

LINKAGE BETWEEN THE FREQUENCY OF MUSCULAR
WEAKNESS AND LOCI THAT REGULATE IMMUNE
RESPONSIVENESS IN MURINE EXPERIMENTAL
MYASTHENIA GRAVIS*

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Myasthenia gravis is a disease characterized by extreme muscular weakness and flaccid paralysis. Many lines of evidence indicate that this condition arises as a consequence of an autoimmune response directed against the nicotinic acetylcholine receptor (AChR)¹ of the neuromuscular junction (1-3). Mice immunized with AChR purified from the electrogenic organ of *Torpedo californica* form anti-AChR antibodies and often develop muscular weakness and flaccid paralysis which closely resembles human myasthenia gravis. This condition, termed experimental myasthenia gravis (EMG), is strain dependent in that the frequency of weakness and paralysis is much greater in some strains than in others (4, 5). Mice of the C57BL/6, SJL, and AKR strains exhibit high susceptibility with 50-70% of the animals immunized developing muscular weakness and paralysis. Other strains such as BALB/c, C3H/He, and SWR display low susceptibility with 0-15% of the animals immunized developing muscular weakness. This strain-dependent difference in susceptibility might reflect differences in the immune system or differences in the neuromuscular junction. The observation (5) that nonimmunized mice from high- and low-susceptibility strains were indistinguishable with respect to their sensitivity toward the cholinergic antagonist *d*-tubocurarine suggested that the strain dependence was a consequence of something other than a difference at the neuromuscular junction. In this paper, we examine the possibility that susceptibility to muscular weakness after immunization with *T. californica* AChR is determined by regions of the mouse genome known to regulate immune responsiveness.

In the mouse, two loci have been identified that participate in determining an individual's ability to respond to any given antigen: the major histocompatibility complex (H-2) and the region that contains the structure genes for the constant region of immunoglobulin heavy chains (IgC_H) (6-9). The H-2 region is located on chromosome 17, and codes for the major histocompatibility antigens, of the mouse (10-

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¹ *Abbreviations used in this paper:* AChR, nicotinic acetylcholine receptor(s); EMG, experimental myasthenia gravis; IgC_H region, region that contains the structural genes for the constant region of immunoglobulin heavy chains; IgV_H region, region that contains the structural genes for the variable region of immunoglobulin heavy chains.

13). This highly polymorphic locus is known to specify gene products that subserve antigen recognition and cell-cell recognition phenomena. The IgC_H region is located on chromosome 12 (14) and contains the structural genes for the constant region of immunoglobulin heavy chains. This locus is smaller than H-2 and is closely linked to the region that contains the structural genes for the variable region of immunoglobulin heavy chains (IgV_H) (15, 16). The availability of H-2- and IgC_H-congenic strains of mice has afforded us an opportunity to examine the influence of both of these regions on the development of muscular weakness and paralysis after immunization with *T. californica* AChR.

Materials and Methods

Mice of the BALB/c, BALB.B10, BALB/K, C.B20, C.B20B, and BAB/14 strains were provided by Dr. Melvin Cohn of The Salk Institute, San Diego, Calif. Mice of the strains CWB/20 and SJA/9 were provided by Dr. Lenore Herzenberg and Dr. Leonard Herzenberg of Stanford University Medical School, Stanford, Calif. Mice of the strain C57BL/Ka.Ig^a (B.C/8) were provided by Dr. Roy Riblet of the Fox Chase Cancer Research Institute, Philadelphia, Pa. B6CF₁ and C3B6F₁ mice were bred at The Salk Institute from breeder stock obtained from The Jackson Laboratory, Bar Harbor, Maine. All other mice used were purchased from The Jackson Laboratory.

The methods used for the purification of *T. californica* AChR and the immunization of mice have been described in detail (5). Mice were judged paralyzed or normal using the assays described previously (5). The significance level of differences in the frequency of EMG between strains of mice was determined by use of a $2 \times 2 \chi^2$ contingency test corrected for continuity as described by Snedecor and Cochran (14). The *P* values reported indicate the probability that differences in the frequency of muscular weakness between two strains of mice occurred as a consequence of sampling error. The frequencies given for the C57BL/6J, A/J, C3H/HeJ, SJL/J, and BALB/cke strains were reported previously (5) and are presented for purposes of comparison.

Results

Effect of Loci That Regulate Immune Responsiveness on the Frequency of Muscular Weakness and Paralysis. The ability of mice to respond to a variety of antigens is known to be determined by immune response (Ir) genes linked to alleles² of the H-2 complex and by genes linked to allotype alleles of the IgC_H region locus. To test the possibility that susceptibility to EMG is similarly determined by alleles of these loci, the frequency of muscular weakness and flaccid paralysis was measured in strains of mice congenic with those previously found to be high or low responders. A list of the strains tested and their relevant characteristics is given in Table I. The first question we considered was whether or not mice constructed with the H-2 and IgC_H haplotypes of high susceptibility strains, and the backgrounds (i.e., all loci not linked to H-2 or IgC_H) of low susceptibility strains, exhibited high susceptibility toward the development of EMG. The C57BL/6 and C57BL/10 strains both exhibit high susceptibility (~70% develop muscular weakness and paralysis) and both possess the H-2^b and Ig-1^b haplotypes. The BALB/c and C3H/HeJ strains, on the other hand, possess the H-2^d and H-2^k haplotypes, respectively, share the Ig-1^a haplotype, and both exhibit low susceptibility (~7–11% develop muscular weakness and paralysis). If susceptibility to myasthenia gravis is determined by regions of the mouse genome that regulate

² The terms "allele" and "haplotype" are used interchangeably to specify a group of genes closely linked on a single chromosome for which phenotypic markers have been defined.

TABLE I
H-2- and Allotype-congenic Strains of Mice

Strain	H-2	IgC _H	Inbred background	Source of IgC _H	Source of H-2	Reference
1 BALB/cke	d	a	BALB/c	BALB/c	BALB/c	12, 15
2 C.B20	d	b	BALB/c	C57BL/Ka	BALB/c	12, 15
3 BAB/14	d	b/a	BALB/c	C57BL/Ka	BALB/c	12, 15
4 C.B20B	b	b	BALB/c	C.B20	BALB.B10	12, 18
5 BALB.B10	b	a	BALB/c	BALB/c	C57BL/10Sn	12, 18
6 BALB.K	k	a	BALB/c	BALB/c	C3H	12
7 C57BL/6J	b	b	C57BL/6J	C57BL/6J	C57BL/6J	12, 15
8 C57BL/10Sn	b	b	C57BL/10Sn	C57BL/10Sn	C57BL/10Sn	12, 15
9 B10.A/SgSn	a	b	C57BL/10	C57BL/10	A/WrSnSg	9, 12
10 B10.D2/nSn	d	b	C57BL/10	C57BL/10	DBA/2	12, 15
11 C57BL/Ka.Ig ^a	b	a	C57BL/Ka	BALB/c	C57BL/Ka	12, 15
12 C3H/HeJ	k	a	C3H/HeJ	C3H/HeJ	C3H/HeJ	12, 15
13 C3H.SW/Sn	b	a	C3H/HeDiSn	C3H/HeDiSn	Swiss	12, 16
14 CWB/20	b	b	C3H.SW/Sn	C57BL/Ka	C3H.SW	12, 16
15 SJL/J	s	b	SJL/J	SJL/J	SJL/J	12, 15
16 SJA/9	s	a	SJL/J	BALB/c	SJL/J	12, 16
17 AKR/J	k	d	AKR/J	AKR/J	AKR/J	12, 15
18 A/J	a	e	A/J	A/J	A/J	12, 15

TABLE II
Effect of the H-2^b and Ig-1^b Haplotypes on the Frequency of Muscular Weakness after Immunization with *T. californica* AChR*

Strain	Background	H-2	Ig-1	Number tested	Number EMG	Frequency of paralysis	
1	C57BL/6J	C57BL	b	b	45	32	0.71
2	C57BL/10Sn	C57BL	b	b	20	14	0.70
3	BALB/cke	BALB	d	a	56	4	0.07
4	C3H/HeJ	C3H	k	a	35	4	0.11
5	C.B20B	BALB	b	b	24	16	0.67
6	CWB/20	C3H	b	b	20	15	0.75

* *P* values for differences in the frequency of paralysis between strains are given in the text.

immune responsiveness, then mice which differ from BALB/c and C3H/He only in that the H-2^b and Ig-1^b haplotypes replace the H-2^d, H-2^k, and Ig-1^a haplotypes should exhibit high susceptibility. Conversely, if susceptibility is determined by regions of the mouse genome other than those regulating immune responsiveness (e.g., those controlling the neuromuscular junction), then mice constructed with the BALB or C3H background and the H-2^b and Ig-1^b haplotypes should exhibit low susceptibility. When the frequency of EMG was determined for two strains of mice that possess these characteristics, C.B20B (BALB background; H-2^b, Ig-1^b) and CWB/20 (C3H background; H-2^b, Ig-1^b), both were found to exhibit high susceptibility (Table II). Thus, the introduction of the H-2^b and Ig-1^b haplotypes onto the BALB background increased ($P < 0.005$) the frequency of muscular weakness and paralysis from 7% for BALB/c (H-2^d, Ig-1^a) to 67% for C.B20B (H-2^b, Ig-1^b). Similarly, the introduction of the H-2^b and Ig-1^b haplotypes onto the C3H background correlated with an increase ($P < 0.005$) in the frequency of myasthenic weakness from 11% for C3H/

HeJ (H-2^k, Ig-1^a) to 75% for CWB/20 (H-2^b, Ig-1^b). Thus, the H-2^b, Ig-1^b genotype is associated with high susceptibility to EMG in four strains of mice (C57BL/6, C57BL/10, CWB/20, and C.B20B) derived from three dissimilar backgrounds (C57BL, C3H, and BALB). These studies demonstrate that susceptibility to EMG is a heritable trait determined by regions of the genome that are linked to, or identical with, those that regulate immune responsiveness, and that by simply inserting the H-2^b- and Ig-1^b-bearing chromosomes, low-susceptibility strains can be converted to high-susceptibility strains.

Effect of the H-2 Locus on the Frequency of Muscular Weakness and Paralysis. Further studies were conducted to assess the relative contributions of the H-2 and IgC_H loci. Initially, we sought to determine whether or not high-susceptibility strains could be converted to low-susceptibility strains by substitutions at the H-2 locus. The C57BL/6 and C57BL/10 strains both exhibit high susceptibility and both bear the H-2^b, Ig-1^b genotype. BALB/c and A/J mice, on the other hand, exhibit low susceptibility and possess the H-2^d and H-2^a haplotypes, respectively. If susceptibility to myasthenic weakness is determined only by the H-2 haplotype, then mice that differ from C57BL/6 or C57BL/10 only in that the H-2^a or H-2^d haplotypes have been substituted for the H-2^b haplotype, should exhibit low susceptibility. The frequency of myasthenic weakness and paralysis was determined for two strains that possess these characteristics: B10.D2/nSn (H-2^d), and B10.A/SgSn (H-2^a). As can be seen in Table III, 47% of the B10.D2/nSn and 38% of the B10.A/SgSn become paralyzed. Thus, although the H-2^a and H-2^d haplotypes probably influenced susceptibility ($P = 0.179$ for B10.D2/nSn; $P = 0.079$ for B10.A/SgSn) by converting high responders to intermediate responders, neither haplotype conferred low susceptibility. These studies suggested that other loci beside H-2 are involved in the regulation of susceptibility to EMG.

In reciprocal studies, we sought to determine whether or not low-susceptibility strains could be converted to high-susceptibility strains as a consequence of substitutions made at the H-2 locus. The BALB/c and C3H/HeJ strains both exhibit low susceptibility (~7-11% developed paralysis) and possess the H-2^d and H-2^k haplotypes,

TABLE III
*Effect of H-2 Haplotypes on the Frequency of Muscular Weakness after Immunization with T. californica AChR**

	Strain	Background	H-2	Number tested	Number EMG	Frequency of paralysis
1	C57BL/6J	C57BL	b	45	32	0.71
2	C57BL/10Sn	C57BL	b	20	14	0.70
3	B10.D2/nSn	C57BL	d	15	7	0.47
4	B10.A/SgSn	C57BL	a	13	5	0.38
5	A/J	A/J	a	15	2	0.13
6	BALB/cke	BALB	d	56	4	0.07
7	BALB.B10	BALB	b	18	9	0.50
8	BALB.K	BALB	k	13	2	0.15
9	AKR/J	AKR	k	28	14	0.50
10	C3H/HeJ	C3H	k	35	4	0.11
11	C3H.SW/Sn	C3H	b	25	15	0.60

* P values for differences in the frequency of paralysis between strains are given in the text.

respectively. If susceptibility is determined by the H-2 locus, then the introduction of the H-2^b haplotype (found in the high-susceptibility C57BL/6 and C57BL/10 strains) onto these backgrounds should confer high susceptibility. As can be seen in Table III, the BALB.B10 strain (BALB background; H-2^b) exhibited intermediate susceptibility and the C3H.SW strain (C3H background; H-2^b) exhibited high susceptibility. Thus the H-2^b haplotype had a significant effect ($P < 0.005$) and increased the frequency of weakness and paralysis from 7% for BALB/c (H-2^d) to 50% for BALB.B10 (H-2^b). In the case of the C3H strain, the substitution of the H-2^b haplotype (C3H.SW) for the H-2^k haplotype (C3H/HeJ) markedly affected susceptibility ($P < 0.005$) and increased the frequency of EMG from 11% (C3H/HeJ) to 60% (C3H.SW).

Previous studies (4, 5) revealed that a high-susceptibility strain (AKR) and a low-susceptibility strain (C3H/HeJ) can share the same haplotype (H-2^k). To further explore this issue, we measured the frequency of paralysis in another strain of mice that possess the H-2^k haplotype, BALB.K. This strain differs from the low responder BALB/c only in that the H-2^k haplotype replaced the H-2^d haplotype. As Table III indicates that only 15% of the BALB.K mice became paralyzed, the substitution of H-2^k for H-2^d on the BALB background, therefore, did not significantly ($P = 0.705$) increase the frequency of muscular weakness or paralysis.

From these studies, it was concluded that the H-2 locus can markedly influence susceptibility. In particular, the H-2^b allele was always found to be associated with intermediate or high susceptibility, and the introduction of the H-2^b haplotype imparted increased susceptibility to the low susceptibility C3H and BALB/c backgrounds. However, the observation that replacement of the H-2^b haplotype on the C57BL/6 and C57BL/10 backgrounds (B10.D2/sSn and B10.A/SgSn) reduced the incidence of myasthenia gravis to intermediate values, but did not confer resistance (low susceptibility) toward the development of muscular weakness, suggested that the H-2 locus is not the only factor involved. Because different inbred strains that share the same haplotype were found to differ significantly with respect to the frequency of muscular weakness and paralysis (e.g., BALB/c and B10.D2/nSn: H-2^d; C3H/HeJ, BALB.K, and AKR: H-2^k; B10.A/SgSn and A/J: H-2^a), it must be concluded that other factors beside the H-2 type participate in conferring susceptibility or resistance to myasthenic weakness.

Effect of Allotype on the Frequency of Muscular Weakness and Paralysis. The high-susceptibility strains C57BL/6, C57BL/10, C.B20B, CWB/20, and SJL/J all possess the Ig-1^b haplotype, which is not present in any of the low-susceptibility strains. Similarly the Ig-1^a haplotype is present on the low-susceptibility strains BALB/c and C3H/HeJ and is found only in high-susceptibility strains that also bear the H-2^b haplotype. To explore the influence of IgC_H alleles on susceptibility to myasthenia gravis, the frequency of muscular weakness was measured in the SJA/9 and C57BL/Ka.Ig^a strains of mice; these strain are identical to the high susceptibility strains SJL and C57BL/6, respectively, with the exception that the Ig-1^a haplotype replaces the Ig-1^b haplotype. If susceptibility to EMG is determined by the IgC_H locus alone, then SJA/9 and C57BL/6Ka.Ig^a mice should exhibit low susceptibility. Conversely, if the IgC_H locus is not involved in susceptibility, then these strains should develop weakness with the same frequency as the high-responder SJL and C57BL/6 strains. If both the H-2 locus and the IgC_H locus determine susceptibility, the C57BL/Ka.Ig^a strain should exhibit intermediate or high susceptibility because it bears the H-2^b allele, and

SJA/9 (H-2^b) could exhibit high, intermediate, or low susceptibility. As Table IV indicates, 27% of the SJA/9 mice became paralyzed, indicating that the substitution of the Ig-1^a haplotype for the Ig-1^b haplotype significantly ($P = 0.009$) reduced the frequency of weakness and paralysis. In the case of C57BL/Ka.Ig^a, 53% of the mice became paralyzed; thus the substitution of the Ig-1^a haplotype for the Ig-1^b haplotype, did not significantly ($P = 0.37$) affect the incidence of weakness and paralysis. This result suggested that the influence of the H-2^b haplotype is greater than that of the Ig-1^a haplotype and can mask any effect resulting from the allotype substitution.

In reciprocal experiments, we sought to determine whether the introduction of the high-susceptibility Ig-1^b haplotype onto the low-susceptibility BALB/c background converted BALB to a high-responder strain. The C.B20 strain is identical to BALB/c with the exception that the Ig-1^b haplotype replaces the Ig-1^a haplotype. As Table IV indicates, 30% of the C.B20 mice became paralyzed, and this represents a fourfold increase ($P = 0.017$) in the frequency of EMG relative to the congenic BALB/c strain. This result again indicated that alleles linked to the IgC_H locus can significantly modulate susceptibility to EMG.

Finally, an effort was made to localize the elements linked to the Ig-1^b allele that correlate with susceptibility to EMG. The BAB/14 strain is identical to the C.B20 strain with the exception that there is a crossover between the IgC_H and the IgV_H locus (9, 19, 20). Thus BAB/14 has the Ig-1^b IgC_H-region allotype markers of C57BL/6 and IgV_H-region idiotypic markers of BALB/c. If susceptibility to EMG is influenced by the IgC_H region only, the frequency of myasthenia gravis in BAB/14 mice should

TABLE IV
*Effect of IgC_H Haplotypes on the Frequency of Muscular Weakness after Immunization with T. californica AChR**

	Strain	Background	Ig-1	Number tested	Number EMG	Frequency of paralysis
1	SJA/9	SJL	a	22	6	0.27
2	SJL/J	SJL	b	45	29	0.64
3	C57BL/Ka.Ig ^a	C57BL/Ka	a	15	8	0.53
4	C57BL/6	C57BL/6	b	45	32	0.71
5	C.B20	BALB/c	b	31	9	0.29
6	BAB/14	BALB/c	b/a	28	0	0
7	BALB/c	BALB/c	a	56	4	0.07

* P values for differences in the frequency of paralysis between strains are given in the text.

TABLE V
*Effect of Heterozygosity on the Frequency of Muscular Weakness after Immunization with T. californica AChR**

	Strain	Maternal Strain	Paternal Strain	H-2	Ig-1	Number tested	Number EMG	Frequency of paralysis
1	CB6F ₁ /J	BALB/cJ	C57BL/6J	d/b	a/b	15	6	0.40
2	B6CF ₁	C57BL/6J	BALB/cke	b/d	b/a	16	6	0.38
3	C3B6F ₁	C3H/HeJ	C57BL/6J	k/b	a/b	33	14	0.42

* P values for differences in the frequency of paralysis between strains are given in the text.

not differ from that in C.B20 mice. Conversely, if susceptibility is determined by the IgV_H region, BAB/14 mice should exhibit low susceptibility, as found in BALB/c mice. As seen in Table IV, not a single BAB/14 mouse developed weakness or paralysis. This result suggests, therefore, that the elements linked to the IgC_H region that correlate with increased susceptibility to EMG are located near the IgV_H region of chromosome 12.

Effect of Heterozygosity on the Frequency of Muscular Weakness and Paralysis. The frequency of muscular weakness and flaccid paralysis was determined for three strains of F₁ mice produced by matings of high-susceptibility strains with low-susceptibility strains. If susceptibility to EMG is a dominant trait, then mice that are heterozygous at the H-2 and IgC_H loci and that bear the high-susceptibility H-2^b and Ig-1^b alleles should exhibit high susceptibility. Conversely, if low susceptibility (resistance) to EMG is a dominant trait, then mice that are heterozygous and that bear the low susceptibility H-2^d, H-2^k, and Ig-1^a alleles should exhibit low susceptibility. The C3B6F₁ strain (C57BL/6 × C3H/HeJ)F₁ bears the H-2^{b/k}, Ig-1^{b/a} genotype. When the incidence of muscular weakness and flaccid paralysis was measured for this strain, it was found that 42% of the animals immunized developed EMG (Table V). Although the frequency of EMG in the C3B6F₁ strain was significantly greater ($P = 0.009$) than the low-susceptibility parental strain C3H/HeJ (11% developed weakness and paralysis) it was lower ($P = 0.022$) than the high-susceptibility parental strain C57BL/6 (71% developed muscular weakness and paralysis). Similarly the CB6F₁ and B6CF₁ strains (C57BL/6 × C3H/HeJ)F₁ bear the H-2^{b/d}, Ig-1^{b/a} genotype. Because 40% of the former and 38% of the latter developed muscular weakness and paralysis, heterozygosity leads to an intermediate responder. Again, the frequency of muscular weakness and flaccid paralysis was significantly greater ($P < 0.007$) than the low-susceptibility parental type BALB/c (7% developed EMG) and was less ($P = 0.068$ for CB6F₁; $P = 0.039$ for B6CF₁) than the high-susceptibility parental type C57BL/6 (71% developed EMG). These studies demonstrate that susceptibility to EMG is a heritable trait and indicate that the heterozygous expression of the H-2^b and Ig-1^b alleles is less effective in conferring increased susceptibility to EMG than the homozygous case. It can be concluded therefore that susceptibility to EMG does not behave as a simple dominant or recessive factor.

Discussion

Several previous studies have considered the matter of differential susceptibility to EMG between inbred strains of mice immunized with *Torpedo* AChR. Fulpius et al. (21) immunized several strains of mice with AChR and found that although all strains tested (BALB/c, DBA/2, and NZB) formed anti-AChR antibodies, none developed EMG. A similar result was reported by Heilbronn et al. (22), who reported that C57BL/6J mice immunized with *Torpedo marmorata* AChR developed anti-AChR antibodies but did not develop EMG. Fuchs et al. (4) reported that mice immunized with *T. californica* AChR did develop paralysis and that EMG was strain dependent in that some strains (e.g., AKR and C57BL/6) developed weakness and paralysis with a much greater frequency than others. These investigators also noted that mice bearing the H-2^s and H-2^q haplotypes appeared particularly resistant to EMG and that mice of all strains tested developed high levels of anti-AChR antibodies. Studies in our laboratory (5) suggested that all mice can develop EMG and demonstrated

that susceptibility is strain dependent in that the probability with which an individual can be expected to develop muscular weakness and flaccid paralysis is much greater for some lineages than others. The observation that strains that share the same H-2 haplotype differed in their susceptibility to EMG suggested that the H-2 locus alone does not determine susceptibility or resistance to EMG. Because the occurrence of muscular weakness and paralysis did not correlate with: (a) the concentration of antibodies reactive with mouse or *T. californica* AChR, (b) the presence of antibodies reactive with cell surface determinants of mouse AChR, (c) the ability of antibodies to increase the rate of receptor degradation on cultured muscle cells, or (d) the presence of antibodies reactive with antigenic determinants unique to mouse AChR, we concluded that if anti-AChR antibodies are responsible for the induction of EMG, then populations of particular structure and/or specificity are required.

Christadoss et al. (23) reported that the ability of lymph node cells obtained from congenic mice immunized with *T. californica* AChR to incorporate [³H]thymidine when stimulated in vitro with limiting receptor was under H-2-linked Ir gene control and concluded that the H-2^b, H-2^f, and H-2^j haplotypes were high responders; the H-2^a and H-2^f haplotypes were intermediate responders; and the H-2^k, H-2^s, H-2^p, and H-2^d haplotypes were low responders. A distinction should be made between the strain-dependent differences in susceptibility described in these studies (23) and those described in the other studies mentioned (4, 5, 21, 22). The former probably reflects strain-dependent differences with respect to the threshold antigen dose necessary for the induction of proliferative response in lymph node cells, whereas the latter deal with the probability of weakness and paralysis after an anti-AChR immune response is well established. From the studies of Christadoss et al. (23) it can be concluded that the threshold necessary for the induction of a proliferative response in cultured lymph node cells against *T. californica* AChR is under H-2-linked Ir gene control. The conclusion that can be drawn from all of the other studies described is that the formation of anti-AChR antibodies in mice does not lead to the induction of EMG in an obligatory fashion.

From our studies it can be concluded that the frequency with which mice develop muscular weakness and flaccid paralysis after immunization with *T. californica* AChR is determined, to a great extent, by regions of the genome that regulate immune responsiveness. Haplotypes of both the H-2 and the IgC_H loci were identified that, in mice derived from dissimilar backgrounds, segregated with high or low susceptibility to EMG. The H-2^b, Ig-1^b genotype was associated with high susceptibility to EMG in four strains (C57BL/6J, C67BL/10Sn, C.B20B, and CWB/20) from three unrelated backgrounds (C57BL, BALB, and C3H). Congenic strains of mice that differ from these high responders only in substitutions at the H-2 and IgC_H loci exhibited reduced susceptibility. For example, the BALB/c (H-2^d, Ig-1^a) and BALB/K (H-2^k, Ig-1^a) strains exhibited low susceptibility (7–15% developed muscular weakness and paralysis), whereas their congenic partner, C.B20B (H-2^b, Ig-1^b), exhibited high susceptibility (67% developed muscular weakness and paralysis). Similarly, C3H/HeJ (H-2^k, Ig-1^a) was a low-susceptibility strain (11% developed muscular weakness and paralysis), whereas its congenic partner, CWB/20 (H-2^b, Ig-1^b), was a high-susceptibility strain (75% developed muscular weakness and paralysis).

The importance of the H-2 locus in determining susceptibility and resistance to EMG can be seen in studies of the H-2^b haplotype. This haplotype was unique in that

it always segregated with increased susceptibility to EMG. The replacement of the H-2^d haplotype, found in BALB/c, with the H-2^b haplotype, as in BALB.B10, was accompanied by an increase in the frequency of muscular weakness and paralysis from 7% (BALB/c) to 50% (BALB.B10). Similarly, the replacement of the H-2^k haplotype, found in C3H/HeJ, by the H-2^b haplotype, found in C3H.SW/Sn, was accompanied by an increase in the frequency of muscular weakness and paralysis from 11% (C3H/HeJ) to 60% (C3H.SW/Sn).

It was not possible, however, to attribute susceptibility or resistance to EMG to the H-2 locus alone because replacement of the high-susceptibility H-2^b haplotype (C57BL/10Sn) by the low-susceptibility H-2^a (A/J) and H-2^d (BALB/c) haplotypes reduced the frequency of EMG to intermediate values, but did not confer low susceptibility. Thus 38% of the B10.A/SgSn (H-2^a) and 47% of the B10.D2/nSn (H-2^d) developed muscular weakness and paralysis as compared to 71% of the C57BL/10Sn (H-2^b), 13% of the A/J (H-2^a), and 7% of the BALB/c (H-2^d). In addition, different strains of mice that bear the same H-2 haplotype were found to differ with respect to the frequency with which they developed muscular weakness and paralysis. For example, three strains that bear the H-2^k haplotype were examined; although the C3H/HeJ and BALB/k strains exhibited low susceptibility (11–15% developed muscular weakness and paralysis), the AKR strain exhibited intermediate susceptibility (50% developed muscular weakness and paralysis). Indeed, strains that share the H-2^a, H-2^d, and H-2^s haplotypes were also found to differ significantly with respect to the frequency with which they developed muscular weakness and paralysis after immunization with *T. californica* AChR. From these studies, it can be concluded that although the H-2 locus exerts a significant effect on susceptibility to EMG, other loci are involved.

Because the Ig-1^b haplotype was always found associated with increased susceptibility to EMG and was not present in any low-susceptibility strains, the possibility that the IgC_H-locus-influenced susceptibility was considered. The SJL strain possessed the H-2^s, Ig-1^b genotype and was the only high-susceptibility strain that did not bear the H-2^b haplotype. Replacement of the Ig-1^b haplotype with the Ig-1^a haplotype (SJA/9) correlated with a lowered incidence of EMG (from 64% for SJL to 30% for SJA/9) and indicated that the IgC_H locus does indeed influence susceptibility to EMG. This conclusion was further supported in studies of mice derived from the BALB background where it was noted that BALB/c (H-2^d, Ig-1^a) exhibited low susceptibility (7% became paralyzed), BALB.B10 (H-2^b, Ig-1^a) exhibited intermediate susceptibility (50% became paralyzed), and C.B20B (H-2^b, Ig-1^b) exhibited high susceptibility (67% became paralyzed). This result suggested that the difference in susceptibility between BALB.B10 and C.B20B is attributable to the Ig-1^b haplotype. This possibility was supported in studies of the C.B20 strain (H-2^d, Ig-1^b) where Ig-1^b replaces the BALB/c Ig-1^a haplotype. This change correlated with an increase in the incidence of muscular weakness and paralysis from 7% (BALB/c H-2^d, Ig-1^a) to 30% (C.B20, H-2^d Ig-1^b).

The observation that the frequency of EMG in the BAB/14 strain (none became paralyzed) differed significantly from that of the C.B20 strain (30% became paralyzed; $P = .007$) suggested that the linkage between allotype loci and susceptibility to EMG involves the IgV_H region. Because this region contains the genes that determine the structure and specificity of the variable region of immunoglobulin heavy chains, the

linkage detected may reflect a particular gene coding for an antibody specificity that is especially effective in inducing muscular weakness and paralysis. Recent studies, however, have demonstrated that BAB/14 mice retain at least one Ig-1^b idiotypic specificity, and the possibility exists that the lack of other Ig-1^b specificities can be attributed to a defect in a regulatory locus that limits the expression of other Ig-1^b alleles (24, 25). Thus, the allotype linkage we have observed cannot be attributed to the IgC_H or IgV_H region with complete confidence.

When the data obtained in these studies is ranked according to the frequency of paralysis, a pattern of linkage between the frequency of muscular weakness and paralysis and the alleles of the H-2 and IgC_H loci is suggested. As can be seen in Table VI, mice that bear the H-2^b, Ig-1^b genotype exhibit the greatest frequency of muscular weakness and paralysis (60–80% became paralyzed). Mice that bear the H-2^b, Ig-1^a genotype exhibited intermediate susceptibility (30–50% became paralyzed). Mice that possess the H-2^x, Ig-1^b genotype (where x corresponds to the H-2^a, H-2^d, H-2^k, or H-2^s haplotypes) also exhibited intermediate susceptibility (30–50% became paralyzed). Mice that bear the H-2^x, Ig-1^a genotype (where x corresponds to the H-2^d, H-2^k, or H-2^s haplotypes) exhibited the lowest frequency of muscular weakness and paralysis (0–15% became paralyzed). Mice that are heterozygous at the H-2 and IgC_H loci and that bear the H-2^{b/d}, Ig-1^{b/a} or H-2^{b/k}, Ig-1^{b/a} genotypes exhibited intermediate susceptibility in that 40–50% became paralyzed. Thus, the frequency of muscular

TABLE VI
Pattern of Susceptibility to EMG after Immunization with T. californica AChR

Genotype	Strain	Background	Frequency of paralysis	Rank order	Classification
H-2 ^b , Ig-1 ^b	CWB/20	C3H	0.75	1	High
	C57BL/6	C57BL	0.71	2	High
	C57BL/10Sn	C57BL	0.70	3	High
	C.B20B	BALB	0.67	4	High
H-2 ^b , Ig-1 ^a	C3H.SW/Sn	C3H	0.60	6	High
	C57BL/Ka.Ig ^a	C57BL	0.53	7	Intermediate
	BALB.B10	BALB	0.50	8	Intermediate
H-2 ^x ,* Ig-1 ^b	SJL/J	SJL	0.64	5	High
	B10.D2/nSn	C57BL	0.47	9	Intermediate
	B10.A/SgSn	C57BL	0.38	13	Intermediate
	C.B20	BALB	0.29	14	Intermediate
H-2 ^{x/b} ,* Ig-1 ^{a/b}	C3B6F ₁	C3H/C57BL	0.42	10	Intermediate
	CB6F ₁ /J	BALB/C57BL	0.40	11	Intermediate
	B6CF ₁	C57BL/C3H	0.38	12	Intermediate
H-2 ^x ,* Ig-1 ^a	SJA/9	SJL	0.27	15	Intermediate
	BALB.K	BALB	0.15	16	Low
	C3H/HeJ	C3H	0.11	17	Low
	BALB/c	BALB	0.07	18	Low

* x = a for B10.A/SgSn; d for B10.D2/nSn, BALB/c, CB6F₁/J, B6CF₁, and C.B20; k for C3H/HeJ, C3B6F₁, and BALB/k; and s for SJL and SJA/9.

weakness and flaccid paralysis after immunization with *T. californica* AChR is determined by at least two loci, and particular haplotypes of the H-2 and IgC_H correlate with increased susceptibility. The strains that are exceptions to the pattern described may be accounted for in several ways. First, they may reflect uncertainties with respect to the size of the H-2 and IgC_H region arising from the lack of suitable markers. Alternatively, they may reflect sampling errors; although it is highly unlikely ($P < 0.005$) that high-susceptibility strains could be mistaken for low-susceptibility strains, the probability that high or low responders could be mistaken for intermediate responders is somewhat greater. In most cases, however, such an error would actually support the conclusions we have drawn and would not change the interpretation of the results. A third possibility is that other loci, yet to be identified, participate along with the H-2 and IgC_H loci in determining susceptibility.

The observation that susceptibility to EMG segregates with H-2 and IgC_H haplotypes was unexpected. Such linkage is usually detected only with simple antigens possessing few antigenic determinants or large multideterminant antigens given in limiting doses. Because AChR (2.0×10^5 – 2.5×10^5 mol wt) is neither a simple antigen, nor was it given the limiting dosages, one would not expect to see linkage between the anti-AChR immune response and immune response loci because all mice should be capable of recognizing at least some of the many antigenic determinants that a molecule the size of AChR must possess. This expectation proved to be correct because mice of all strains tested developed high concentrations of antibodies reactive with mouse and *T. californica* AChR (average concentration of antibodies to *T. californica* AChR, 2.2×10^{-5} M; average concentration of antibodies to mouse AChR, 3.4×10^{-7} M; [5]). Thus, the linkage we have found is only between the development of muscular weakness and immune response loci, and does not apply to the magnitude of the humoral immune response to mouse or *T. californica* AChR. The H-2 and IgC_H linkage may be accounted for by several alternative mechanisms: (a) The linkage may reflect antigenic specificities of T cell- or B cell-derived effector molecules that are particularly effective (or necessary) for the induction of muscular weakness and paralysis. (b) The linkage may reflect regulatory mechanisms that favor particular modes of immune attack (e.g., antibody-dependent cytotoxicity, killer T cells, complement-mediated cytotoxicity). (c) Mouse AChR or neuromuscular junctions may be polymorphic and their structure may be determined by alleles linked to the H-2 or IgC_H regions.

The hypothesis that not all antibodies reactive with autologous AChR can induce myasthenia gravis and that particular specificities are required, may account for several unexplained aspects of human myasthenia gravis: (a) Although 90% of all myasthenics possess humoral antibodies reactive with AChR, there is an imperfect relationship between the concentration of anti-receptor antibodies and the severity of the disease (26–28). This observation may be accounted for if it is assumed that only a limited population of anti-receptor antibodies can induce muscular weakness and flaccid paralysis. (b) Only 12% of neonates from myasthenic mothers develop neonatal myasthenia (29), and nonmyasthenic neonates possessing anti-AChR antibodies have been reported (30). This observation can also be explained by assuming that populations of a particular structure and/or specificity are required for the induction of myasthenia. (c) Appel and Elias (31) have reported that some myasthenics undergoing remission and who are relatively free of clinical symptoms, possess high concentrations

of anti-AChR antibodies that can increase the rate of AChR degradation on cultured muscle cells. This observation also is consistent with the idea of myasthenogenic specificities and suggests a distinction between antibodies that induce myasthenia gravis and those that increase the rate of AChR degradation. Such a distinction was suggested in our studies of EMG (5) where many animals that did not exhibit weakness and paralysis were found to possess antibodies that could increase the rate of AChR degradation on cultured muscle cells.

In conclusion, immunization of mice with *T. californica* AChR induces the formation of anti-AChR antibodies and often results in extreme muscular weakness and flaccid paralysis closely resembling the human disease myasthenia gravis. Knowledge of the concentration of antibodies reactive with mouse or *T. californica* AChR, the presence of antibodies that are able to increase the rate of AChR degradation, or the presence of antibodies reactive with determinants unique to mouse AChR does not permit an estimation of the probability of muscular weakness and paralysis. However, knowledge of the strain of mouse or of the haplotypes present at the H-2 and IgC_H loci does allow for an estimation of paralysis and provides strong evidence that susceptibility to EMG is a heritable trait linked to regions of the mouse genome that regulate immune responsiveness. That one genotype (H-2^b, Ig-1^b) was associated with high susceptibility on three dissimilar backgrounds suggests that despite the complex mechanisms that underlie neuromuscular transmission and muscle contraction, it is the alleles present at the H-2 locus on chromosome 17 and the IgC_H locus on chromosome 12 that are of major importance in determining whether or not a mouse immunized with *T. californica* AChR will become paralyzed. At this time, it is not known whether the H-2- and IgC_H-linked effects on susceptibility are additive or synergistic, and the possibility remains that additional alleles at these and other loci may modulate susceptibility or resistance to EMG. Further genetic analysis employing recombinant-inbred strains of mice and progeny resulting from backcrosses of F₁ mice with parental types should help to clarify these issues.

Summary

Mice immunized with acetylcholine receptor (AChR) purified from *Torpedo californica* form anti-AChR antibodies and often develop muscular weakness and flaccid paralysis closely resembling the human disease myasthenia gravis. This condition, termed experimental myasthenia gravis (EMG), is strain dependent in that the frequency of paralysis is much greater in some strains than in others. Differences in the frequency of EMG might result from differences in the immune system or the neuromuscular junction. In these studies, we have identified two loci, the major histocompatibility complex (H-2) region on chromosome 17 and the region that contains the structural genes for the constant region of immunoglobulin heavy chains (IgC_H region) on chromosome 12, which significantly effect the probability with which a mouse immunized with *T. californica* AChR can be expected to become paralyzed. One genotype (H-2^b, Ig-1^b) correlated with high susceptibility to EMG in four strains with three dissimilar backgrounds. These studies demonstrate that susceptibility to EMG is a heritable trait determined by at least two distinct loci that are linked to regions of the mouse genome that regulate immune responsiveness.

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