PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 499-502 (1976)

Congenic strains of Mice Susceptible and Resistant to Mouse Hepatitis Virus[1] (39426)

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Ever since Snell (1) established congenic strains of mice which differ on the basis of the major histocompatibility antigen, the usefulness of such lines for both genetic and physiological studies has grown in importance (2-4). We here report the establishment of the line of C_3HSS mice which are susceptible to a standard strain of mouse hepatitis virus, derived from PRI mice (MHV-PRI) and which are congenic to the established inbred line of resistant C_3H mice. These strains of mice should be useful not only in analyzing the locus for susceptibility, but also should allow for lymphocyte and macrophage transfer so that the factors involved in pathogenesis may be more accurately analyzed.

A virulent variant of the original virus (MHV-PRI) which is capable of killing both susceptible and resistant strains of mice was isolated by Shif and Bang (5). This complex interdependence of virulence and susceptibility is represented diagramatically in quadrate analysis (Fig. 1), a presentation which is similar to that used by plant pathologists (6). In this there is a matching of one gene difference in host susceptibility against the presumed one-step conversion of virus from restriction to PRI mice to adaption to C_3H mice. The present paper is limited to a study of susceptibility to MHV-PRI.

Materials and methods. Mouse strains. Princeton (PRI) and C_3H mice were used. PRI mice were originally obtained from Dr. John Nelson in 1951 and have since been maintained by brother-sister inbreeding in our laboratory. This strain of mice is genetically susceptible to mouse hepatitis virus infection. C_3H, a resistant strain, was obtained from Dr. H.B. Andervont on August 10, 1956 and has also been maintained through inbreeding.

Virus. MHV-2 strain of mouse hepatitis virus was obtained from Dr. John Nelson (7). It was passed in our laboratory by intraperitoneal inoculation of one-month old PRI mice. A 10% (wt/vol) liver homogenate was prepared by grinding up livers of moribund mice. This preparation was used as stock virus and kept at −70°.

Macrophage cultures. Cultures of peritoneal macrophages were prepared in the following manner: Mice were stimulated by injecting 3.0 ml of sterile thioglycollate medium (Difco) intraperitoneally. Three days later, 5.0 ml of warmed phosphate buffered solution (pH = 7.2) containing 20 U.S.P. units of sodium heparin (Upjohn) per milliliter was again injected intraperitoneally. Fluid was withdrawn and cells were counted. The cell suspension was centrifuged at 1000 rpm for 10 min and the cellular pellet was then resuspended in Chang's medium which is 90% horse serum, 8% Hanks BSS, and 2% beef embryo extract. The medium was seeded with 1-1.5 × 10^6 cells in Wassermann tubes which were then closed with siliconized stoppers. After attachment, they were transferred to a roller drum in a 37° incubator.

Tests of histocompatibility. Histocompatibility was tested by reciprocal skin grafting and mixed lymphocyte culture (MLC) reaction.

Method for obtaining a congenic susceptible line of mice. Inbred PRI mice were crossed with inbred C_3H mice to produce F_1 hybrids. The hybrids were then mated to the resistant strain (C_3H) to produce back-cross generation-1 (BC-1). The progeny of this back-cross were tested for cell susceptibility, and only the susceptible ones were chosen and mated again to C_3H mice to produce BC-2. This was continued until BC-20 was achieved. During each generation, susceptibility to MHV was tested by inoculating cultured macrophages with MHV at a dilu-

[1] This study was supported by a grant from the National Science Foundation (No. 31479).

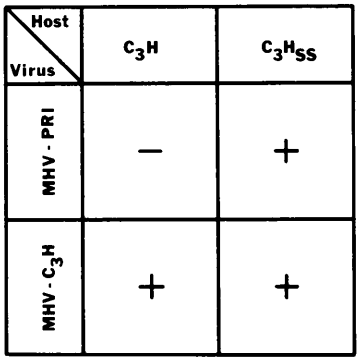

FIG. 1. Quadrate analysis. Plus signs indicate that the virus and the host are compatible, whereas the negative sign indicates that they are not compatible. C_3HSS = C_3H mice carrying the gene for susceptibility to mouse hepatitis virus (MHV). MHV-PRI = original strain of MHV, derived from the livers of moribound PRI mice; MHV-C_3H = virulent viriant virus.

tion of 10^{-3}, since Bang and Warwick (8) had found that cultured peritoneal macrophages expressed the genetic response to MHV of their donor animals. Mice with susceptible macrophages were kept for further breeding.

Method for obtaining an homogenous C_3H-susceptible (C_3HSS) line of mice. Susceptible mice from BC-20 were bred by brother-sister mating: BC-20 × BC-20 = Ss × Ss → SS,2Ss,ss. ss was eliminated by testing cultivated peritoneal macrophages and discarding all mice that were resistant. Homozygosity for susceptibility was tested by back-crossing mice with susceptible macrophages to resistant C_3H mice. Mice whose descendants were 100% susceptible were kept as parents for subsequent inbreedings.

SS × C_3H = SS × ss → Ss --------100% susceptible
Ss × C_3H = Ss × ss → Ss,ss -------- 50% susceptible

By selection of presumed homozygotes, C_3HSS was developed.

Results and Discussion. The ratio of susceptible to resistant mice among 20 back-crosses. The ratio of susceptible to resistant mice among the offspring of back-crosses was rather constant, close to 1:1 (Table I). This suggests that the genes segregated in a typical Mendelian fashion with one locus responsible for susceptibility. However, the overall percentage of mice yielding positive cultures was 209/455 or 45.9. If the first back-cross which still contains a considerable mixture of genes is eliminated, there was no significant difference in the expected and the actual ratios of susceptible to resistant mice.

TABLE I. RESULTS OF *IN VITRO* INOCULATION OF MOUSE MACROPHAGE CULTURES FROM SUCCESSIVE BACK-CROSS MATING OF SUSCEPTIBLE MICE TO A RESISTANT STRAIN.

Generation	Susceptible/ total mice	Susceptible (%)	Expected susceptible (%) on hypothesis of one dominant gene
F_1 (PRI × C_3H)	21/21	100	100
Back-cross-1	6/27	22.2	50
Back-cross-2	2/5	40.0	50
Back-cross-3	11/18	61.1	50
Back-cross-4	3/7	42.9	50
Back-cross-5	3/10	30.0	50
Subtotal	25/67	37.3	
Back-cross-6	15/32	46.9	50
Back-cross-7	14/30	46.7	50
Back-cross-8	25/43	58.1	50
Back-cross-9	7/15	46.7	50
Back-cross-10	9/17	52.9	50
Subtotal	70/137	51.1	
Back-cross-11	3/6	50.0	50
Back-cross-12	18/32	56.3	50
Back-cross-13	16/36	44.4	50
Back-cross-14	3/7	42.9	50
Back-cross-15	15/31	48.4	50
Subtotal	55/112	49.1	
Back-cross-16	6/13	46.2	50
Back-cross-17	16/38	42.1	50
Back-cross-18	11/23	47.8	50
Back-cross-19	6/17	35.3	50
Back-cross-20	20/48	41.7	50
Subtotal	59/139	42.4	
Grand total	209/455	45.93	

Test for histocompatibility. (i) *Skin graft.* After 10 generations of C_3HSS and C_3H, 20 reciprocal skin grafts were exchanged between C_3H and C_3H_{SS}. Ten mice were kept for 45 days while others were kept for 75 days. There was no rejection between them. Exchanges between PRI and C_3H, PRI and C_3HSS were rejected within 9 to 12 days. (ii) *Mixed lymphocyte culture reaction.* The MLC reaction was kindly performed by the Immunogenetics Laboratory of The Johns

Hopkins University School of Medicine. There was no stimulation between C_3H and C_3HSS mice.

Further testing of susceptibility of C_3HSS mice to MHV. Even though C_3HSS was developed after four generations of inbreeding from presumed homozygotes and no heterozygotes were found thereafter (Table II), a routine check-up of our inbred C_3HSS mice colonies has been done to ensure the homogenicity. Forty-five mice, 4 to 6 weeks of age, were given 0.2 ml of 4.5 log ($TCID_{50}$) of virus intraperitoneally. They all died in 4 days. Macrophage cultures prepared from 50 individual mice were also tested by inoculating 10^{-5} to 10^{-7} dilutions of virus and were found susceptible. When a complete virus assay was performed, the viral titer was the same as from PRI mice.

Change of susceptibility in C_3HSS mice. Gallily *et al.* (9) demonstrated that the ability to resist the lethal dose of MHV-PRI infection of C_3H mice was a characteristic of the adult. That is, MHV-PRI infection was lethal to infant C_3H mice; their macrophages supported viral growth and were destroyed by the virus. Since C_3HSS and C_3H are congenic, it was of interest to determine whether there was any change in susceptibility of C_3HSS with increasing age. Different ages of mice were tested by infection of macrophage cultures and/or by intraperitoneal injection of MHV-PRI. There was a decrease in susceptibility as the mice passed 7 weeks of age (Table III). However, mice between 12 and 13 months old apparently lost most of this reduced susceptibility and became susceptible again. But when 44 macrophage cultures from various ages of mice were tested, they were found uniformly susceptible.

Summary. A congenic strain of C_3HSS mice, which is histocompatible with C_3H mice but differs from them in susceptibility to mouse hepatitis virus (MHV), has been developed by introducing the gene for susceptibility to the MHV-PRI virus from the PRI mice. This was accomplished by continual back-crossing of the hybrids to the C_3H mice, but at the same time by selection of susceptibility by use of macrophage culture tests. After 20 back-crosses, a strain homozygous for susceptibility was produced by brother-sister mating of individual mice whose potential for carrying the recessive gene for resistance was tested in progeny. Since the original choice of mice for breeding was based on *in vitro* macrophage susceptibility, and since highly susceptible mice were developed on the same basis, it seems evident that macrophage susceptibility is an integral aspect of mouse susceptibility.

The continued production of almost 50% susceptible mice in the back-crosses is further evidence of the dominant one-locus explanation of genetic susceptibility to this agent. Incomplete penetrance may also be present in 8 and 9 week old mice of the C_3HSS strain since there was a sharp decrease in susceptibility of these mice even though their macrophages in culture maintained full susceptibility.

TABLE II. RESULTS OF PROGENY TEST FROM PRESUMED HOMOZYGOTE SUSCEPTIBLE C_3H MICE.

Generations[a]	Susceptible/total mice	Susceptible (%)
F_1	50/81	61.7
F_2	70/90	77.8
F_3	324/358	90.5
F_4	76/77	98.7

[a] F_1 and F_2 were tested *in vitro*; F_3 and F_4 were tested *in vivo*.

TABLE III. RESULTS OF *IN VIVO* INOCULATION OF VARIOUS AGE C_3HSS WITH MHV-PRI.[a]

Age of mice	Number tested	Number died	Number died/number tested (%)
4 weeks	10	10	100
5 weeks	10	10	100
6 weeks	27	27	100
7 weeks	18	18	100
8 weeks	22	9	41
9 weeks	10	1	10
12–13 months	34	32	94

[a] Macrophages from survived mice were tested individually and found susceptible.

We wish to thank Dr. Ilan Shif for his contribution in testing for susceptibility.

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Received November 17, 1975. P.S.E.B.M. 1976, Vol. 152.