INFECTION AND IMMUNITY, Nov. 1979, p. 764-766
0019-9567/79/11-0764/03$02.00/0

Vol. 26, No. 2

NOTES

Respiratory Syncytial Virus Infection in Inbred Mice

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Received for publication 13 August 1979

Respiratory syncytial virus infected the nose and lungs of each of 20 strains of inbred mice, with viral titers varying 100-fold from least permissive to most permissive strains. Viral titers appeared to be under genetic control, but did not correlate with the *H-2* haplotype.

The recent development of animal models of respiratory syncytial (RS) virus infection has opened new opportunities for research on pathogenesis and immunity. Experimental infection has now been described in ferrets (8), chimpanzees (1), cebus monkeys (10), owl monkeys (9), and cotton rats (7). Each of these models has unique features which make it valuable for RS virus research. For instance, the ferret shows an age dependence of viral replication in the lung (8) and provides a model for passive protection via breast-feeding (13). The chimpanzee develops upper respiratory tract disease most nearly resembling RS virus disease in humans (1). The extreme scarcity of chimpanzees, however, severely limits experimental work with this species. For this reason the owl monkey, the only other nonhuman species known to develop clinically evident RS virus-induced disease (9), has become a major resource because this primate is more readily available than the chimpanzee. The cebus monkey does not develop signs of upper respiratory tract disease during RS virus infection; however, transtracheal inoculation of a large quantity of virus (10^8 plaque-forming units) induces extensive pulmonary pathology (10). In this situation, it is necessary to sacrifice these scarce primates in order to study RS virus pneumonia. Finally, the cotton rat exhibits uniform susceptibility to infection throughout life (7), making it a desirable animal for long-term studies. Cotton rats are readily available, are relatively simple to maintain and manipulate, and have become the most widely used animal in our studies of RS virus.

None of these species, however, is inbred, thus precluding genetic manipulation and certain types of immunological study. Furthermore, few, if any, specific immunological reagents are available to allow study of certain aspects of RS virus pathogenesis and virus-host immunological interaction. In an effort to develop a model for experimental RS virus infection in an inbred species, we examined 20 strains of inbred mice. Pregnant mice of the following strains were obtained from the Animal Production Unit, Veterinary Resources Branch, Division of Research Services, National Institutes of Health: A/HeN, AKR/N, AL/N, BALB/cAnN, C3H/HeN, C57BL/6N, C57BL/10ScN, C57L/N, CBA/N, CBA/CaHN, CBA/CaHN-T6, DBA/2N, GR/N, NFR/N, NFS/N, NZB/N, NZW/N, P/N, SJL/N, and WB/ReN-$W^{+/v}$. At 3 days of age, infant mice were anesthetized with ether and inoculated intranasally with $10^{3.3}$ to $10^{3.7}$ plaque-forming units of RS virus in a volume of 0.01 ml. A single suspension of the Long strain of RS virus, prepared in HEp-2 cell cultures, was used to infect each of the mouse strains.

At 4 days after inoculation, the time of peak virus titer in other animals experimentally infected with RS virus, the infant mice were sacrificed by decapitation. The lungs and nose (including nasal passages and turbinates) were removed, weighed, and homogenized in Hanks balanced salt solution, which was enriched with sucrose, phosphate, and glutamate (2). Homogenates were clarified by centrifugation at 400 × *g* for 5 min, decanted, and stored at −70°C until assayed.

The quantity of virus present in homogenized tissues was determined by plaque assay on HEp-2 cell monolayers (12).

A minimum of 10 infants from two litters was used to determine the response of each mouse strain to RS virus. When geometric mean titers of viral replication in the lungs were ordered by magnitude (Fig. 1), a spectrum of growth pat-

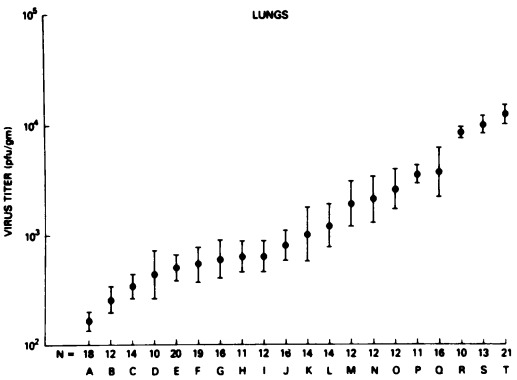

FIG. 1. *Geometric mean titer of RS virus recovered from lungs (± standard error). The number of animals used for each strain is indicated on the abscissa. Letters refer to inbred strains, as follows: (A) CBA/CaHN; (B) C3H/HeN; (C) CBA/CaHN-T6; (D) WB/ReN-W$^{t/v}$; (E) NFR/N; (F) NZW/N; (G) NFS/N; (H) CBA/N; (I) A/HeN; (J) NZB/N; (K) C57BL/6N; (L) GR/N; (M) SJL/N; (N) BALB/cAnN; (O) C57BL/10ScN; (P) AL/N; (Q) AKR/N; (R) P/N; (S) C57L/N; (T) DBA/2N. pfu, Plaque-forming units.*

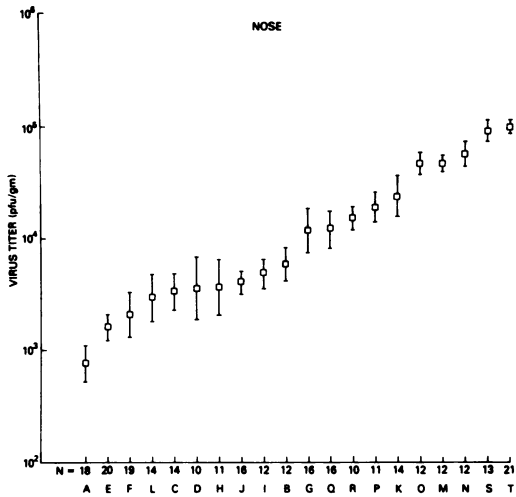

FIG. 2. *Geometric mean titer of RS virus recovered from nose (± standard error). See the legend to Fig. 1 for strain designations. pfu, Plaque-forming units.*

terns was seen. The most resistant strain, CBA/CaHN, yielded only $10^{2.2}$ plaque-forming units per g, whereas the most permissive strain, DBA/2N, yielded $10^{4.1}$ plaque-forming units per g, a nearly 100-fold increase over the CBA/CaHN strain. The temporal course of viral replication in the nose and the lungs was determined for C57L/N and CBA/CaHN mice to verify that both high-yielding and low-yielding strains achieved peak viral titer on post-inoculation day 4. Animals were sacrificed throughout the course of infection (12 days), and the maximal viral titers for both strains were observed on day 4.

A plot of geometric mean titer of virus in the nose (Fig. 2) in order of increasing magnitude demonstrated a pattern closely resembling that seen in the lungs. There was a gradual, incremental increase in nasal titer from relatively resistant to relatively permissive strains. Again, the difference between the most resistant strain (CBA/CaHN, $10^{2.8}$ plaque-forming units per g) and the most permissive strain (DBA/2N, $10^{5.0}$ plaque-forming units per g) was approximately 100-fold. However, a consistent relationship between nasal and pulmonary titers was not seen for each strain.

The relatively small variability in the level of viral replication within any individual strain was in contrast to the considerable variability between different strains. This suggests that the interstrain differences were genetically determined. If the level of viral replication were controlled by a single gene with few alleles, one would not expect to observe the shallow linear array seen when the inbred strains were ordered by level of viral titer in tissue homogenates. This suggests that response to RS virus infection is determined by a combination of genes or perhaps by a single gene with multiple alleles. Furthermore, the lack of a consistent relationship between nasal and pulmonary viral titers within each strain suggests that the growth of virus in these two organs may be independently controlled.

The association of the *H-2* haplotype with susceptibility to viruses has been documented with leukemia virus (4), mammary tumor viruses (5), and lymphocytic choriomeningitis virus (6). However, such a relationship was not seen in the 20 strains of mice that we examined. The most permissive strains (DBA/2N and C57L/N) did not share *H-2* haplotypes with each other; furthermore, other strains carrying the same haplotype as either DBA/2N or C57L/N did not support viral growth to a high level. Similarly, other strains bearing the same haplotype as the lowest viral growth strain (CBA/CaHN) showed wide variation in the level of viral replication.

The nature of the genetic factors governing susceptibility to RS virus infection in mice is not known. Our preliminary survey suggests that multiple factors are involved, but does not distinguish between control by several genes and multiple-allele control within a single gene. Since there was no overlap between the virus titers observed for the strains that exhibited the lowest and the highest levels of viral growth, it should be possible to analyze the genetic control of viral replication by the appropriate crossbreeding techniques.

In addition to the opportunity to study genetic control of viral replication, the availability of an inbred animal model for RS virus infection offers possibilities for studies which were not feasible previously. For instance, in vivo experiments using adoptive transfer of immunological components, including immune cells, can now be performed. In addition, a large number of specific immunological reagents exists for mice, for example, antisera directed against mouse immunoglobulins (with heavy- or light-chain as well as whole-molecule specificity), interferon, theta antigen, Ly 1, 2, and 3 T-cell antigens, specific histocompatibility antigens, and macrophages. Finally, the existence in certain inbred mice of well-defined immunological defects gives this model a unique advantage. Mice lacking T-cell-dependent (3), B-cell-dependent (11), and both T- and B-cell-dependent (C. T. Hansen, H. A. Azar, and J. Costa, Fed. Proc. 38:929, 1979) immune functions have been described. Efforts to introduce such specific defects into strains of mice most susceptible to RS virus infection are currently in progress.

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