Viral Enhancement of Nasal Colonization with *Haemophilus influenzae* Type b in the Infant Rat

RICHARD H. MICHAELS[16] AND RICHARD L. MYEROWITZ

Children's Hospital of Pittsburgh and the Departments of Pediatrics and Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Summary

Infant rats infected with influenza A virus, Sendai (parainfluenza 1) virus or rat coronavirus were used to determine whether viral infection increases the intensity of nasal colonization with *Haemophilus influenzae* type b (HIB). Intranasal inoculation of HIB in rats previously infected with each of these viruses resulted in nasal HIB titers at least 100-fold higher than those for controls during the first 2 wk after HIB inoculation, and as much as 10,000-fold higher during the first week. Children with cough, sneezing, or rhinorrhea could be effective disseminators of HIB if they were as heavily and persistently colonized as these virus-infected animals.

Abbreviations

cfu, colony forming units
EID_{50}, 50% egg infectious dose endpoint
HIB, Haemophilus influenzae type b
$TCID_{50}$, 50% tissue culture infectious dose endpoint

Attention has recently been focused on the potential for spread of meningitis and other invasive diseases due to HIB among young children in close contact (2, 14). HIB disease is often associated with a much increased frequency of pharyngeal HIB colonization in family members or day-care center contacts, but little is known about factors that favor communicability. Because viral respiratory infection has often occurred in patients with HIB meningitis (4, 5) it is conceivable that viruses could play a role in the pathogenesis and spread of HIB disease. One possibility is that viral infection of the upper respiratory tract increases the intensity of pharyngeal HIB colonization, thus facilitating dissemination of the organism to other children. An infant rat model was employed to help examine this hypothesis.

MATERIALS AND METHODS

The strains of HIB and influenza A virus, experimental animals and methods of animal inoculation, tissue homogenation and culture have all been described previously (11). Briefly, suckling Sprague-Dawley rats were inoculated intranasally with a 100 μl precision syringe (Hamilton Company, Whittier, CA) attached to the plastic tubing of a pediatric infusion set (Butterfly Short 25-G, Abbott Laboratories, North Chicago, IL). Great care was employed to inoculate atraumatically by slowly ejecting 5 μl of fluid, which hung as a drop on the edge of the needle, and gently touching the drop to the external nares. The fluid was usually quickly sucked into the nasal cavity during inspiration. The procedure was repeated on the same side after several minutes so that a total of 10 μl of viral or bacterial suspension was inoculated.

The strain of influenza virus used was an isolate from the pharynx of a Pittsburgh child in 1976 and identified as A/Victoria (H_3N_2). Strains of Sendai (parainfluenzae 1) virus (VR-105) and rat coronavirus (VR-635) were obtained from the American Type Culture Collection (Rockville, MD). Nasal washings were performed by allowing inhalation of 20 μl of normal saline and collecting the effluent from the nostrils with a capillary pipet. Washings (both undiluted and a 1:100 dilution in saline) were inoculated with a 1 μl calibrated loop on antiserum agar (10). A radial streaking technique was employed that allowed enumeration of up to 300 colonies on a plate.

RESULTS

Initially, the duration and intensity of nasal HIB colonization were studied in infant rats that had been inoculated with approximately 10^5 EID_{50} (50% egg infectious dose endpoint) of influenza A virus at age 3 days and with 10^5 colony-forming units (cfu) of HIB 48 h later. Five animals were then sacrificed every 2–3 days until HIB could no longer be detected in homogenized nasal (snout) tissue. Using this procedure, approximately 10^4 cfu of HIB/μg of nasal tissue could be demonstrated throughout the first week after HIB inoculation, with a decline to $10^{2.9}$ cfu/μg by the 14th day. There was no detectable HIB by the 19th day (less than 1 cfu/μg). In contrast, nasal HIB became undetectable within 24 h after the same intranasal dose of HIB in animals that were not previously infected with influenza virus.

Other viruses were then studied to determine whether they would produce similar effects. Sendai (parainfluenza 1) virus and the rat coronavirus were selected because these agents are known respiratory pathogens for rodents (1). Nasal washings were substituted for nasal tissue homogenates to allow for repeated culture of large numbers of animals, and to provide quantitative data that would better reflect the portion of the nasal HIB population available for spread. In all subsequent experiments two litters, each with 15 randomly-assorted infant rats, were utilized. One litter received 10^3–10^4 $TCID_{50}$ (50% tissue culture infectious dose endpoint) of virus intranasally and the other normal saline 48 h before the intranasal inoculation of 10^5 cfu of HIB.

Table 1 summarizes the results of four experiments with these two respiratory viruses. It can be seen that the effect of either Sendai virus or rat coronavirus inoculated at age 3 days was similar to that of influenza A virus, resulting in high titers of HIB (10^3–10^4 cfu/μl of nasal washings) during the 2–3 wk after intranasal HIB inoculation of virus-infected animals. HIB became undetectable during the first week after bacterial inoculation of animals that had not received virus, but there was an unexplained appearance of HIB in low titer (not exceeding 10^2 cfu/μl) between the 14th and 24th days. Similar results were obtained when Sendai virus was administered at age 8 days, and when rat coronavirus was given at age 14 days. Most animals could not be colonized with HIB when sequentially inoculated with coronavirus and HIB at the age of one month, but nasal cultures of both virus-infected and control rats revealed heavy growth of other bacteria at this time.

DISCUSSION

Halsey and associates (3) have demonstrated nasal HIB colonization for up to 24 days in infant rats repeatedly inoculated with

Table 1. *Titer of H. influenzae type b in nasal washings from infant rats given either virus or normal saline intranasally, followed in 48 h by 10^5 cfu of H. influenzae type b intranasally*

Viral inoculation		Days after inoculation of *H. influenzae* type b						
Virus	Age	3	5	7	11	14	18	24
Sendai Virus	3 days	4.0[1]	4.0	4.1	3.9	3.0	0.2	<0.1
None (saline)	3 days	1.2	0.3	<0.1	<0.1	0.4	<0.1	<0.1
Sendai Virus	8 days	4.2				4.2		3.9	1.6
None (saline)	8 days	<0.1			<0.1	3.9 / 1.6	3.9	1.5	0.9
Rat Coronavirus	3 days	4.2	4.3	4.2	4.3		3.5	2.6
None (Saline)	3 days	<0.1	<0.1	<0.1	<0.1	3.6 / 0.6	2.0	0.4
Rat Coronavirus	14 days	4.1		4.0				
None (saline)	14 days	1.1		<0.1				

[1] Geometric mean titer, representing nasal washings from 15 rats and expressed as log 10 cfu/μl.

high doses of HIB (10^5–10^7 cfu intranasally twice daily for 2 days). A single intranasal dose of 10^5 cfu produced inconsistent results in our hands; however, this same dose of HIB given 48 h after inoculation of a respiratory virus resulted in prolonged and intense colonization. Quantitative study demonstrated HIB titers for nasal washings from virus-infected rats that were at least 100-fold higher than those for controls during the first 2 wk after HIB inculation, and as much as 10,000-fold higher during the first week. There was no coughing, sneezing or apparent rhinorrhea among the virus-infected rats, but children with these manifestations of upper respiratory infection might well be effective disseminators of HIB if they were as heavily and persistently colonized as the virus-infected animals that we studied.

Both intensity (10) and frequency (8, 13) of pharyngeal HIB colonization are increased in siblings of patients with HIB meningitis or epiglottitis. We are not aware of any published virologic study of such siblings, but it is likely that respiratory viruses circulate freely among close contacts of virus-infected patients with HIB meningitis (4, 5). Furthermore, Sell and her coworkers (12) isolated HIB and other typable *H. influenzae* much more often from children with acute respiratory illnesses than from those who were not ill. A previous Pittsburgh study showed a higher pharyngeal HIB colonization rate in children with coryza than in those who were well, although the difference was not statistically significant (9).

The mechanism responsible for the enhancing effect of viral infection on HIB colonization in infant rats is unknown, although it might involve pathogenic mediators released from virus-infected cells or virus-induced suppression of humoral or cell-mediated immune responses. There is reason to suggest that the explanation may involve a virus-induced mucosal inflammation with consequent elaboration of growth factors for HIB (11). At any rate, the demonstrated intense and prolonged nasal HIB multiplication could well be a factor in the development of meningitis and other invasive HIB disease.

Our initial investigation was concerned with a possible viral contribution to the pathogenesis of HIB meningitis rather than with the role of viruses in the communicability of HIB. We demonstrated that the intranasal dose of HIB required to produce meningitis in infant rats was significantly reduced if the animals were pre-infected with influenza A virus (7). Subsequent studies in rats by Krasinski and Nelson have shown that the respiratory syncytial virus as well as parainfluenza 1 and 2 viruses may also potentiate the development of HIB meningitis (data presented at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy in New Orleans, 1980). Continued work in our laboratory has shown that some strains of influenza B virus and the rat coronavirus may have a similar effect (6).

In summary, recent investigations have shown that various respiratory viruses may potentiate invasive HIB disease in an infant rat model. The present study has demonstrated that respiratory viruses may also greatly increase the intensity of nasal HIB colonization in rats, with possible epidemiologic as well as pathogenetic implications for human HIB infection.

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16. Requests for reprints should be addressed to: Dr. Richard H. Michaels, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania 15213.
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