INFECTION AND IMMUNITY, Jan. 1979, p. 45–48
0019-9567/79/01-0045/04$02.00/0

Vol. 23, No. 1

Histocompatibility-Linked Genetic Control of Susceptibility to Age-Dependent Polioencephalomyelitis in Mice

DOUGLAS MARTINEZ

Division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486

Received for publication 23 October 1978

Susceptibility to induction of immune polioencephalomyelitis (IPE) was found to be controlled by a gene that is closely linked to the *H-2* complex. Whereas mice of the AKR (*H-2*^{k}) strain were susceptible to IPE induction, *H-2*-congenic mice, AKR.*H-2*^{b} (*H-2*^{b} from C57BL/6) and AKR.M (*H-2*^{m}), were resistant. However, susceptibility to IPE may be under additional control by a gene(s) outside of the *H-2* region, since both C57BL/6 (*H-2*^{b}) mice and congenic B6.*H-2*^{k} mice (*H-2*^{k} from AKR) were resistant to IPE induction. F_1 hybrid mice derived from AKR (susceptible) and DBA/2 (resistant) mice were susceptible to IPE induction, indicating that susceptibility is dominant in at least one gene, but susceptibility developed at a later age in the hybrid mice than in AKR mice. B6.PL-*Ly-2*^{a}*Ly-3*^{a}/Cy, C57BR, C57L, PL, and RF strain mice were resistant to IPE induction. Thus, of the 12 inbred strains tested so far, only two (C58 and AKR) are susceptible to IPE.

During studies of the immune response of C58 mice to syngenic line I_b leukemia (I_b cells), Murphy et al. (10) found that the injection of inactivated I_b cells induces a paralytic central nervous system disease in old (9 months or older) C58 mice. Histopathologically, the disease is characterized by a mononuclear cell infiltration in the gray matter of the spinal cord and brain stem (5, 7). The disease was thought to result from an autoimmune response of old C58 mice to an antigen common to central nervous system tissue and I_b cells (10, 11). Therefore, it was designated immune polioencephalomyelitis (IPE). However, it was later determined that IPE is induced by a filterable replicating agent that is probably a virus (9). That is to say, IPE was serially transmitted in immunosuppressed C58 mice with filtered extracts of spleens from diseased animals. Moreover, extracts of I_b cells and of spinal cords from mice with IPE contained a virus-like particle, 40 nm in diameter, which may be the IPE agent.

Susceptibility of mice to the induction of IPE was found to be very strain specific (3, 11), suggesting a genetic control. Of the seven standard inbred mouse strains tested, only C58 and AKR/J mice were susceptible to IPE induction (3). In the current study, a survey for susceptibility to IPE was performed on additional selected inbred mouse strains, including congenic strains that differ from either the AKR (susceptible) or C57BL/6 (resistant) strain only at the *H-2* region. The data presented indicate that susceptibility to IPE is controlled, at least in part, by a gene closely linked to the *H-2* complex.

MATERIALS AND METHODS

Mice. AKR/J, C57BL/6J, C57BR/cdJ, C57L/J, C58/J, DBA/2J, and (AKR/J × DBA/2J)F_1 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Congenic AKR.M/nSn mice, congenic B6.PL-*Ly-2*^{a}*Ly-3*^{a}/Cy mice and PL/J mice (obtained from The Jackson Laboratory), and C58/Wm mice (obtained from W. H. Murphy, The University of Michigan, Ann Arbor) were maintained as a closed colony for Merck & Co. by Buckshire Corp., Perkasie, Penn. AKR/Cum mice were purchased from the Cumberland View Farms, Clinton, Tenn. Caesarian-originated, barrier-sustained C3H/HeNCr1BR mice were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. C58/Boy and congenic strains, AKR/Boy, AKR.*H-2*^{b}, C57BL/6Boy, and B6.*H-2*^{k}, were obtained from E. A. Boyse, Memorial Sloan-Kettering Cancer Center, New York, N.Y.

Immunosuppression. Mice were given a single intraperitoneal injection of cyclophosphamide (Cytoxan, Meade-Johnson & Co., Evansville, Ind.) at 150 mg/kg. The average weight of each group of mice was determined, and then the concentration of cyclophosphamide (freshly dissolved in water) was adjusted with phosphate-buffered saline (pH 7.2) to deliver the appropriate dose in 1 ml.

Test for susceptibility to IPE. A previous study showed that old C58 mice are normally susceptible to IPE induction, whereas old AKR mice and young C58 mice are susceptible only when immunosuppressed (3). Accordingly, groups of 20 mice each were given cyclophosphamide intraperitoneally 24 h before intraperitoneal challenge with IPE agent. Mice were chal-

lenged with a suspension of 10^6 γ-irradiated (10,000 R) I_b cells in 0.1 ml Hanks balanced salt solution, which contained ca. 10^5 IPE-inducing units. Each strain was tested at 6 months and 12 months of age. C58/Wm or C58/J were used as positive controls. Mice were observed 35 days for paralysis (IPE).

RESULTS

Strain specificity of IPE induction. The two strains of mice that were known to be susceptible to IPE induction, C58 and AKR, have a high incidence of spontaneous leukemia. These strains also have a common non-*H-2* histocompatibility antigen, Ly-3.1, which is not present in the resistant strains that had been tested (3). Therefore, other mouse strains that have a relatively high incidence of spontaneous leukemia (PL and RF) or have the Ly-3.1 antigen (PL, RF, and B6.PL-*Ly-2^{a}Ly-3^a*) were tested for susceptibility to IPE induction. Mice were immunosuppressed and then challenged with IPE agent. None of these mice were susceptible to IPE induction. C3H/He mice, DBA/2 mice, and mice that have a common, but distant, ancestry to C58 mice (C57BL/6, C57BR/cd, and C57L) were also resistant to IPE induction. Mice of the substrain AKR/Cum, which differ from AKR/J and AKR/Boy at the *Thy-1* locus, were susceptible. Like AKR/J and AKR/Boy, AKR/Cum mice were susceptible only when immunosuppressed. F_1 hybrid mice derived from AKR/J (susceptible) and DBA/2J (resistant) mice were also susceptible to IPE induction when immunosuppressed, but not as early as the AKR/J mice. At 6 months of age, hybrid mice had only a 40% incidence of IPE instead of the 90 to 100% usually obtained with AKR/J mice. At 12 months of age, the hybrid mice also had a 100% incidence. These results and previous studies are summarized in Table 1.

***H-2*-linked genetic control of IPE susceptibility.** Since resistance of mice to several viruses has been found to be regulated, in part, by *H-2*-linked genes (6), the relationship of the *H-2* region to susceptibility to IPE was investigated. Congenic mouse strains (1, 6), differing from either the AKR (susceptible) or C57BL/6 (resistant) strain only at the *H-2* region, were tested for susceptibility to IPE induction. Mice were immunosuppressed and then challenged with IPE agent. Unlike mice of the parental AKR/J and AKR/Boy (*H-2^k*) strains, the congenic AKR.M (*H-2^m*) and AKR.*H-2^b* (*H-2^b* from C57BL/6Boy) mice were resistant to IPE induction. With the congenic strains used, there is a strong probability of AKR-contributed homozygosity at loci that are relatively close to the *H-2* complex (6). For instance, a locus that is only 20 map units away from the *H-2* complex would have a probability of AKR-contributed homozygosity equal to 0.914 in the AKR.M strain (11 backcrosses) and 0.965 in the AKR.*H-2^b* strain (15 backcrosses). Therefore, the combined probability that the resistance gene introduced into these congenic strains lies farther than 20 map units from the *H-2* region is <0.003 (26 backcrosses total). Thus, the results obtained with the congenic mice indicate that susceptibility to IPE is controlled by a gene which is closely linked to the *H-2* complex. Congenic B6.*H-2^k* mice (15 backcrosses), like mice of the parental C57BL/6Boy strain, were resistant to IPE induction, although they received the *H-2^k* from the susceptible AKR/Boy strain. These results indicate that susceptibility to IPE is either controlled by more than one gene or is controlled by a single passenger gene that, by chance, remained with the *H-2* complex during the development of the AKR.M and AKR.*H-2^b* congenic strains, but was separated from the *H-2* complex by recombination during the development of the B6.*H-2^k* congenic strain. These two possibilities are illustrated in Fig. 1.

TABLE 1. *Strain specificity of IPE induction in immunosuppressed mice*[a]

Mouse strain	*H-2* type	IPE S or R[b]
AKR/Boy, AKR/Cum, AKR/J	*k*	S
BALB/cWm	*d*	R
CBA/J	*k*	R
C3H/HeJ, C3H/HeNCr1BR	*k*	R
C57BL/6Boy, C57BL/6J B6.PL-*Ly-2^{a}Ly-3^a*/Cy	*b*	R
C57BR/cdJ	*k*	R
C57L/J	*b*	R
C58/Boy, C58/J, C58/Wm	*k*	S
DBA/2J	*d*	R
NZB	*d*	R
PL/J	*u*	R
RF/J	*k*	R

[a] Summary of the current and previous (3) studies. Six- to twelve-month-old mice of the indicated strains were X-irradiated (3) or given cyclophosphamide 24 h before the intraperitoneal injection of IPE agent. Mice that developed paralysis (IPE) are referred to as susceptible.

[b] S, Susceptible; R, resistant.

DISCUSSION

In a previous study (3), mice from only two of the four *H-2^k* strains challenged with IPE agent (C58 and AKR) developed clinical and histopathologic signs of IPE. Thus, it was suggested that susceptibility to IPE is *H-2^k*-linked, but not *H-2^k*-limited. The results obtained from the *H-*

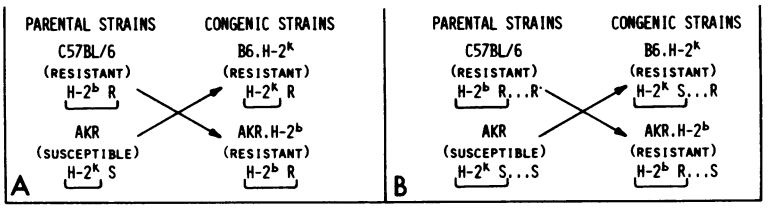

FIG. 1. *Two possible explanations for resistance of the H-2-congenic mouse strains to IPE induction (A and B). Each parental strain was used both as gene donor and recipient (1). The brackets indicate the genes that were transferred. (A) One gene codes for resistance (R) or susceptibility (S) and is closely linked to the H-2 complex. The gene remained with the H-2 complex during the development of the AKR.H-2^b congenic strain, but was separated by recombination from the H-2 complex during the development of the B6.H-2^k congenic strain. (B) Two or more genes code for R or S, one of which is closely linked to the H-2 complex. Only mice that have S at each gene are susceptible. The H-2-linked gene remained with the H-2 complex during the development of both the AKR.H-2^b and B6.H-2^k congenic strains.*

2-congenic strains, in the present study, indicate that susceptibility to IPE is controlled, at least in part, by a gene that is closely linked to the *H-2* complex (Fig. 1). The probability of there being a map distance of greater than 20 units between the IPE resistance gene and *H-2* is small (<0.003); the probability of no linkage at all is minute ($<10^{-6}$). Appropriate cross-breeding experiments could be used to confirm this linkage and to estimate linkage strength and the number of genes involved (6). If the genetic control of IPE susceptibility follows the pattern of other investigated viral systems (6), it will be found to be controlled by two or more genes. Whatever the number of genes, susceptibility is dominant in at least one gene, since F_1 hybrid mice derived from AKR (susceptible) and DBA/2 (resistant) mice are susceptible to IPE induction.

So far, the inbred mouse strains tested for susceptibility to IPE induction (12 total) fall into three categories: those that become susceptible by aging or by immunosuppression (C58), those that become susceptible only by immunosuppression (AKR), and those that are resistant even when immunosuppressed (e.g., C57BL/6). The existence of these categories may be explained as follows: upon aging, C58 mice lose the ability to immunologically suppress the amount of IPE agent in the central nervous system tissue (unpublished data), and, therefore, are susceptible to induction of the paralytic disease. Similarly, after immunosuppressive (lympholytic) therapy, young C58 mice and AKR mice lose the ability to suppress the amount of IPE agent in the central nervous system tissue (9). On the other hand, the mouse strains that are completely resistant to IPE induction apparently are not dependent on immunological (lymphocyte-mediated) restriction of the IPE agent for resistance to disease induction, since the resistance of these strains is unaffected by immunosuppression. Therefore, the resistant strains must possess a genetically determined resistance factor(s) that is non-immunological in nature. The *H-2*-linked gene that controls susceptibility to IPE induction probably governs such a non-immunological resistance factor, since the AKR.*H-2*^{b} and AKR.M strains are resistant to the immunosuppression-challenge regimen.

Resistance to IPE induction in C58 mice is thymus dependent. This is substantiated by the fact that neonatal thymectomy makes young C58 mice susceptible to IPE induction (2). Moreover, old C58 mice are deficient in T-cell functions (8, 10). Since C58 mice make antibodies against the IPE agent (9), susceptibility to IPE induction may be the result of loss of thymus-dependent antibody production. Although the thymus-dependent functions of normal old AKR mice are depressed (4), apparently the loss in thymus-dependent resistance to IPE, whatever its source, is not sufficient to make them fully susceptible. However, after challenge these mice do have incipient disease which can be detected histopathologically and produces a slight loss in their spreading reflex (3).

Much remains to be answered concerning the genetics of IPE susceptibility, e.g., the number of genes involved, the dominance of susceptibility or resistance, the location of the genes involved, and the possible relationship of high spontaneous leukemia incidence to IPE susceptibility. The genetic study of the various strains may also provide a useful tool for identifying the mechanisms of resistance and disease induction.

ACKNOWLEDGMENTS

I thank Robert Lynch and Patricia Ann Dennison for expert technical assistance, Zoilo A. Zayas (Memorial Sloan-Kettering Cancer Center) for providing congenic mice, Jeff B. Meeker for statistical advice, and Marianna Cherry and Edward A. Boyse for stimulating discussions and for reviewing the manuscript.

LITERATURE CITED

1. **Boyse, E. A.** 1977. The increasing value of congenic mice in biomedical research. Lab. Anim. Sci. **27**:771–781.
2. **Duffey, P. S., O. A. Lukasewycz, D. Martinez, and W. H. Murphy.** 1976. Pathogenetic mechanisms in immune polioencephalomyelitis: quantitative evaluation of protective and pathogenetic effects of lymphoid cells. J. Immunol. **116**:1332–1336.
3. **Duffey, P. S., D. Martinez, G. D. Abrams, and W. H. Murphy.** 1976. Pathogenetic mechanisms in immune polioencephalomyelitis: induction of disease in immunosuppressed mice. J. Immunol. **116**:475–481.
4. **Gershwin, R. J., M. E. Gershwin, A. D. Steinberg, A. Ahmed, and T. Ochiai.** 1976. Relationship between age and thymic function in the development of leukemia in AKR mice. Proc. Soc. Exp. Biol. Med. **152**:403–407.
5. **Homburger, H., G. D. Abrams, J. W. M. Lawton, and W. H. Murphy.** 1973. Histopathology of serum-transmitted immune polioencephalomyelitis in C58 mice. Proc. Soc. Exp. Biol. Med. **144**:979–982.
6. **Klein, J.** 1975. Biology of the mouse histocompatibility-2 complex. Principles of immunogenetics applied to a single system. Springer-Verlag New York, Inc., New York.
7. **Lawton, J. W. M., and W. H. Murphy.** 1973. Histopathology of immune polioencephalomyelitis in C58 mice. Arch. Neurol. **28**:367–370.
8. **Lawton, J. W. M., and W. H. Murphy.** 1975. Characterization of the blastogenic response of C58 spleen cells: age-dependent changes. Immunology **26**:1093–1099.
9. **Martinez, D., B. Wolanski, A. A. Tytell, and R. G. Devlin.** 1979. Viral etiology of age-dependent polioencephalomyelitis in C58 mice. Infect. Immun. **23**: 133–139.
10. **Murphy, W. H., M. R. Tam, R. L. Lanzi, M. R. Abell, and C. Kauffman.** 1970. Age dependence of immunologically induced central nervous system disease in C58 mice. Cancer Res. **30**:1612–1622.
11. **Sager, M. A., J. W. M. Lawton, and W. H. Murphy.** 1973. Serum transmissibility of immune polioencephalomyelitis in C58 mice. J. Immunol. **110**:219–226.