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Genetic Control of Serum Neutralizing-Antibody Response to Rabies Vaccination and Survival after a Rabies Challenge Infection in Mice

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Quantitative differences in serum neutralizing-antibody (SNAb) responses to rabies vaccination and survival after a rabies challenge infection between two inbred mice strains, C3H/J and C57BL/6J, were shown to be under genetic control. A 99% confidence limit calculated from the SNAb response titers of 14 C57BL/6J mice resulted in an upper limit for the SNAb respone titer of C57BL/6J mice at 50.63. A SNAb titer ≤50.63 in response to rabies vaccination was assigned the phenotype of hyporesponder, and a SNAb titer >50.63 in response to rabies vaccination was assigned the phenotype of hyperresponder in this study. The hyper-SNAb response to rabies vaccination and the higher frequency of survival after rabies challenge infection behave as Mendelian dominant alleles in F_1 hybrids (C3H/J × C57BL/6J) and backcross (BC) (F_1 [C3H/J × C57BL/6J] × C57BL/6J) progeny. Both a relatively hyper-SNAb response and a higher frequency of vaccine-inducible survival phenotypes occur in C3H/J mice. On the other hand, both the relatively hypo-SNAb response and a lower frequency of vaccine-inducible survival phenotypes behave as Mendelian recessive alleles and occur in C57BL/6J mice. C3H/J mice are *H-2* K^k, and C57BL/6J mice are *H-2* K^b. All three phenotypic traits (H-2 type, SNAb response, and survival after rabies challenge infection) segregate as independent (unlinked) monogenic traits in BC progeny (F_1 [C3H/J × C57BL/6J] × C57BL/6J). The genetically controlled survival trait is inducible by rabies vaccination, but SNAb response is not a parameter that measures successful vaccine induction of preexposure protection from a rabies challenge infection in the BC progeny. The essential role of vaccination in developing preexposure protection in genetically responsive mice is confirmed, but indicates that in vitro measurements other than SNAb titers need to be developed to identify mice that have failed to achieve preexposure protection by rabies vaccination. This study confirms Lodmell's findings (D. L. Lodmell and B. Chesebro, J. Virol. 50:359–362, 1984; D. L. Lodmell, J. Exp. Med. 157:451–460, 1983) that susceptibility to rabies infection is genetically controlled in some mice strains. Additionally, this study indicates that conventional rabies vaccination even with more potent vaccines may not induce protection from infection in some genetically susceptible individuals.

The greatest scientific contribution to the treatment of rabies infection in humans was Pasteur's report of the development in 1884 of the first rabies vaccine, which consisted of dessicated spinal cord from rabies-infected rabbits (13). The application of rabies vaccine to animals, especially domestic dogs, for preexposure protection has been the primary reason for the reduction of the incidence of human rabies infection (23). Shortly after Pasteur's successful human rabies vaccination, Babès and Lepp showed that antibody to rabies correlated with protection from rabies infection (1). Therefore, most investigators have sought the protection of a rabies vaccination that is better at inducing antibody production specific for rabies virus either pre- or postexposure (14). This search has resulted in a sequential development of various rabies vaccines produced in either mammalian central nervous system tissue or avian embryos. The problems associated with these earlier rabies vaccines have been detailed elsewhere (14). Rabies vaccines derived from diploid cell culture were developed to overcome the side effects or variability of antigen content experienced with the mammalian brain- or avian-embryo-derived rabies vaccines. These diploid vaccines stimulate a high rate of seroconversion with minimal side effects (2, 15, 24). It appears that in the future, rabies virus external-surface glycoprotein analogs that might be better neutralizing-antibody stimulators can be biosynthesized from cloned rabies DNA sequences in *Escherichia coli* (26) and used in vaccines.

Although the newer rabies vaccines stimulate seroconversion at a high frequency, there still remain a few humans in some trials who do not produce a high titer of serum neutralizing-antibody (SNAb) after vaccination (2, 4, 19). Because of conflicting results in animals, it is not clear if these individuals are more susceptible to rabies infection than are individuals that seroconvert with a high titer after vaccination. With animals, some data indicate that a SNAb titer is the fulcrum for protection from rabies infection (6, 12, 25). In contrast, other available data show that SNAb titers are not consistent indicators of protection from rabies infection in animals (3, 17, 20).

In a preliminary study of human SNAb response to a duck embryo-derived rabies vaccine, we observed a significant association of HLA-B7 with a low level (titer of <1:4) of SNAb response (R. M. Sharp, C. A. Holmberg, L. H. Russell, and J. W. Templeton, unpublished data). The study could not be concluded because the production of duck embryo-derived rabies vaccine for human use was discontinued. Since considerable homology of the human *HLA* complex and mouse *H-2* complex exists (5), our interest was to develop a genetic model for SNAb hypo- and hyper-

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responses to rabies vaccination in inbred mice strains. With a genetic model, we wanted to determine if mice that were genetically controlled SNAb hyporesponders to rabies vaccination were more susceptible to infection from a rabies virus challenge. The advantages of utilizing mice for these studies are that (i) inbred strains allow the best opportunity to determine the mode of inheritance of genetically controlled immune mechanisms of resistance to rabies infection in a mammalian species; and (ii) mice can be challenged with live virus with the least amount of risk to personnel and with the accompanying expectation that the experimental findings are relevant to field situations of rabies exposure.

The data reported here indicate that in this model system, both SNAb response to rabies vaccination and resistance to rabies challenge infection are controlled by single genes. SNAb hyper- and hyporesponses to rabies vaccination are controlled by dominant and recessive alleles, respectively, and survival after rabies challenge infection is dominant to nonsurvival. These genes are not linked to each other, and they are not linked to *H-2*.

MATERIALS AND METHODS

Mice. AKR/J, C3H/J, and C57BL/6J mice were obtained from Roscoe B. Jackson Laboratories, Bar Harbor, Maine. BALB/cAnTexAm and DBA/2TexAm mice were obtained from Texas Inbred Mouse Co., Houston, Tex. Outbred Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. B10.SM (70NS)/Sn mice were obtained from James E. Womack, Department of Veterinary Pathology, Texas A&M University, College Station. The F_1 and backcross (BC) progeny were produced in our laboratories. The mice used in these experiments were 7 to 12 weeks of age at the time of vaccination.

Vaccine. The rabies vaccine used in this study was provided by G. H. Burgoyne, Michigan Department of Public Health. The vaccine is derived from a rhesus diploid cell line (2). In a preliminary experiment, the dose used was 60 μl of a 1:7.5, 1:15, 1:30, 1:60, or 1:90 dilution of the vaccine in Dulbecco modified minimal essential medium. In the experiments with parental strains (C3H/J and C57BL/6J) and F_1 (C3H/J × C57BL/6J) mice, 60 μl of a 1:30, 1:60, or 1:90 dilution of vaccine was used. A 60-μl volume of 1:60 dilution of vaccine was used to inoculate the BC progeny (F_1 [C3H/J × C57BL/6J] × C57BL/6J). Two inoculations 7 days apart were used for immunization. All immunizations were given intramuscularly in the hind limb. The various vaccine doses could discriminate between hypo- and hyper-SNAb responders with no significant variation in SNAb titers in the parental strains and F_1 mice, so the data for the different vaccine doses were pooled.

SNAb titers. The SNAb response to rabies vaccination was quantitated by a modification of the rapid fluorescent-focus inhibition test, with the BHK-13s cell line as target indicator cells (22). The test was modified by using a 24-well microtiter plate instead of an 8-well culture slide. The reagent volumes were doubled to correspond to the increased culture surface area. The rabies strain used was CVS-11, obtained from the Texas Department of Health. The BHK-13s cells were inoculated with CVS-11 at a virus titer ranging from 40 to 68 50% tissue culture infective doses per 0.1 ml. The test sera were diluted 1:10, 1:50, 1:500, and 1:5,000. The serum from each experiment was assayed for SNAb titer in the same test. A U.S. standard rabies immune globulin, obtained from Division of Control Activities, Bureau of Biologics, Food and Drug Administration, Public Health Services, Department of Health, Education, and Welfare, Rockville, Md. 20852, was utilized as a control antiserum in each test. The inoculated BHK-13s cells were stained with a fluorescein-conjugated antibody to rabies virus obtained from BBL Microbiology Systems, Cockeysville, Md. (lot no. AZGCOF). The cells were fixed with a mixture of 85 parts acetone, 15 parts ethanol, and 5 parts water. The 50% serum neutralization endpoint titer was determined by the method of Reed-Muench (18) from the number of fluorescent foci in 20 fields. The SNAb titers were determined for each mouse in the study, with ear tags to identify individual mice. All titers presented in this paper are reciprocal titers.

***H-2* serotyping.** The *H-2* antigens on peripheral blood lymphocytes were determined by a two-stage microcytotoxicity test (11). Low-Tox-M rabbit complement was obtained from Accurate Chemical and Scientific Corp., Westbury, N.Y. The *H-2* K^k and K^b antisera were obtained from S. Rich, Baylor College of Medicine, Houston, Tex., and J. VandeBerg, Southwest Foundation for Biomedical Research, San Antonio, Tex. The *H-2* K^b reagents are National Institutes of Health antisera designated E-28b and la. 9,20 (catalog no. Y1-8-03-29-02). The *H-2* K^k reagents are National Institutes of Health antisera designated anti-K^k control serum (catalog no. Y1-9-03-15-03) and D-25. The antisera were used at dilutions of 1:100 and 1:200.

Rabies challenge infection. The viral challenge was done with a CVS-11 rabies virus harvested from BHK-13s cells grown in Dulbecco modified minimal essential medium with 10% fetal calf serum. The challenge procedure developed was the subcutaneous inoculation in the ventral cervical region of a single virus lot containing 3.5×10^5 tissue culture infective doses in 0.1 ml. The animals were observed daily for a minimum of 28 days for symptoms of rabies infection. Nonvaccinated mice died between 8 and 14 days post-challenge inoculation.

Statistical analysis. The chi-square test, Fisher exact test, and Wilcoxon test for two-sample rank testing were used to determine statistical significance.

RESULTS

Selection of rabies vaccine dose and inbred strains for study. In a preliminary study in BALB/cAnTexAM mice with five dilutions of the human vaccine (1:7.5, 1:15, 1:30, 1:60, and 1:90), it was determined that the 1:60 and 1:90 vaccine dilutions discriminated between response and nonresponse, respectively, of SNAb in this strain. The optimal bleeding times for two intramuscular inoculations 7 days apart of 60 μl of the 1:60 and 1:90 dilutions were 14 and 21 days post-second immunization (data not shown).

Utilizing this immunization schedule, another preliminary study was conducted in five inbred strains (AKR/J, BALB/cAnTexAm, C3H/J, C57BL/6J, and DBA/2TexAm), one cogenic strain [B10.SM (70NS)/Sn] and one group of Swiss outbred mice. Each strain and the Swiss mice were divided into two groups of four mice. One group was inoculated two times 1 week apart with 60 μl of a 1:60 dilution of vaccine, and the other group was inoculated two times 1 week apart with 60 μl of a 1:90 dilution of vaccine. The SNAb titers were measured at 14 days post-second immunization. Two inbred strains (C3H/J and C57BL/6J) were selected for further studies. The geometric mean titers (GMT) of the C3H/J in the 1:60 and 1:90 vaccine dilution groups were 113.8 and 195.4, respectively. The C57BL/6J mice had GMT of 27.3 and 19.1 with 1:60 and 1:90 vaccine

TABLE 1. SNAb response of C3H/J and C57BL/6J parental strains and F_1 (C3H/J × C57BL/6J) mice to three dilutions of rabies vaccine

Mouse strain	SNAb GMT at vaccine dilution of:		
	1:30	1:60	1:90
C3H/J[a]	158.0	157.8	96.4
C57BL/6J	14.1	22.3	15.4
F_1 (C3H/J × C57BL/6J)[b]	107.4	110.0	98.3

[a] SNAb titers at each dilution are significantly higher ($P < 0.01$) for C3H/J mice than for C57BL/6J mice but not significantly different from F_1 mice.
[b] SNAb titers at each dilution are singificantly higher ($P < 0.01$) for F_1 mice than for C57BL/6J mice.

dilutions, respectively. Four mice from each strain (two from the 1:60 and two from the 1:90 vaccine dilution groups) were immunized a third time, 31 days after the second immunization, with a 1:60 dilution of vaccine (data not shown). The SNAb titers were determined at 14-days post-immunization. The SNAb responses of the two strains were comparable to the SNAb responses from the first immunizations, although the titers uniformly increased. The GMT was 1,428 for the C3H/J mice and 89 for the C57BL/6J mice. These latter data indicate that the relatively hyper- and hyporesponses of the C3H/J and C57BL/6J mice, respectively, do not indicate a difference in immunization timing requirements for effective antibody respone to the vaccine by the two strains.

The parental strains (C3H/J and C57BL/6J) and the F_1 (C3H/J × C57BL/6J) mice were clearly distinguishable as hyper- or hyporesponders with a 1:30, 1:60, or 1:90 dilution of the vaccine dose (Table 1). There is no significant difference in the SNAb response of the parental strain, C3H/J, and the F_1 (C3H/J × C57BL/6J) mice to the various dilutions of rabies vaccine, but both groups have significantly higher SNAb responses compared with the other parental strain, C57BL/6J.

Genetics of SNAb response. The C3H/J and C57BL/6J strains were assigned the phenotypes of hyper-SNAb responder, respectively. With these two strains as parents, the F_1 hybrids (Table 2) and the BC (F_1 × C57BL/6J) mice (Table 3) were produced. The SNAb titer responses of the F_1 hybrids were not significantly different from those of the C3H/J parental strain (Table 2). These data indicate that a dominant gene(s) controls SNAb response. Based on the data in Table 2, the BC matings were made to the C57BL/6J strain, and the SNAb titers were determined. The BC progeny were assigned the phenotype of hyper- or hyporesponder based on their individual SNAb titers. A 99% confidence limit calculated from the SNAb titers of 14 C57BL/6J mice resulted in an upper limit for SNAb titer hyporesponsiveness of 50.63 in the C57BL/6J strain. A

TABLE 2. SNAb titers in C3H/J and C57BL6J parental strains and F_1 (C3H/J × C57BL/6J)

Mouse strain	No. of mice tested	SNAb GMT (range)
C3H/J[a]	15	134.34 (12–616)
C57BL/6J[b]	14	17.8 (10–50)
F_1 (C3H/J × C57BL/6J)	23	108.8 (22–1,390)

[a] SNAb titers of the C3H/J mice are significantly higher than those of the C57BL/6J mice ($P < 0.001$), but not significantly different than those of the F_1 mice ($0.0735 < P < 0.0749$).
[b] SNAb titers of the F_1 mice are significantly higher than those of the C57BL/6J mice ($P < 0.001$).

TABLE 3. Segregation of SNAb titers in BC progeny of F_1 (C3H/J × C57BL/6J) × C57BL/6J mice

Phenotype	No. of BC progeny	SNAb GMT (range)
Hyperresponder	19	153.3 (62–406)
Hyporesponder[a]	18	24.9 (22–50)

[a] BC mice were considered hyperresponder if they had a SNAb titer of >50.63, which is the upper 99% confidence limit of SNAb titer response to human rabies vaccination in 14 C57BL/6J mice.

SNAb titer ≤50.63 was assigned the phenotype of hyporesponder. In the BC progeny, 17 of 19 mice assigned the hyperresponder phenotype had SNAb titers >100, and of the two BC progeny with SNAb titers of <100, one had a SNAb titer of 97, and one had a SNAb titer of 62. In the BC progeny assigned the phenotype of hyporesponder, 16 of 18 mice had SNAb titers of <23, and of the two with SNAb titers >23 but <50.63, one had a SNAb titer of 38, and one had a SNAb titer of 50. If these two hyporesponders with SNAb titers of >23 but <50.63 were misassigned and should have been assigned phenotypes of hyperresponders, the BC segregation ratio is still not altered enough to reject the hypothesis that a single dominant allele is controlling the SNAb response to the rabies vaccine. The data in Table 3 fit a segregation ratio of 1:1, which is expected if SNAb response to the rabies vaccine is controlled by a monogenic dominant allele ($\chi^2 = 0.03$, 1 df).

Survival after rabies challenge infection. A series of preliminary trials of live-virus peripheral challenge was performed (data not shown). It was determined that a subcutaneous inoculation of live challenge virus in the ventral cervical area resulted in a uniform death rate for all nonvaccinated mice. After vaccination, the C3H/J and F_1 (C3H/J × C57BL/6J) mice were relatively resistant, and the C57BL/6J mice were relatively susceptible to the live-virus challenge (Table 4). The percentage of immunized F_1 (C3H/J × C57BL/6J) survivors of the rabies challenge infection is not statistically different ($P = 0.6737$) from the survival rate of the immunized C3H/J parental strain, indicating that a dominant gene(s) controls survival.

Mode of inheritance of SNAb response to rabies vaccination and of survival after rabies challenge infection and linkage of *H-2*. In the first experiment, the linkage of *H-2* to survival was tested in a BC of the F_1 (C3H/J × C57BL/6J) to the C57BL/6J parental strain (Table 5). We reject the hypothesis of linkage between *H-2* and survival after a rabies challenge infection ($\chi^2 = 1.45$, 1 df). Based on the facts that *H-2* K^k and *H-2* K^b are codominant gene traits and that SNAb response to rabies vaccination and survival after rabies challenge infection behave as dominant gene traits in the F_1, a trihybrid

TABLE 4. Survival of C3H, C57BL/6J, and F_1 (C3H/J × C57BL/6J) mice after challenge infection with rabies virus

Mouse strain	No. of mice surviving/no. of mice challenged	
	Not vaccinated	Vaccinated
C3H/J[a]	0/8	9/12
C57BL/6J[b]	0/8	1/12
F_1 (C3H/J × C57BL/6J)	0/11	4/5

[a] The survival rate of vaccinated C3H/J mice is significantly higher than that of vaccinated C57BL/6J mice ($P = 0.0014$), but not significantly different than that of the vaccinated F_1 mice ($P = 0.6737$).
[b] The survival rate of the vaccinated C57BL/6J mice is significantly lower than that of the vaccinated F_1 mice ($P = 0.0097$).

TABLE 5. Segregation of *H-2* and survival after a rabies challenge infection in BC [F_1 (C3H/J × C57BL/6J) × C57BL/6J] progeny

Genotype	No. of mice	
	Surviving[a]	Not surviving
$H\text{-}2^{k/b}$	25[b]	16
$H\text{-}2^{b/b}$	16	18

[a] $\chi^2 = 0.65$ 1 df, for rejection of a 1:1 segregation ratio of both *H-2* K^k and survival versus death after rabies challenge infection.

[b] $\chi^2 = 1.45$, 1 df, for acceptance of linkage between *H-2 K* and survival after rabies challenge infection.

BC between F_1 (C3H/J × C57BL/6J) × C57BL6/J was made, and the phenotypes of the BC progeny were scored (Table 6). The chi-square test is partitioned to test for goodness of fit of each individual trait to a 1:1 segregation ratio and for linkage between any of the traits. The results of these statistical tests allow us to accept the hypotheses that *H-2*, SNAb response to vaccination, and survival after rabies challenge infection segregate as monogenic, unlinked traits in the BC (F_1 [C3H/J × C57BL/6J] × C57BL6/J) progeny. Thus, SNAb hyper- and hyporesponses are controlled by dominant and recessive alleles, respectively, and survival after rabies challenge infection is dominant to nonsurvival.

DISCUSSION

In this study we found that the quantitative difference in SNAb response to rabies vaccination between two inbred strains of mice (C3H/J and C57BL/6) is controlled by a single gene, and the hyper-SNAb response to rabies vaccination is controlled by a dominant allele. This SNAb response gene is not linked to *H-2*. Survival after a rabies challenge infection in these two strains is controlled by a dominant allele, and the rabies survival gene is not linked to the *H-2* or the SNAb response gene.

The evidence for genetic control of SNAb response to rabies vaccination and survival after rabies challenge infection was obtained by genetic studies between two inbred strains. The strains selected for genetic analysis were C3H/J and C57BL/6J. In surveying several different inbred strains to determine if there were quantitative differences in SNAb responses to rabies vaccination, the C3H/J strain was selected as consistently responding to vaccination with high SNAb titers compared with the other inbred strains tested, and the C57BL/6J strain was selected as a consistently low responder compared with the other inbred strains tested in a series of preliminary studies (data not shown). Based on these data, the C3H/J strain was assigned the phenotype of hyper-SNAb responder, and the C57BL/6J strain was assigned the phenotype of hypo-SNAb responder. With these two inbred strains as the parental strains, F_1 and BC progeny were produced for genetic analysis of the SNAb response to rabies vaccination. The phenotypic ratios of SNAb responses in the F_1 and BC progeny (Tables 2 and 3) fit the expected ratios for a monogenic mode of inheritance for SNAb response to rabies vaccination. The hyper-SNAb response behaves as a dominant allele, and the hypo-SNAb response behaves as a recessive allele.

TABLE 6. Analysis of segregation[a] and linkage[b] of *H-2* haplotypes, SNAb response to rabies vaccination, and survival after rabies challenge infection in BC [F_1 (C3H/J × C57BL/6J) × C57BL/6J] progeny

H-2 haplotype[c] and SNAb response[d]	Response to challenge infection				
	Survival		Death		
	No. of BC progeny	GMT	No. of BC progeny	GMT	Days until death
b/k					
+	6	123	8	157	8–14
−	5	27	4	23	10
b/b					
+	1	149	4	148	10–12
−	2	23	7	25	10–12

[a] χ^2 values for 1:1 segregation ratios of: $H\text{-}2^k$ and $H\text{-}2^b = 2.189$ with 1 df; SNAb hypo and hyperresponses = 0.027 with 1 df; survival or death after rabies challenge infection = 2.189 with 1 df.

[b] χ^2 values for linkage of: *H-2* with SNAb response = 2.189 with 1 df; *H-2* with survival after rabies challenge infection = 0.243 with 1 df; SNAb response with survival after rabies challenge infection = 0.027 with 1 df. χ^2 value for independent assortment of alleles at three loci = 0.676 with 1 df.

[c] *H-2* haplotypes: C3H/J = $H\text{-}2^k = k$, and C57BL/6J = $H\text{-}2^b = b$.

[d] +, SNAb hyperresponders with individual SNAb titers of >50.63; −, SNAb hyporesponders with individual SNAb titers of ≤50.63.

The question we addressed was whether or not the survivability of an individual challenged with a rabies infection is dependent on the SNAb response to rabies vaccination. The method of challenge infection adopted for this study was uniformly lethal in all unimmunized mice tested, regardless of strain (data not shown). In the strains we tested, it was also shown that 100% of the unimmunized C3H/J, C57BL/6J, and F_1 (C3H/J × C57BL/6J) mice died by our method of rabies challenge infection (Table 5). The C3H/J and F_1 (C3H/J × C57BL/6J) mice were protected from rabies challenge infection by rabies vaccination at a higher frequency than the C57BL/6J mice. The phenotype of hyper-SNAb response to rabies vaccination and a higher frequency of inducible protection from rabies challenge infection by vaccination occur together in the C3H/J and segregate in the F_1 (C3H/J × C57BL/6J) strains as a dominant gene(s) trait. C3H/J mice are *H-2* K^k, and C57BL/6J mice are *H-2* K^b. However, in the BC (F_1 [C3H/J × C57BL/6J] × C57BL/6J), these phenotypes (hyper-SNAb response, *H-2 K* locus, and inducible survival after rabies challenge infection) segregate as unlinked monogenic traits (Table 5 and 6). Therefore, these data indicate that inducible survival after rabies challenge infection and hyper-SNAb response in these two strains of mice are separate, independent functions.

The role of genetic control of survivability in inbred strains of mice after rabies challenge infection has been extensively studied by Lodmell (7, 8). Data from both of his studies and this study are consistent with few genes controlling survivability after rabies infection in mice. Due to the different challenge methods and lack of appropriate genetic studies, it is not known if the same genes are controlling survival in Lodmell's challenge experiments and in these reported challenge experiments. A contrasting point is that we do not observe any difference in survivability between sexes as reported by Lodmell (8), but this finding may be due to the fact that our challenge infection procedure is more rigorous, since vaccination is essential for survival in our studies (Table 4), and we may not have investigated the appropriate strains. Lodmell (8) reported that many of the mice in his study did not die from the challenge infection, but did suffer from secondary effects of the challenge infection, principally paralysis. He labeled these mice nonrecovered survivors. Those mice which recovered and demonstrated no secondary effects of the challenge infection he labeled recovered survivors. All of the mice in our challenge infection studies exhibited behavioral changes. These changes initially included agitation, marked by increased motion, and

some exhibited depression characterized by loss of appetite and recumbency. The agitation was first observed on day 6 after challenge. None of the survivors exhibited agitation or depression after day 12 postchallenge. There was no obvious permanent neurological damage exhibited by the survivors, whereas Lodmell (8) observed a large variation between strains in terms of secondary effects of the challenge infection.

Other investigators have questioned the role of SNAb in protection (3, 17, 20), and our data support the concept that SNAb alone does not afford immune protection to rabies infection. These data do not provide arguments against the current prophylaxis of rabies vaccination for preexposure protection or postexposure treatment. In fact, rabies vaccination was crucial for protection from rabies infection. The data do support, however, a need to study the mechanisms of immune protection to rabies infection to find an in vitro parameter that correlates better than SNAb titer with immune protection. This need seems obvious, as we have mice that have been vaccinated preexposure, have responded with high SNAb titers, and yet were vulnerable to rabies challenge infection (Table 6).

The complexity of host resistance to rabies infection has been stated by others (8). The essential role of cytotoxic-T-cells in protection from rabies infection has been demonstrated (9, 10, 16, 21). Although our studies did not provide information concerning immune mechanisms other than the role of SNAb, the protection induced by vaccination to our challenge method is long term, since comparable survival rates were observed in mice challenged 1, 2, or 6 months after the second vaccination (data unpublished). These data indicate that immunologic memory is being developed by the vaccine-induced protection. This finding does not argue for a pure cytotoxic-T-cell-derived protection, but supports the evidence of others (9, 10, 21) that a cytotoxic-T-cell response is a crucial component of immune protection to rabies infection.

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