed plates (Corning Glass Works, Corning, NY) at 37°C in 5% CO_2 and 95% humidity. The supernatants were collected after 48 h of in vitro boosting. An ELISA kit was used for detection of IFN-γ (PharMingen, San Diego, CA). Concentrations of IFN-γ were determined using a standard curve based on known quantities of mouse recombinant IFN-γ (Genzyme, Cambridge, MA).

Induction and Assessment of EAMG. Groups of mice (n = 13 to 15) were immunized subcutaneously in both hind footpads and at two shoulder regions with 20 μg of *Torpedo californica* AChR in CFA (*Mycobacterium tuberculosis*, H37Ra) (Difco Laboratories, Detroit, MI). Each site received ~5 μg of AChR in 50 μl of emulsion. Mice were boosted on days 30 and 75 with 20 μg of AChR in CFA at selected sites in the shoulders and also in thigh regions (four sites). In a blind study, these mice were assessed daily for the characteristic symptom of EAMG: muscle weakness graded from 0 to 3 as described earlier (3). Muscle weakness attributable to MG was further confirmed by administering intraperitoneally the anticholinesterase, neostigmine bromide, combined with atrophine sulfate, and assessing for temporary improvement in muscle strength (4).

Radioimmunoassay for Anti–mouse-AChR (M-AChR) Abs. Serum anti–M-AChR Ab levels were determined by using an established protocol (7). M-AChR (1×10^{-9}) was incubated at 4°C in Triton buffer with [^{125}I]α-bungarotoxin (2×10^{-9} M) for 4 h. To 1 ml of labeled M-AChR, 1 μl sera from experimental mice was added. Normal mouse serum (NMS) was used as a control. After overnight reaction at 4°C, rabbit anti–mouse Ig (100 μl) was added. After 4-h incubation at room temperature, the tubes were centrifuged, and the pellet was washed with 1 ml Triton buffer, centrifuged again, and counted in a γ counter. The difference in AChR counts precipitated in the experimental versus the control samples was used to calculate the Ab response in nanomoles of toxin binding sites per liter of serum.

ELISA for IgG Isotype Determination. Anti–M-AChR responses were measured as described earlier (9). The 96-well flat-bottomed polysterene plates (Corning Glass Works, Corning, NY) coated with M-AChR (0.5 μg/ml) in 0.1 M carbonate–bicarbonate buffer (pH 9.6) were incubated overnight at 4°C. The wells were blocked with 2% BSA in PBS at room temperature for 30 min. Serum samples (diluted 1:4,000 for IgG1, and IgG2b; 1:200 for IgG2a) were added and incubated at 37°C for 90 min. After four washes, horseradish peroxidase (HRPO)–conjugated goat anti–mouse IgG isotypes (1:2,000) (CALTAG Labs., San Francisco, CA) were added and incubated at 37°C for 90 min. After washing the plates, 0.3 mg/ml 2.2′-Azino-di 3-athyl benzthiazolinsulfonat (Boehringer Mannheim GmbH, Mannheim, Germany) substrate solution was added and allowed to develop color at room temperature in the dark. Serially diluted anti-AChR and NMS were used as positive and negative controls, respectively. Plates were read at OD_{410} nm and results were expressed as OD values.

Measurement of the Ab Response to KLH. Mice (n = 6) were primed with 100 μg KLH in CFA on day 0 and boosted on days 30 and 75 as in the AChR immunization protocol. In brief, we coated the 96-well flat-bottomed plates (Corning Glass Works, Corning, NY) with 5 μg/ml KLH in PBS overnight at 4°C. Later, the wells were blocked for 2 h at 37°C with PBS containing 1% BSA, 10% heat-inactivated fetal bovine serum, and 0.05% Tween-20. Immune sera (diluted 1:800,000 for IgG1; 1:6,400 for IgG2a) were added and incubated for 2 h at room temperature. For IgG isotype measurement, HRPO-labeled Ab to murine IgG isotypes was used at 1:2,000 dilution in plates incubated for 2 h at room temperature. After three washes, color was developed with the substrate, *o*-phenylene-diamine dihydrochloride (Sigma Chemical Co.), and OD read at 492 nm. Serially diluted anti-KLH sera and NMS were used as positive and negative controls, respectively. The results were expressed as OD values.

Results

The IFN-γ Gene Disruption Prevents the AChR-induced Clinical EAMG. To test the hypothesis that IFN-γ plays a

Table 1. *Ablation of Endogenous IFN-γ Prevents the Development of AChR-induced EAMG in Mice*

In vivo	No. of mice per group	Muscle weakness (grade) 0	1	2	3	No. of mice died due to severe disease	Disease incidence
IFN-gko (−/−)	14	14	−	−	−	−	0/15 (0%)
Wild type (+/+)	13	1	7	3	2	2	12/13 (92.3%)
C57BL/6 (+/+)	15	2	6	4	1	2	13/15 (86.7%)

Clinical manisfestation of EAMG was graded as follows: grade 0 indicated no muscle weakness even after exercise consisting of 20–30 consecutive paw grips on cage top steel grids. Grade 1 was defined as normal at rest, but weak with chin on the floor and inability to raise head after exercise. Grade 2 was defined as grade 1 weakness at rest. Grade 3 was moribund, dehydrated, or paralyzed. Clinical EAMG was further confirmed by assessing for temporary improvement after administering a combination neostigmine bromide and atrophine sulfate (4).

role in the development of AChR-induced EAMG of susceptible H-2^b mice, we immunized C57BL/6 (+/+) (n = 15), WT (+/+) (n = 13), and IFN-gko (−/−) (n = 14) mice with 20 μg of AChR in CFA on day 0 and again on days 30 (first boost) and 75 (second boost). After the first boost, the mice were monitored daily for clinical symptoms (muscle weakness) of EAMG. The final results appear in Table 1. At 39 d after the first immunization, 7 of 15 C57BL/6 (+/+) mice and 8 of 13 WT (+/+) mice developed muscle weakness, but none of the 14 IFN-gko (−/−) mice exhibited such weakness. At 42 d after first immunization, one C57BL/6 (+/+) mouse died from severe clinical EAMG; then 11 and 14 d later, two more died. Although none of the WT (+/+) mice died as a result of severe disease, two were killed for humanitarian reasons because of their severe muscle weakness 11 d after the second boost. However, the IFN-gko (−/−) mice remained resistant to clinical EAMG (P values: IFN-gko versus WT = <0.001; P value: IFN-gko versus C57BL/6 = <0.001). These results indicate that IFN-γ influences the genesis of EAMG.

The IFN-γ Gene Disruption Does Not Impair the Proliferation of Lymphocytes in Response to AChR and Its Dominant Epitope α146–162. $CD4^+$ T cells reactive to AChR and its dominant α146–162 peptide are pivotal in the ability of B cells to generate pathogenic anti-AChR Abs (3, 10–12). To address whether the resistance of IFN-gko (−/−) mice results from their inability to evoke T cell responses to AChR and its α146–162 peptide, we immunized WT (+/+) and IFN-gko (−/−) mice with 20 μg of AChR in CFA. 5 d later, proliferation of their draining LNC was assayed. As illustrated in Fig. 1 *A*, the AChR-primed LNC from C57BL/6 and WT (+/+) mice proliferated strongly in response to AChR, as expected. LNC from IFN-gko (−/−) mice also proliferated significantly against the AChR. Significant proliferative responses (SI = 25.5 ± 1.8) to α146–162 peptide of AChR were also observed with AChR-primed LNC from IFN-gko mice (data not shown). A similar magnitude of proliferative responses were observed with LNC from IFN-gko and control group mice after 14 d of AChR priming (data not shown). Interestingly, when

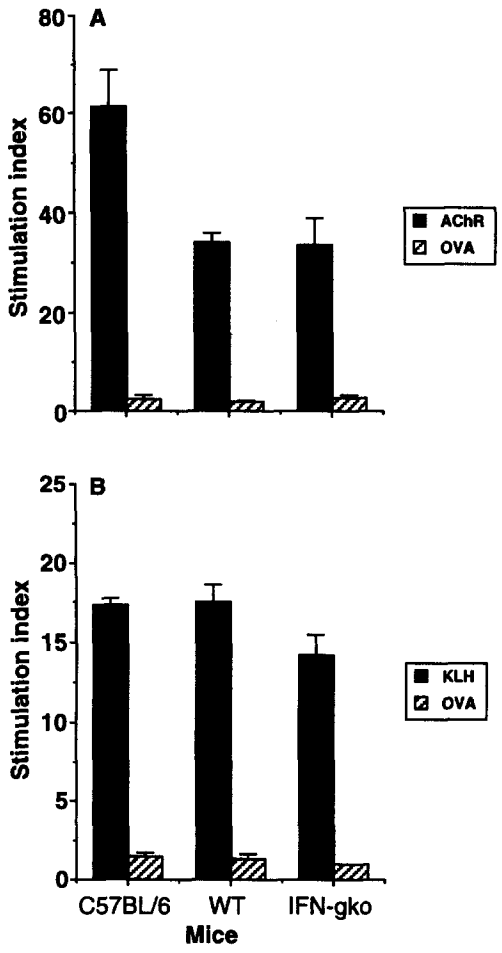

Figure 1. (*A*) Effect of IFN-γ gene disruption on in vitro lymphocyte proliferation in response to AChR (10 μg/ml) and control antigen (OVA; 20 μg/ml) on day 5 after immunization with 20 μg AChR in CFA. Results were expressed as a stimulation index. Background cpm in the absence of antigen for C57BL/6, WT, and IFN-gko mice are as follows: 1,019, 1,039, 3,443. (*B*). Effect of IFN-γ gene disruption on in vitro lymphocyte proliferation in response to KLH (50 μg/ml) and control antigen (OVA; 20 μg/ml) on day 5 after immunization with 100 μg KLH in CFA. Background cpm in the absence of antigen for C57BL/6, WT, and IFN-gko mice are as follows: 15,184, 14,223, 17,095.

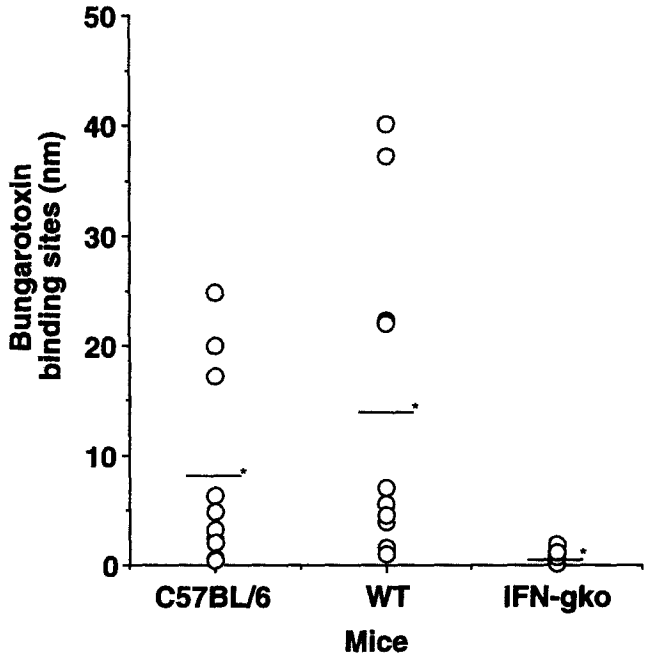

Figure 2. RIA of serum anti-AChR Ab to mouse muscle AChR. Serum samples were collected on day 89 after immunization. The difference in anti-AChR antibody levels between WT and IFN-gko mice (P = 0.002) and between C57BL/6 and IFN-gko mice (P = 0.003) was statistically significant. The difference in anti-AChR antibody levels between WT and C57BL/6 mice (P = 0.222) is not statistically significant. —*, the mean value.

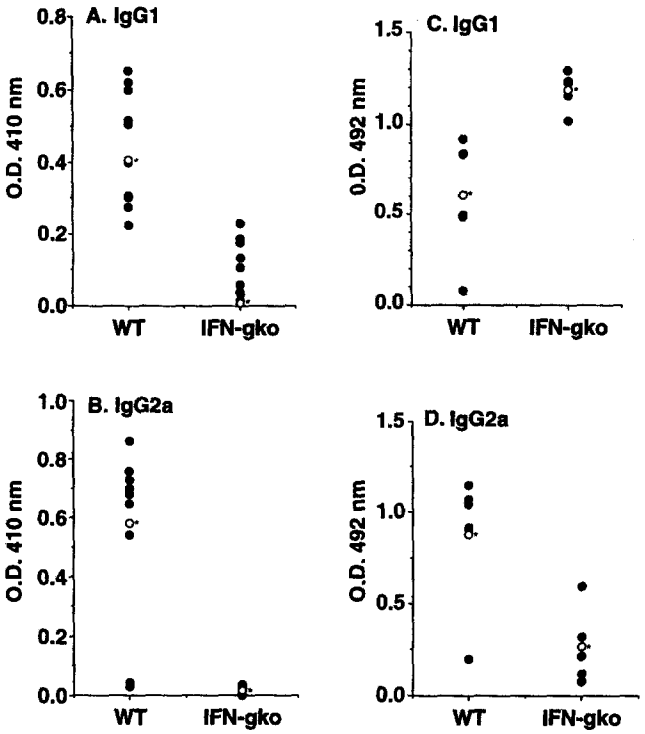

Figure 3. The effect of IFN-γ gene disruption on anti-AChR Ab isotypes (*A*) IgG1, (*B*) IgG2a and anti-KLH Ab isotypes, (*C*) IgG1, (*D*) IgG2a. Serum samples were collected on day 89 after the first immunization with AChR or KLH. The differences in IgG1 and IgG2a responses between AChR-immunized WT and IFN-gko mice were statistically significant; for IgG1 response, P = 0.000; for IgG2a response, P = 0.000. *, the mean value.

the mice were immunized with KLH, the intensity of the proliferative response of draining LNC from IFN-gko (−/−) and WT (+/+) mice appear similar (Fig. 1 *B*). Further examination of IFN-γ levels in the culture supernatants collected 48 h after an in vitro boost with AChR revealed the presence of IFN-γ in cultured LNC of the C57BL/6 and WT (+/+) mice but not of the IFN-gko (−/−) mice (data not shown).

The IFN-γ Gene Disruption Dramatically Affects Serum Anti-AChR Ab Response. The primary pathology of EAMG, the end plate AChR loss, stems from the deleterious effect of pathogenic AAbs to the AChR (2). Therefore, to learn whether a reduced anti-AChR Ab response underlies the resistance of IFN-gko (−/−) mice to EAMG, we used RIA to compare their levels of serum anti-M-AChR Ab to those in C57BL/6 (+/+), and WT (+/+) mice on day 89 after immunization with AChR in CFA. The results appear in Fig. 2. The results indicate that the resistance of the mutants (−/−) to EAMG correlated with their dramatic decrease in the level of AAbs to AChR compared with significantly higher levels in the C57BL/6 (+/+) and WT (+/+) mice. The individual AChR-immunized C57BL/6 (+/+) and WT (+/+) mice responded heterogeneously. A wide heterogeneity in anti-AChR response has earlier been documented in inbred C57BL/6 mice (13). However, we did not find a correlation between the anti-AChR antibody response magnitude of individual mice (C57BL/6 and WT) and severity of EAMG. Our findings further support the earlier findings that no correlation exists between the magnitude of the anti-AChR antibody response and severity of EAMG (8, 13–15).

The IFN-γ Gene Disruption Dramatically Affects Serum Anti-AChR Isotype Response. Because IFN-γ has been associated with the IgG2a response (16), we examined AChR-immunized mice for levels of IgG2a and IgG1 with an IgG isotype-specific ELISA. Comparison of immune sera from IFN-gko (−/−) mice and WT (+/+) mice revealed dramatic reductions in both IgG1 and IgG2a Ab levels in the IFN-gko (−/−) mice (Fig. 3, *A–B*). Similarly, the IgG2b and IgG3 Ab levels of the latters were significantly reduced (data not shown). Therefore, AChR priming of IFN-gko (−/−) mice apparently failed to elicit significant amounts of Ab in any IgG subclass.

Finally, to assess whether the observed IgG subclass distribution was unique to AChR stimulation, we used KLH as the immunogen. Sera from mice immunized with KLH on the same schedule used for AChR priming were subsequently analyzed for IgG isotypes. As the results in Fig. 3, *C–D*, show, immune sera from IFN-γ–containing WT (+/+) mice, when compared with such sera from IFN-gko (−/−) mice, had decreased levels of IgG2a and increased levels of IgG1, confirming the association between IFN-γ and the IgG2a response (17).

Discussion

Our report documents that IFN-γ is essential for the development of EAMG in mice. Herein, we demonstrate that the lack of endogenous IFN-γ dramatically affected the humoral autoimmune response to AChR and prevented clinical disease in H-2^b mice. However, T cell responses to AChR were found relatively normal. Unexpectedly, our findings also demonstrate a dichotomy of the requirement for IFN-γ in the induction of humoral immune response to self (AChR) and foreign (KLH) antigens.

The EAMG-resistant IFN-gko mice failed to mount significant M-AChR–specific Ab responses (Fig. 2). The importance of this lapse may be that their AChR-specific Ab levels were too low (*a*) to provoke antigenic modulation at the motor end plate (18, 19); (*b*) to competitively block AChR function (20); and/or (*c*) to destroy postsynaptic membranes through complement fixation (21, 22), thereby preventing immune destruction.

The IFN-gko (−/−) and the control WT (+/+) mice used in our study were derived from intercrossing of (129/SvEv × C57BL/6)F1 mice and (129/SvJ × C57BL/6)F1 mice, respectively. Therefore, the genetic origin (129/Sv substrain) of the embryonic stem cells used provides a source of genetic heterogeneity in mice, although the SvEv and SvJ substrains are very (99.99%) much similar (see Materials and Methods). However, this genetic heterogeneity may have little influence, if any, on the dramatic effect that we were measuring. This interpretation stems from three pieces of observations: (*a*) the complete resistance (100%) of IFN-gko mice to clinical EMAG and a higher incidence (92.3%) of disease in WT mice (Table 1); (*b*) a dramatic reduction in anti–M-AChR response in all of the IFN-gko mice (Fig. 2); and (*c*) a similar heterogeneous humoral immune response among individual WT (+/+) and C57BL/6 mice (Fig. 2). Thus, the simplest and most direct interpretation of our findings is that the phenotype (resistance to EAMG) reflects an effect of IFN-γ. Therefore, the resistance of IFN-gko mice to EAMG is attributed to the absence of IFN-γ.

The dichotomy observed in the humoral immune response to AChR versus KLH-immunized IFN-gko (−/−) mice is unexpected. Because a significant AChR-specific T cell proliferative response is observed in WT (+/+) and IFN-gko (−/−) mice, the loss of M-AChR–specific Ab responses in IFN-gko (−/−) mice probably results from defective T cell–B cell cognate interactions. What role could IFN-γ play in that process? It is known that T cells responding to self-antigen(s) are generally of low affinity compared with those responding to foreign antigens (23, 24). Additionally, the expression of AChR in the thymus (25) may enhance negative selection of higher affinity autoreactive T cells, resulting in a particularly low affinity AChR T cell repertoire. During cognate T cell–B cell interactions, these AChR-specific low affinity T cells may require a higher level of costimulation to achieve the threshold for contact-dependent signals to cause stimulation and differentiation of B cells and subsequent Ab production. Therefore, we speculate that mounting an effective humoral immune response to this (AChR) self-antigen is IFN-γ dependent, because the cytokine regulates the expression of costimulatory molecules (26, 27). On the other hand, the KLH-specific high affinity T cells do not require IFN-γ–dependent costimulation by B cells to effectively participate in humoral response. The hypothetical model is shown in Fig. 4. The data reported in our manuscript are suggestive of differential requirements for IFN-γ in the induction of humoral immune response to self (AChR) and foreign (KLH) antigens; however, the study of other self-antigens would substantiate this notion. The findings of humoral immune responses observed in KLH-immunized IFN-gko (−/−) mice are further supported by previous findings in which IFN-gko (−/−) mice produce antibodies to a wide variety of pathogens; influenza (17), *Leishmania major* (28), and Herpes simplex virus (29).

In support of our hypothesis, it has long been known that cognate T cell–B cell interactions involve several reciprocal receptor–counterreceptor interactions. One of such interactions is CD28–CTLA-4/B7 signaling. A role for CD28/B7 signaling in humoral immune response has been documented; human CTLA-4Ig therapy (30) and anti-B7-2 mAb treatment (31) of mice profoundly blocked antibody responses to a nominal antigen priming. This notion is further supported by the report that transgenic expression of soluble murine CTLA4-H γ 1 in mice profoundly blocked antibody response to a protein antigen without affecting the T cell responses (32). It is likely that IFN-γ by virtue of its ability to upregulate the basal levels of B7-2 on B cells (33–35) may influence the CD28/B7 costimulation during cognate T cell–B cell interactions, which in turn affect the humoral immune response for a self-antigen (M-AChR).

The fact that anti–self-humoral responses require additional cytokine inducible signals seems evolutionarily appropriate. In the case of the AChR, even extremely low levels of autoantibodies are highly detrimental to the functioning of the neuromuscular junction. Our findings suggest that under nonstimulatory circumstances the process of cognate help by low affinity anti-AChR T cells is not favored.

Our results demonstrate that the lack of IFN-γ converts an otherwise EAMG-susceptible mouse strain to a disease-resistant one; that is, in H-2^b mice, IFN-γ confers suscepti-

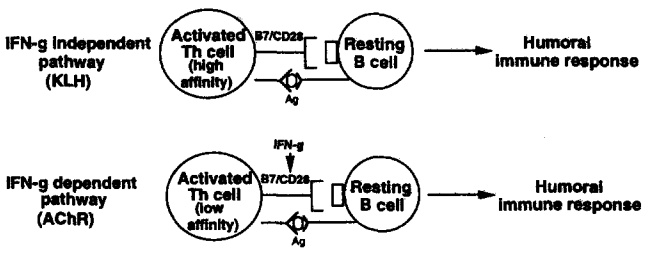

Figure 4. Hypothetical model: how IFN-γ influences the humoral immune response to self (AChR) and foreign (AChR) antigens in vivo.

bility to EAMG (Table 1). The freedom from EAMG in IFN-gko mice was somewhat surprising for two reasons: first, EAMG is an Ab-mediated disease (36–38), and second, the proinflammatory Th1 cytokine IFN-γ is strongly associated with cell-mediated autoimmune diseases (39–41). However, elsewhere it was shown that IFN-γ is not required for the cell-mediated autoimmune diseases, autoimmune encephalomyelitis and diabetes (42, 43), or confers resistance to them (44). Thus, it appears that IFN-γ plays a more essential role in antibody-mediated than in cell-mediated autoimmune diseases.

We have shown in this study that the endogenous absence of IFN-γ in the periphery prevented the development of EAMG in mice to AChR challenge. Work from our laboratory has shown earlier that the localized expression of IFN-γ transgene in the neuromuscular junction elicited a humoral autoimmune response to an unidentified 87-kD antigen and provoked a MG-like syndrome in mice of the H-2^d haplotype (4). These findings, as demonstrated by diverse approaches in mice of disparate haplotypes, implicate IFN-γ as a pivotal player in the pathogenesis of humoral autoimmunity in the neuromuscular junction.

In conclusion, the results of this study reveal a differential requirement for IFN-γ (*a*) in humoral immune response to self (AChR) versus foreign (KLH) antigens, and (*b*) the cytokine plays an important immunomodulatory role in the pathogenesis of EAMG. Therefore, IFN-γ antagonists may prove beneficial in the treatment of Ab-mediated autoimmune diseases such as MG.

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