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Resistance/Susceptibility to Lethal Sendai Virus Infection Genetically Linked to a Mucociliary Transport Polymorphism

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Linkage was tested between a mucociliary transport polymorphism and resistance/susceptibility to lethal Sendai virus infection in segregant hybrid mice of C57BL/6J and DBA/2J parents. The distribution of paired phenotypes for tracheal mucociliary transport rates and susceptibility to lethal Sendai virus infection in 171 F_1 × DBA/2J mice showed strong interaction of the parental phenotypes.

Sendai virus, a parainfluenza 1 virus, is a naturally occurring respiratory pathogen of laboratory rodents (10, 11). In mice, Sendai virus causes a descending infection of conducting airways (1, 4, 5). The spectrum of disease varies from mild to severe depending on the degree of lower respiratory tract involvement (4, 7, 11). Studies of inbred strains of mice indicate that the course of Sendai virus infection is genetically determined. Genetically resistant strains are up to 40,000-fold more resistant to the lethal effects of Sendai virus than are genetically susceptible strains (7, 11). Mice with resistance genotypes (SJL, RF, and C57BL) abrogate the infection while it is still confined primarily to the airways, whereas mice with susceptibility genotypes (129 and DBA) permit the infection to spread from airway to lung parenchyma (7, 11). The host mechanisms through which these genetic differences are expressed are unknown.

An inherited difference in tracheal mucociliary transport rates (TMTR) between C57BL/6J and DBA/2J mice has recently been described. C57BL/6J mice have sixfold faster TMTR than DBA/2J mice and transmit this functional polymorphism as an autosomal incomplete dominant trait (D. G. Brownstein, Exp. Lung Res., in press). In this study, I tested the hypothesis that TMTR polymorphism mediates variations in resistance to the lethal effects of Sendai virus infection.

Male DBA/2J and female (C57BL/6J × DBA/2J)F_1 mice aged 6 to 8 weeks were obtained from Jackson Laboratory, Bar Harbor, Maine. Backcross mice were produced at this laboratory with F_1 female and DBA/2J male mice under specific-pathogen-free conditions to exclude adventitious infections. Microbiological and serological monitoring confirmed specific-pathogen-free status throughout the study. TMTR were determined in individually identified (toe clip) backcross mice at 6 to 8 weeks of age by measuring clearance rates of epi-illuminated fluorescent latex microspheres through surgically exposed tracheas as described elsewhere (Brownstein, in press). By this technique, the mean TMTR for C57BL/6J and DBA/2J mice were 2.29 and 0.40 mm/min, respectively. The lower 95% confidence interval for C57BL/6J mice and the upper 95% confidence interval for DBA/2J mice was 1.25 mm/min. Backcross mice with TMTR above 1.25 mm/min were classified as rapid transporters, and those with TMTR below that value were classified as slow transporters. Because of incomplete dominance, about 30% of backcross mice were expected to be rapid transporters.

After a minimum 2-week recovery from surgery, mice were exposed to 3.0 median aerosol infectious doses (2.3 DBA/2J and 0.02 C57BL/6J median lethal doses) of Sendai virus with a modified Henderson aerosol generator as previously described (8). The strain of Sendai virus (771076) and its passage history have been described elsewhere (6). Previously, the dose of virus used caused 0 to 20% mortality in C57BL/6J mice, 80 to 100% mortality in DBA/2J mice, and 40 to 60% mortality in F_1 × DBA/2J backcross mice 6 to 12 weeks old (unpublished data). Deaths in backcross mice began 9 days postinfection, peaked on day 12, and ended on day 19. Mice that succumbed were classified as susceptible, and mice that survived were classified as resistant to Sendai virus. Infection was confirmed in survivors by seroconversion to Sendai virus with a fluorescent-antibody assay (12). Mice that died were necropsied to confirm lung consolidation, and lungs from selected mice were examined histologically for changes characteristic of Sendai virus infection (1, 7).

Using the phenotypic criteria for TMTR and response to Sendai virus infection, 76 to 100% of C57BL/6J mice were classified as rapid/resistant, and 76 to 100% of DBA/2J mice were classified as slow/susceptible. If the two phenotypic classes were causally linked, the parental combinations would persist in a segregant hybrid generation and therefore exceed predictions based on a random association of phenotypes.

In three experiments, a total of 171 F_1 × DBA/2J mice were classified as to their combined phenotypes for TMTR and response to Sendai virus (Table 1). The interaction chi-square (13) of the observed distribution of mice among the four classes, adjusted for 1 degree of freedom, was 15.40, $P < 0.001$. There were 30% more mice with parental combinations and 29% fewer mice with new combinations than were expected based on predictions for noninteraction. Mice with slow TMTR were 2.33 times as likely to succumb to Sendai virus infection as were mice with rapid TMTR. A homogeneity chi-square of 0.36 ($P > 0.75$, 2 degrees of freedom) was justification for combining the results of the three experiments.

These results support an etiologic link between genetic polymorphism in TMTR and genetic resistance/susceptibility to lethal Sendai virus pneumonia. The alternative interpretation, that the phenotypes were not etiologically linked but were controlled by linked genes, seems less likely since mucociliary epithelium is the principal site of Sendai virus replication and also presumably expresses TMTR polymorphism. TMTR polymorphism alone cannot explain why F_1 mice, which express intermediate TMTR, are highly resistant to lethal Sendai virus pneumonia (6). Heterosis or

TABLE 1. Contingency table showing observed distribution of backcross mice according to phenotype combinations and expected distribution based on noninteraction

TMTR phenotype	No. of mice (expected distribution) with the following phenotype:	
	Resistant	Susceptible
Rapid	42 (29.5)[a]	14 (26.5)
Slow	48 (60.5)	67 (54.5)

[a] Expected number = (total number resistant or susceptible × total number rapid or slow)/(total number of mice).

additional genetic mechanisms must decide heterozygote resistance.

Movement of the mucous blanket by ciliary action is an important protective mechanism, but the magnitude of its role in antiviral defense has not been clearly defined. The mucociliary escalator is believed to account for lower susceptibility to viral infections of mucociliary epithelium in intact animals versus organ cultures (3). In intact animals, reducing mucus TMTR by as little as one-half by using topical or systemic antagonists can increase the susceptibility of respiratory epithelium to viral infections 40-fold (2). The evidence presented here provides further support for a major role of mucociliary transport in respiratory antiviral defense and implicates genetic regulation of TMTR as an important variable in genetically heterogeneous populations. Genetic polymorphisms of TMTR are probably not only important in respiratory viral pathogenesis in mice; there is evidence that heritable factors exert considerable control over TMTR in humans (9).

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