

Persistence of viral RNA in 2 rat strains differing in susceptibility to postbronchiolitis airway dysfunction

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After viral bronchiolitis at an early age, a chronic asthma-like syndrome develops in BN, but not F344, rats. We hypothesized that the BN strain is less effective at clearing virus from the involved tissues. Weanling BN and F344 rats were inoculated with Sendai virus, and lung and peribronchial lymph nodes were harvested from each strain at 5 to 84 days after infection; control tissues were obtained from noninfected rats. Lung viral titers were similar for the 2 strains, with no infectious virus detectable by day 10. However, viral RNA was detected consistently by means of RT-PCR analyses in lungs and lymph nodes of both strains from days 10 to 27 and was still present at day 84 in some of the tissues from each strain. In contrast, there were strain-related differences in immune responses because IL-13 levels remained increased in the lung secretions of BN rats at 4 weeks after inoculation. Thus although Sendai virus could persist for at least 3 months after an acute infection in rats, this did not differ with strain. The persistent increase in IL-13 suggests instead that the strain-related variability in virus-associated airway pathology might be determined by the host response to infection rather than by the intensity or duration of infection. (*J Allergy Clin Immunol* 2002;110:607-9.)

Key words: Asthma, parainfluenza virus

Severe viral bronchiolitis in infancy is a risk factor for chronic airway dysfunction, but a mechanism has not been identified, and the debate continues regarding the causality of the association.¹ One potential mechanism for a chronic inflammatory process after resolution of an acute viral illness is a latent viral infection that produces a sustained immune response and amplifies the inflammatory response to environmental stimuli.²

After recovering from viral bronchiolitis at an early age, the BN rat strain has chronic airway dysfunction that resembles asthma (airway hyperresponsiveness, episodic

obstruction, bronchiolar inflammation, and remodeling).^{3,4} In contrast, F344 rats recover from bronchiolitis without sequelae,⁴ and the BN rats have attenuated sequelae when IFN- γ is administered early in the viral illness.⁵ We hypothesized that persistent or latent viral infection might contribute to the development of postbronchiolitis sequelae in BN rats and designed this study to determine whether BN and F344 strains differ in regard to persistence of viral RNA after infection with Sendai virus.

METHODS

All procedures were approved by the University of Wisconsin Animal Care and Use Committee and conform to the Guide for the Care and Use of Laboratory Animals (1996). Weanling male inbred BN and F344 rats (Charles River Laboratories, Raleigh, NC) were inoculated with aerosolized parainfluenza type 1 (Sendai) virus, as described previously.³⁻⁵ On days 5, 6, 7, 10, 13, 20, 27, and 84 after inoculation, 3 to 4 rats of each strain were killed. After aseptic thoracotomy, one lung was frozen in liquid nitrogen for viral plaque assays (days 5-10), and one lung and a peribronchial lymph node were each placed in RNAlater (Ambion, Austin, Tex) for RNA extraction (days 10-84). Tissues from noninfected rats were obtained for control studies. Care was taken to avoid cross-contamination between lungs and lymph nodes of individual animals and among tissues harvested from different animals. Bronchoalveolar lavage fluid was obtained⁶ to measure IL-13 to detect the presence of a T_H2-biased inflammatory process (rat IL-13-specific ELISA, sensitivity of 1.5 pg/mL; Biosource, Camarillo, Calif).

Plaque assays were used to quantify virus in lung tissue at postinoculation days 5, 6, 7, and 10.^{5,7} Lung samples were thawed, homogenized in cold buffer, and centrifuged to obtain supernates that were diluted serially and inoculated onto confluent monolayers of Madin-Darby bovine kidney cells. An agarose overlay containing trypsin was removed from the plate after 3 days' incubation, and the monolayers were stained with crystal violet to accentuate the plaques. Viral titers were expressed as plaque-forming units per gram of lung.

RNA was extracted from lung or peribronchial lymph node tissue by using a phenol-chloroform reagent (Tri-Reagent; Cinna/Leedo Medical Laboratories, Houston, Tex), according to the manufacturer's instructions, and resuspended in 50 μ L of water. The RNA suspension (8 μ L) was incubated (20- μ L volume at 37°C for 1 hour) with 200 U of reverse transcriptase (Superscript II; Gibco BRL, Grand Island, NY) in reaction buffer, 0.01 mol/L dithiothreitol, 40 U of recombinant Rnasin (Gibco), 0.5 μ g of random primers (Promega, Madison, Wis), and 0.5 mmol/L deoxyribonucleoside triphosphates (Promega).

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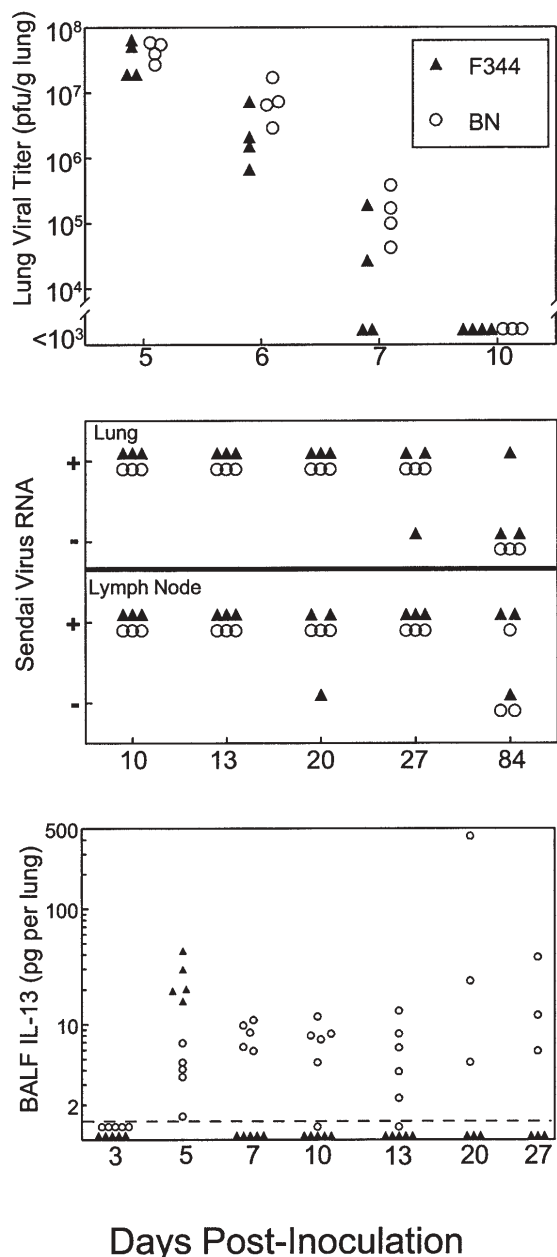


FIG 1. Lung viral titers (upper), qualitative detection of Sendai virus RNA (middle), and bronchoalveolar lavage fluid IL-13 levels (lower) at 3 to 84 days after inoculation with virus. Each symbol represents tissue or fluid from one rat. Viral RNA was present (+) or not detected (–) after 40 cycles of PCR. The dotted line on the IL-13 plot represents the lower limit of sensitivity for the assay, and the day 13 data include samples from days 13 to 14.

For PCR, the cDNA suspension was diluted 5-fold, and 4- μ L samples were transferred to 650- μ L PCR tubes along with 2 U of *Taq* DNA polymerase (Promega), reaction buffer, 6 μ L of $MgCl_2$ (25 mmol/L), and 1 μ L of each primer (20 μ mol/L) in a total reaction volume of 50 μ L. The primers (upstream, 1838-1861; downstream, 2240-2220) from within the Sendai virus polymerase gene⁸ were predicted to amplify a 0.4-kb cDNA, and glyceraldehyde-3-phosphate dehydrogenase primers (Clontech, Palo Alto, Calif) were

used as a control for RNA isolation. The PCR mixture was denatured (at 94°C for 5 minutes) and subjected to 40 cycles (Sendai virus) or 26 cycles (glyceraldehyde-3-phosphate dehydrogenase) of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 2 minutes.

Southern blots were performed with specific cDNA probes labeled with horseradish peroxidase (ECL System; Amersham, Arlington Heights, Ill). PCR products were blotted onto a nylon membrane (Nytran; S&S, Keene, NH), washed, baked at 80°C for 30 minutes, and then hybridized and developed with a chemiluminescence detection kit (ECL System, Amersham). Sendai virus cDNA from as few as 10 infected HeLa cells was detectable with this technique. Controls in each batch of samples included RNA from virus-infected HeLa cells (positive control) and a sample of buffer alone (negative control).

RESULTS

Peak viral titers at postinoculation day 5 were similar for the BN and F344 strains, averaging 4×10^7 plaque-forming units per gram of lung (Fig 1). During the rapid clearance of virus on days 6 and 7, the BN rats tended to lag slightly behind the F344 rats, but viral titers were not significantly different between strains on either day, and by day 10, no virus was detectable on plaque assay from lungs of either strain (Fig 1, upper). Lungs and peribronchial lymph nodes from both strains were consistently positive for Sendai virus RNA in the first month after inoculation: all the rats had detectable viral RNA in at least one of the tissue samples, and 22 of 24 had both lung and lymph node RNA (Figs 1 and 2). However, only the BN rats exhibited persistent IL-13 in bronchoalveolar lavage fluid after the first week (Fig 1), indicating a difference between the strains with regard to pulmonary inflammatory cell populations after resolution of the acute illness. At 12 weeks after inoculation, 1 of 3 BN rats and 2 of 3 F344 rats still had at least one tissue with positive results for viral RNA (Figs 1 and 2). Uninfected rats had no detectable Sendai virus RNA (Fig 2, lane 27).

DISCUSSION

The results of the study indicate that Sendai viral RNA persists in lung and peribronchial lymph node tissue for weeks after replicating virus is no longer detected from lung homogenates. The postbronchiolitis abnormalities in airway morphology and physiology are present in BN rats by 4 weeks after inoculation,^{4,5} so that the time course of development of sequelae coincides with the period of latent viral infection. However, latent virus infection cannot be implicated as the sole cause of postbronchiolitis airway dysfunction, in that the F344 rats, which are not susceptible to postbronchiolitis sequelae,⁴ had persistence of Sendai virus RNA similar to that of the BN rats.

Intact, but not UV-inactivated, Sendai virus can persist in nerve tissue for at least 6 months after nasal infection.⁹ Persistence requires that a virus can maintain a functional genome, limit cytolysis, and avoid removal by means of innate and adaptive immune mechanisms¹⁰; however, the specific mechanisms for persistent Sendai virus infection are unknown.

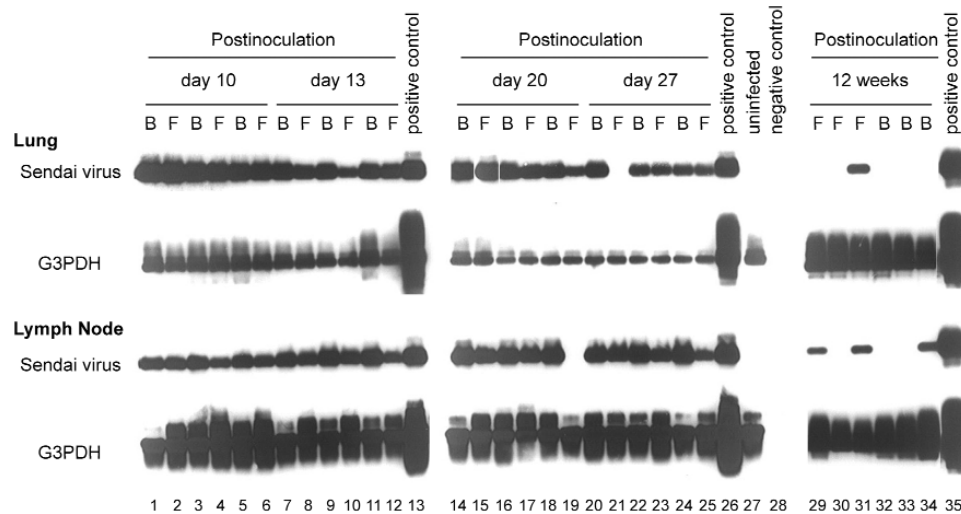


FIG 2. Southern blots of Sendai virus- and glyceraldehyde-3-phosphate dehydrogenase-specific PCR fragments obtained by means of RT-PCR analysis of lung tissue (A) and peribronchial lymph node tissue (B) RNA of individual BN (B) and F344 (F) rats. Positive control, RNA from Sendai virus-infected HeLa cells; negative control, primers alone (no cDNA).

Young BN rats respond differently than do F344 rats to acute Sendai viral infection, as indicated by lower IFN- γ levels in bronchoalveolar lavage fluid⁶ and increased expression of transforming growth factor β_1 .⁴ The current study revealed that IL-13 also is elevated persistently in the lungs of BN rats, suggesting a prolonged T_H2 or T_C2 inflammatory response after bronchiolitis. The importance of the initial immune response to the development of airway dysfunction was illustrated by the attenuation of postbronchiolitis sequelae in BN rats that received aerosolized IFN- γ during the acute viral illness, despite the treatment having no effect on the intensity of the acute illness.⁵ These observations support the hypothesis that differences in host response to infection, rather than quantitative differences in the infection itself, might determine whether postbronchiolitis sequelae develop.

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