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Antibody Responses After Sendai Virus Infection and Their Role in Upper and Lower Respiratory Tract Disease in Rats

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Background and Purpose: **Sendai virus infection in rats is an excellent model for studying development and role of host defenses throughout the respiratory tract after this infection. Therefore, development of serum antibody responses and disease were studied.**

Methods: **Forty-two anesthetized pathogen-free 3- to 4- week-old LEW/NCr rats were inoculated intranasally with Sendai virus. At postinoculation days 0, 2, 3, 5, 8, 10, and 14, rats were euthanized by administration of a pentobarbital sodium overdose followed by exsanguination. Serum was obtained from all animals, and nasal wash and bronchoalveolar lavage specimens were collected during selected experiments. An ELISPOT assay was used to measure numbers of Sendai virus-specific antibody-forming cells in respiratory tract lymphoid tissue.**

Results: **Recovery from disease and clearance of virus from respiratory tract tissues coincided with development of serum antibody responses. Upper respiratory tract lymph nodes were the initial and major sites of appearance of antibody-forming cells. Immunoglobulin G was the predominant subtype of these cells during recovery from the infection and in rats resistant to infection. Passive transfer of antisera or specific IgG protected the lower but not the upper respiratory tract.**

Conclusions: **Circulating components of immunity have a major role in resistance and recovery from disease in the lower respiratory tract, whereas local responses are likely involved in protection of the upper respiratory tract. Local lymphoid tissues are the major production sites of IgG, which contributes to resistance to and recovery from respiratory tract diseases.**

Development of adaptive immune responses is important in recovery from viral respiratory tract diseases, and an understanding of the mechanisms involved in resistance to infection and disease will help in developing more effective vaccines against lung disease. The role of humoral immunity against acute respiratory tract infection has been intensively investigated. On the basis of results of our previous studies (1, 2), it is likely that the development of antibody responses after infection will vary along the respiratory tract. Most studies have examined the presence of specific antibody in serum or secretions of the upper respiratory tract or lungs, or the distribution of plasma cells, without regard to antigen specificity (3–10). Although our previous studies (1, 2) examined the appearance of antibody-forming cells (AFC) in upper and lower respiratory tissues in rats with chronic mycoplasmal infection, little is known about different isotypes of specific AFC in these lymphoid tissues after acute respiratory tract infection and their contribution to prevention of or recovery from disease.

Sendai virus, paramyxovirus type 1, is a natural respiratory tract pathogen in rats (10, 11). The disease is most severe 4 to 5 days after infection and resolves within 2 weeks (12). Similar to many respiratory tract infections including influenza, Sendai virus disease involves all tissues of the respiratory tract including nasal turbinates, trachea, and lungs. Thus, Sendai virus infection in rats is an excellent model for studying the development and role of host defenses throughout the respiratory tract (11).

The purpose of the study reported here was to characterize the development and potential role of antibody responses against Sendai virus in infected rats. Although a role for humoral immunity has been documented in Sendai disease in mice (13), little is known about the development of antibody responses in respiratory tract lymphoid tissues after infection in mice or rats. We found that the development of serum antibody responses corresponded with recovery from Sendai virus disease and infection and major sites of production of antibody-forming cells. Antibody-forming cells of the IgG class were predominant during recovery from disease, and they remained the major isotype in animals, which were resistant to re-infection by Sendai virus. Passive intravenous transfer of antisera and immune IgG protected the lower respiratory tract from disease but did not provide any protection from development of infection or disease in the upper

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respiratory tract. Thus, components of immunity found in the circulation play a major role in resistance and recovery from disease in the lower respiratory tract, while local immune responses are likely involved in the protection of the upper respiratory tract. In addition, local lymphoid tissues are the major site of IgG production after viral infection, and IgG contributes to resistance to (and probably recovery from) lung disease.

Materials and Methods

Animals: Pathogen-free, 3- to 4-week-old LEW/NCr rats (n = 42) were obtained from the Frederick National Cancer Research and Development Facility, National Cancer Institute, Frederick, Md. Male rats were used in all studies, except that pooled immune sera for passive immunization studies were obtained from female rats. The parent colony was periodically monitored for mycoplasmal, viral, and bacterial pathogens by use of serologic testing and culturing techniques, and was consistently test negative for all pathogens for more than 5 years. Serum samples from four rats 3 weeks after arrival were tested by use of a commercial enzyme-linked immunosorbent assay (ELISA) for antibody to Sendai virus, pneumonia virus of mice, coronavirus (sialodacryonadenitis virus or rat coronavirus), Kilham rat virus, H-1 virus, reovirus type-3, mouse adenovirus, and *Mycoplasma pulmonis* (Charles River Professional Services, Wilmington, Mass.). All results were negative except those for inoculated rats, which had positive results for the inoculated virus only.

Rats were intranasally inoculated with 50 µl of Sendai virus after anesthesia was induced with a combination of ketamine hydrochloride (100 mg/ml; Warner-Lambert Co., Morris Plains, N.J.) and xylazine (100 mg/ml; Mobay Corp., Shawnee, Kans.). At 0, 2, 3, 5, 8, 10, and 14 days after inoculation, rats were euthanized by administration of an overdose of pentobarbital sodium followed by exsanguination. Serum was obtained from all animals. In selected experiments, nasal wash and bronchoalveolar lavage specimens were collected as described (14).

The animal care and use program at the University of Alabama at Birmingham is accredited by AAALAC, International. Rats were housed in microisolators (Lab Products, Inc., Mayberry, N.J.), on autoclaved hardwood bedding (Betachip; Northeastern Products Corp., Warrensburg, N.Y.), and were provided autoclaved water and food. Animal rooms had 15 air changes/h and were maintained at 23 ± 2°C and a 12/12-h light/dark cycle. The Institutional Animal Care and Use Committee of the University of Alabama at Birmingham approved all animal procedures.

Virus preparation: Sendai virus (paramyxovirus type 1, strain Sendai/52) was obtained from the American Type Culture Collection, Rockville, Md. As described (12), Sendai virus was passed four times in BHK-21 cells (American Type Culture Collection) and six times in BALB/c mice to increase its pathogenicity in animals; it was then grown once more in BHK-21 cells, which were incubated in McCoy 5A medium (modified; Sigma Chemical Co., St. Louis, Mo.) with 2 mM glutamine, 10 mM HEPES buffer, 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, Utah), and 1% of an antibiotic-antimycotic mixture containing penicillin, streptomycin, and Amphotericin B (Grand Island Biological Co. [GIBCO], Grand Island, N.Y.). Cells were negative for viral and mycoplasmal contamination on the basis of culture results, for fluorochrome staining (15), and by use of a genetic probe (Mycoplasma T. C. Rapid Detection System; Gen-Probe Inc., San Diego, Calif.). The virus was negative for mycoplasmal contamination by results of genetic probe testing. Two virus stocks, identically produced, were used in these studies. For the initial experiments, a virus stock containing $10^{6.0}$ median tissue culture infective doses ($TCID_{50}$)/ml was used, whereas later studies involved use of a Sendai virus stock containing 10^4 $TCID_{50}$/ml. Both stocks were frozen at -70°C, and both were pathogenic to rats and mice.

Sendai virus titration: Nasal turbinates, trachea, and lungs were collected and homogenized in minimal essential medium (MEM; GIBCO) supplemented with 10% FBS, using Ten Broeck tissue grinders, and were frozen at -70°C until needed. For determination of virus titer, frozen homogenates were thawed and sonicated for 30 sec. Serial tenfold dilutions of tissue suspensions were inoculated in triplicate onto BHK-21 cells in 24-well culture plates. Four days after inoculation, cells were inspected for cytopathic effect (syncytia), and viral titer was determined by hemadsorption with 0.3% guinea pig red blood cells as described (12). Virus titer was calculated according to the method of Reed and Muench (16) and was expressed as $TCID_{50}$/g of tissue.

Histologic examination: Tracheas and lungs were collected and fixed in buffered alcoholic formalin (10% formalin in 80% ethanol) for histologic sectioning. Heads were fixed in buffered 10% formalin, then were de-calcified. Sections of each nose, trachea, and lung lobe were scored subjectively for the characteristic lesions of Sendai virus as described (12). Lesions in nasal turbinates and trachea were scored 0 for normal; 1 for necrotizing lesions involving <10% of the luminal epithelium with small numbers of submucosal lymphocytes and submucosal, intraepithelial, or luminal neutrophils; 2 for necrosis of 10 to 30% of the luminal epithelium with moderate numbers of neutrophils and lymphocytes; and 3 for necrosis of >30% of the luminal epithelium with large numbers of lymphocytes and neutrophils. Lesions in each lung lobe were evaluated separately. Airway lesions were scored similarly to those of the upper respiratory tract, except that peribronchial lymphoid cell accumulation also was included, with a score of 1 for sparse, multifocal collections adjacent to bronchi or bronchioles; 2 for multifocal coalescing accumulations of moderate numbers of cells; and 3 for extensive accumulations along bronchi and complete cuffs several cells thick around many bronchioles. The pulmonary parenchyma was scored 0 for normal; 1 for a few foci of alveolitis <25 µm in diameter; 2 for one or two foci of alveolitis >25 µm in diameter or many smaller foci; and 3 for three or more foci >25 µm in diameter or extensive areas of alveolitis. All lung lobes were examined microscopically, and for each lung lobe, the score was summed and weighted according to its relative contribution to total lung weight. The weighing factors were 3:2:1:1:1 for left, right caudal, right middle, right cranial, and accessory) lobes, respectively. Lesion indices were calculated by summation of the score for each individual lobe and division of the sum by the maximal

score possible. A lesion index of 1 was the most severe change possible. Slides were coded so that the examiners did not know the experimental group.

Serologic testing: Virus-specific IgG, IgM, and IgA antibodies were quantified in sera, and nasal wash and bronchoalveolar lavage specimens, using a modification of a described ELISA (17). Briefly, serial dilutions of each serum were added in triplicate to microtitration wells coated with Sendai virus antigens (Charles River Professional Service). After overnight incubation, the unbound antibody was removed by washing the wells with phosphate-buffered saline (PBS) containing 0.2% Tween 20 (Sigma Chemical Co.). Alkaline phosphatase-labeled, goat anti-rat secondary antibodies (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md., and Cappel, Malvern, Pa.) were then added to each of the wells at a dilution documented to have minimal nonspecific binding to antigen-coated wells. After 5 h incubation, the plates were washed, substrate (*p*-nitrophenyl phosphate, Sigma Chemical Co.) was added, and the plates were incubated for 60 min at 37°C. Absorbence at 405 nm was read, using a Titertek Multiskan ELISA reader (Flow Laboratories, Inc., Mclean, Va.), and relative antibody activities were determined, using ELISANALYSIS Program V 5.01 (18). In all assays, a serum sample from a rat that recovered from Sendai virus disease was used as the standard in the ELISA throughout these studies. This standard serum was given an arbitrary activity of 1,000, and for each assay, a curve was obtained from serially diluted standard serum. Each of the sample dilutions was compared with the standard curve, and values located on the linear portion of the standard curve were multiplied by their dilution factor, averaged, and reported as the relative titer of that sample (17).

For lung and nasal passage lavage specimens, presence of antibody was determined, using the previously described ELISA, except that lavage specimens were not serially diluted, and OD values, not titers, were used for comparative purposes.

Cell isolation: Mononuclear cells were collected from lungs, draining lymph nodes, and spleen of rats inoculated with Sendai virus 14 days earlier and allowed to recover, as described (1, 2). Briefly, lung and spleen lymphocytes were isolated by use of a Ficoll-sodium diatrizoate (LymphoPaque; Pharmacia, Piscataway, N.J.) density gradient after mechanical desegregation of tissue. Upper and lower respiratory tract lymph nodes (URN and LRN, respectively) were mechanically desegregated. The URN comprised the deep cervical and retropharyngeal lymph nodes, whereas LRN were the tracheobronchial lymph nodes. All cells were washed, using RPMI-1640 medium (GIBCO) supplemented with 10 mM HEPES buffer and 5% FBS.

Determination of antibody-forming cells (AFC): The ELISPOT assay for Sendai virus AFC in lymphoid tissues was based on the method described by Czerkinsky et al. (19), as modified by Simecka et al. (1, 17). Sendai virus antigen was prepared as described (20). Briefly, Sendai virus was grown in chicken eggs, collected from allantoic fluid, and centrifuged at 500 X g for 20 min to remove red blood cells. The supernatant was centrifuged for 60 min at 65,000 X g (SW-28 rotor; Beckman Instruments, Fullerton, Calif.) at 4°C. The pellet was mixed with a small volume of sterile Hanks' balanced saline solution and sonicated for 20 sec to disrupt the pellet. After sonication, the virus pellet was purified by centrifugation in a linear sucrose gradient (20 to 60%) for 2 h at 65,000 X g (SW-28 rotor; Beckman Instruments) at 4°C. The visible virus band was collected and dialyzed in PBS overnight at 4°C. After dialysis, the virus was UV-light inactivated. Inactivation of virus was confirmed by use of hemadsorption with guinea pig blood after inoculation of BHK-21 cells with the antigen preparation. The protein concentration of the inactivated virus was determined by use of the micromethod of Bradford (Bio-Rad Laboratories, Richmond, Calif.) (21).

For the ELISPOT assay, Sendai virus antigen was used at an optimal concentration of 3 μg of protein/well. Nonspecific binding was minimized by use of cell culture medium (RPMI-1640 medium, 10 mM HEPES buffer, L-glutamine, 0.2% FBS) to block the plate. After washing with PBS/5% Tween 20, endogenous peroxidase activity was removed by incubating the plates with 0.5% H_2O_2. Mouse monoclonal anti-rat IgA (MARA-2; Bioproducts for Science, Inc./Serotec, Indianapolis, Ind.), anti-rat IgM (MARM-4), horseradish peroxidase (HRP)-labeled goat anti-mouse IgG, and anti-rat IgG (Kirkegaard & Perry Laboratories, Inc.) were used to reveal antibody reactions. Monoclonal antibodies were reacted with HRP-labeled goat anti-mouse antibody, and HRP substrate (25 mg/97 ml of 3-amino-9-ethyl carbazole; AEC, Sigma Chemical Co., in 0.05 M sodium acetate buffer, pH 5.0, plus 0.04% H_2O_2) was used to detect antibodies (1). The spots representing AFC were counted, using a dissecting stereomicroscope illuminated indirectly by use of a high-intensity lamp. The cell concentrations yielding about 30 to 50 spots/well were counted and averaged. Values were expressed as the absolute number of AFC in each tissue or AFC/10^6 lymphocytes; the numbers of AFC were corrected for the percentage of lymphocytes in the initial cell population.

Antisera and IgG purification: Immune sera were obtained from rats that had recovered from Sendai virus infection. Eight-week-old female LEW rats were intranasally inoculated with 5 x $10^{2.0}$ $TCID_{50}$ of live virus. Antisera were obtained from rats 14 days after inoculation. Normal sera were obtained from uninfected animals. Immune and normal sera were separately pooled, aliquoted, and frozen. Immune sera had virus-specific antibody titers of 2,400 for IgG, 1,200 for IgA, and 300 for IgM.

To purify serum IgG, we used the nonchromatographic procedure described by McKinney and Parkinson (22). Briefly, antisera obtained from convalescent rats (immune) and normal LEW rat serum were treated with caprylic acid (Sigma Chemical Co.) to precipitate non-IgG and albumin. The IgG fraction in the supernatant was precipitated with 50% ammonium sulfate (Sigma Chemical Co.). After dialysis with PBS overnight at 4°C, the dialyzed IgG fraction was collected.

Protein concentrations of immune or normal IgG were determined by use of the micromethod described by Bradford (21) (Bio-Rad Laboratories). The purity of IgG was confirmed by the Ouchterlony double immunodiffusion method (23), using (AffiniPure) goat anti-rat IgG (γ chain specific; Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.), goat anti-rat IgM (μ chain specific; Jackson ImmunoResearch Labo-

ratories, Inc.), and goat anti-rat IgA (α chain specific; Sigma Chemical Co.). Relative antibody titer was determined, using the ELISA described previously.

Statistical analysis: Statistical analysis was performed, using the SYSTAT program (Systat, Inc., Evanston, Ill.). Antibody and virus titers and the numbers of AFC were transformed to common logarithms prior to statistical analysis, and lesion indices were transformed to arcsines. The transformed data were analyzed by use of analysis of variance (ANOVA) followed by post-hoc tests for multigroup comparisons, as needed (24). A probability (P) of 0.05 or less was accepted as significant.

Results

Antibody responses in rats after Sendai virus infection: To compare serum antibody responses with lesion severity and virus titers, rats were intranasally inoculated with 5 x $10^{4.0}$ $TCID_{50}$ of Sendai virus and were sacrificed 2, 3, 5, 8, 10, or 14 days after inoculation. At each time point, lesions in the lungs, virus titers in lungs and nasal turbinates, and antibody titers in serum were determined. After infection, airway and parenchymal lesions were first detected on day 2 (Figure 1A). Lesion severity peaked on day 5 and rapidly resolved thereafter. In nasal turbinates, trachea, and lungs, virus was first detected 2 days after inoculation and remained high in all three tissues until day 8 (Figure 1B). Anti-Sendai virus IgG and IgM were detected in serum by day 5 after inoculation. The IgM concentration did not significantly increase after postinoculation day 5. However, serum IgA and IgG titers against Sendai virus increased markedly between 8 and 10 days after inoculation (Figure 1C).

In addition to presence in serum, antibody was determined in lungs and nasal passage lavage specimens from Sendai virus-infected rats (Table 1). High titer of anti-Sendai virus IgG was present in nasal and bronchoalveolar lavage specimens from infected rats 10 and 14 days after inoculation. The IgA antibodies were detected earlier in nasal passage lavage (10 days) than lung lavage (14 days) specimens. Very low concentration of IgM was detected in nasal passage lavage specimens 10 and 14 days after inoculation, whereas in lung lavage specimens, significant titer of Sendai virus-specific IgM was not detectable until day 14.

Development and distribution of Sendai virus-specific AFC: To characterize the development and tissue distribution of antibody responses in the rats after virus inoculation, we determined the numbers of Sendai virus-specific AFC in the URN and LRN and the systemic (spleen) lymphoid tissues. At 5, 8, 11, and 14 days after inoculation, cells from each of the tissues were collected, and the numbers of Sendai virus-specific IgM, IgG, and IgA AFC were determined (Figure 2). At 5 days after inoculation, AFC were detected principally in URN, whereas AFC in LRN or spleen were not detected until 8 or 14 days after inoculation. The numbers of IgG and IgA AFC were consistently higher in URN than in LRN or spleen, whereas IgM AFC numbers in URN were greater only on day 5 ($P < 0.001$).

Antibody responses of each isotype developed after inoculation. In the URN, IgM, IgG, and IgA, responses were detected at 5 days after inoculation (Figure 3). Beginning at 8 days after

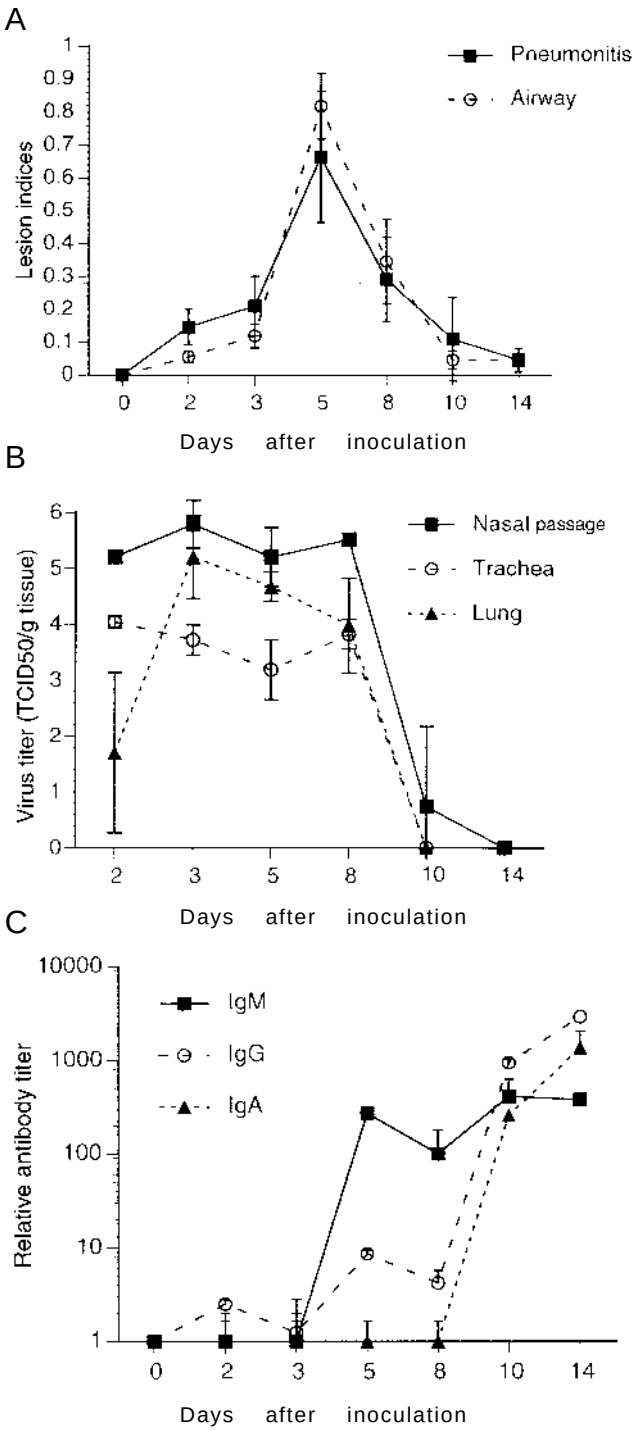


Figure 1. Viral disease and serum antibody responses after intranasal inoculation of LEW rats with Sendai virus. **(A)** Development of lung parenchyma and airway lesions; **(B)** recovery of Sendai virus from respiratory tissues (nasal passage, trachea, and lungs) and expressed as median tissue culture infective dose ($TCID_{50}$) per gram of tissue; and **(C)** serum antibody titers (IgM, IgG, and IgA). Each point represents the mean and SD (n = 3) lesion index at each time point.

inoculation, the contribution to the total antibody response of IgG was significantly greater than that of IgM and IgA in all

Table 1. Sendai virus-specific antibody in lavage specimens from the upper respiratory tract and lungs of infected rats

Lavage	Isotype	Time after inoculation (days)[a] 0	3	5	8	10	14
Nasal	IgG	0.002 (0.003)	0.001 (0)	0.024 (0.011)	0.007 (0.007)	0.984 (0.657)[b]	1.315 (0.394)[b]
	IgM	0.012 (0.001)	0.005 (0.006)	0.007 (0.008)	0.001 (0.001)	0.040 (0.012)[b]	0.063 (0.024)[b]
	IgA	0.014 (0.002)	0.008 (0.001)	0.016 (0.012)	0.007 (0.003)	0.147 (0.024)[b]	0.222 (0.072)[b]
Lung	IgG	0.005 (0.001)	0.004 (0.001)	0.035 (0.035)	0.093 (0.150)	0.282 (0.267)[b]	1.756 (0.423)[b]
	IgM	0.007 (0.008)	0.004 (0.006)	0.019 (0.013)	0.016 (0.006)	0.007 (0.003)	0.110 (0.082)[b]
	IgA	0.008 (0.005)	0.007 (0.010)	0.007 (0.005)	0.011 (0.001)	0.030 (0.036)	0.134 (0.007)[b]

[a]The ELISA antibody data are presented as the mean OD_{405nm} (SD) for three lavage specimens per time point.
[b]Denotes values that are significantly ($P < 0.05$) different from values obtained for control (uninfected) rats.

the tissues examined ($P < 0.001$). The IgM AFC were present at an early stage but became minimal in number or absent in local (URN and LRN) and systemic tissues. Although IgA AFC were detected during the entire study, their numbers were significantly lower than those of IgG AFC in each of the tissues ($P < 0.001$). Thus, IgG was the predominant isotype in each of the tissues examined after Sendai virus inoculation.

Antibody responses in rats that are resistant to re-infection: Rats that recovered from infection were resistant to re-infection with Sendai virus. The LEW rats were intranasally inoculated with 5 x $10^{2.0}$ $TCID_{50}$ of Sendai virus. Fourteen days later, the rats were given the same dosage of virus via the intranasal route. After an additional 5 days, nasal turbinates and lungs were collected for histologic examination and determination of virus titer. Airway and parenchymal lesions and rhinitis were less severe in rats that had recovered from previous virus infection than in rats that had not previously been exposed to Sendai virus ($P < 0.001$) (Figure 4A). Virus was not detected in the upper or lower respiratory tract of re-infected rats, whereas virus was recovered from both locations in immune naive rats inoculated with the virus ($P < 0.001$) (data not shown). Thus, rats that had recently recovered from Sendai virus disease were resistant to re-infection and developed milder or no disease throughout the respiratory tract.

We also examined antibody responses in immune rats after re-infection. Rats were inoculated with Sendai virus and allowed to recover from disease. Fourteen days after inoculation, these rats were intranasally inoculated with Sendai virus, and 5 days later, the numbers of IgM, IgG, and IgA AFC in cells from URN, LRN, and the spleen were determined. Simultaneously, we determined the numbers of AFC in these tissues from an additional group of rats inoculated 19 days previously. Similar to our previous experiments, virus was not recovered from lungs or nasal turbinates of animals that were re-infected (data not shown). The number of IgG AFC was significantly greater than that of the other isotypes in rats 19 days after inoculation ($P < 0.001$) and in rats that were re-infected with virus ($P < 0.009$) (Figure 4B). Few IgM AFC were detected in these rats. There was no significant difference in the responses between rats re-infected with virus and those animals inoculated 19 days previously. Thus, IgG re-

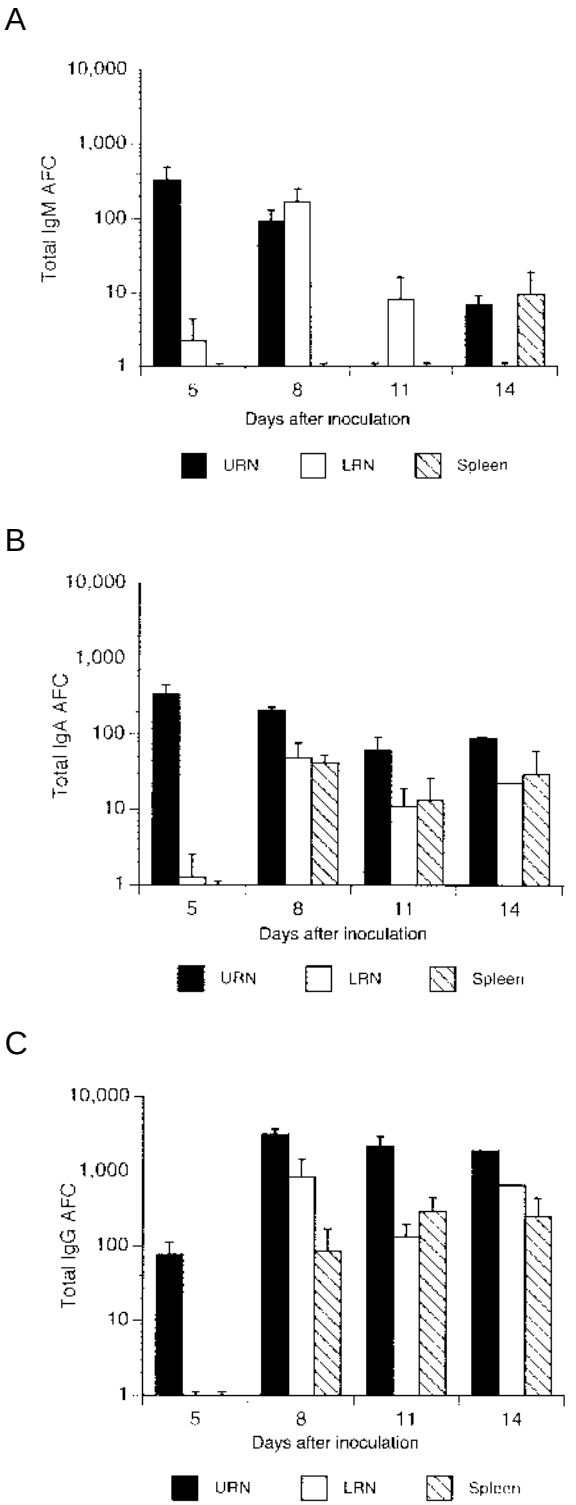


Figure 2. Tissue distribution of the developing virus-specific antibody responses. Antibody-forming cells (AFC) in upper respiratory tract lymph nodes (URN), lower respiratory tract lymph nodes (LRN), and spleen were determined. Assays were done on days 5, 8, 11, and 14 after inoculation. The URN were the initial and major site for production of **(A)** IgM-, **(B)** IgA-, and **(C)** IgG-forming cells. The data are expressed as mean ± SD (n = 6) total number of AFC recovered from each tissue.

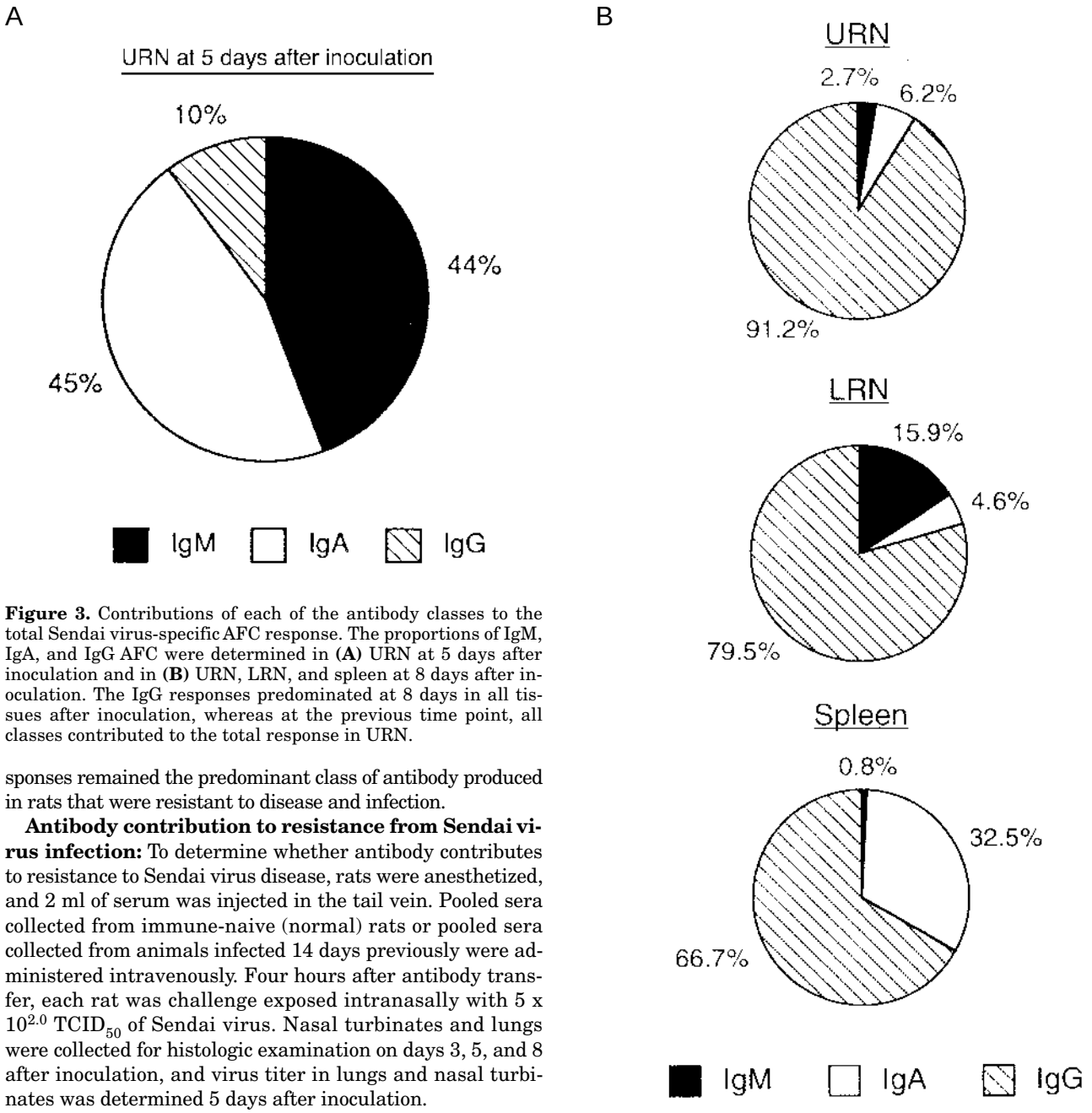


Figure 3. Contributions of each of the antibody classes to the total Sendai virus-specific AFC response. The proportions of IgM, IgA, and IgG AFC were determined in **(A)** URN at 5 days after inoculation and in **(B)** URN, LRN, and spleen at 8 days after inoculation. The IgG responses predominated at 8 days in all tissues after inoculation, whereas at the previous time point, all classes contributed to the total response in URN.

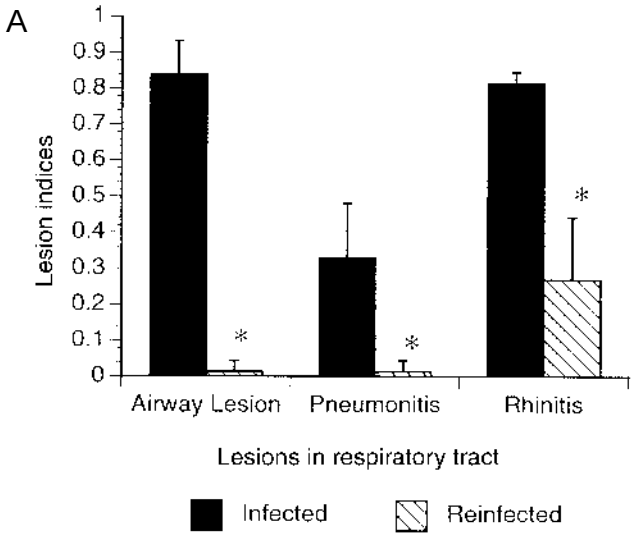
sponses remained the predominant class of antibody produced in rats that were resistant to disease and infection.

Antibody contribution to resistance from Sendai virus infection: To determine whether antibody contributes to resistance to Sendai virus disease, rats were anesthetized, and 2 ml of serum was injected in the tail vein. Pooled sera collected from immune-naive (normal) rats or pooled sera collected from animals infected 14 days previously were administered intravenously. Four hours after antibody transfer, each rat was challenge exposed intranasally with 5 x $10^{2.0}$ $TCID_{50}$ of Sendai virus. Nasal turbinates and lungs were collected for histologic examination on days 3, 5, and 8 after inoculation, and virus titer in lungs and nasal turbinates was determined 5 days after inoculation.

Intravenous administration of antisera protected the rats against pneumonitis ($P < 0.001$), airway lesions in the lungs ($P < 0.001$), and tracheitis ($P < 0.001$), compared with rats given sera from immune-naive rats (Figure 5). However, there was no significant decrease in severity of the lesions in the nasal turbinates when rats were given immune sera intravenously. There was no significant difference in lesion severity between untreated rats and those given normal sera intravenously (data not shown).

Virus titer was consistent with the distribution of lesions in both experimental groups. Virus titer also were significantly lower in the lungs of rats given antisera intravenously ($P < 0.001$) (Table 2). There was no significant decrease in the severity of lesions or virus titer in the nasal turbinates when rats were given immune sera intravenously. However, virus was not detected in nasal turbinates or lungs from rats inoculated 5 days previously with virus pre-incubated with immune sera ($P < 0.001$), whereas virus was recovered from rats given virus pre-incubated with normal sera. Although these experiments suggest that virus-specific antibodies in immune sera were capable of protecting against infection, there was no significant decrease in virus titer in the nasal turbinates of rats who were given virus-specific immune sera intravenously.

Effect of passive transfer of Sendai virus-specific IgG on development of lung disease: To further examine the role of IgG in disease, we examined the ability of Sendai



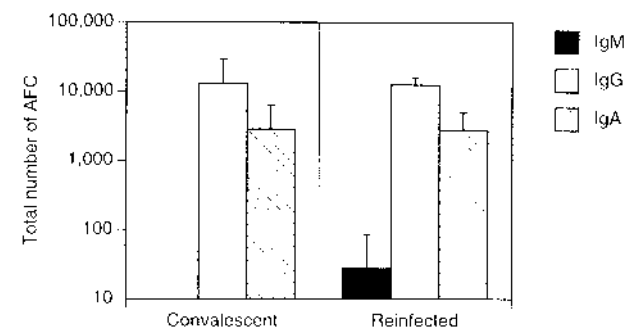


Figure 4. Resistance of rats to re-infection and their virus-specific antibody responses. **(A)** Rats that had recently recovered from Sendai virus infection (14 days after primary infection) were re-infected and developed less severe lesions than did rats undergoing primary infection. Bars represent mean and SD (n = 6) lesion indices at 5 days after re-infection/infection. Statistical significance, compared with rats having a primary infection, is denoted by an asterisk. **(B)** Numbers of IgM, IgG, and IgA AFC in the URN at 5 days after re-infection (as above) and in convalescent rats (19 days after primary infection). Similar results were found in the LRN and spleen. The IgM AFC were not detected in convalescent rats, and numbers of IgG AFC were significantly greater than those of IgA AFC ($P < 0.05$). The bars represent mean and SD (n = 6).

virus-specific IgG to confer resistance to disease. Sendai virus-specific antiserum or Sendai virus-specific IgG was administered intravenously to rats. For comparison, control rats were given serum from immune-naive rats or IgG. There were four experimental groups: rats given 2 ml of Sendai virus-specific antiserum; rats given purified anti-Sendai virus IgG containing the same amount of specific IgG ELISA activity (2,400 relative titer) as that given to the first group; rats given 2 ml of immune-naive (normal) rat serum; and rats given the same amount of normal rat serum IgG on the basis of amount of protein (3.5 mg) as that of rats given anti-Sendai virus IgG. Four hours after antibody treatment, each rat was challenge exposed intranasally with 5 x $10^{2.0}$ $TCID_{50}$ of Sendai virus. Nasal turbinates and lungs were

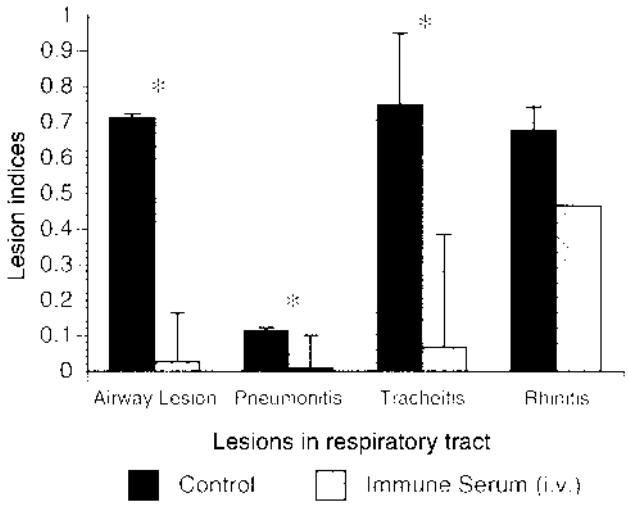


Figure 5. Severity of respiratory tract lesions in Sendai virus-infected rats after passive transfer of antibody prior to Sendai virus inoculation. Bars represent mean and SD (n = 6) of the lesion indices. Rats given Sendai virus-specific serum antibody (cross-hatched bars) intravenously were resistant to tracheitis, lung airway lesions, and pneumonitis ($P < 0.001$), but there was no protection against rhinitis attributable to the intravenously administered antibody. Statistical significance, compared with control rats (filled bars), is denoted by asterisk.

Table 2. Virus titers in nasal wash specimens and lungs of Sendai virus-infected rats administered virus-specific immune serum or negative control serum intravenously

Variable serum	Virus titers ($TCID_{50}$/g tissue)	
	Nasal turbinates	Lungs
Negative-control serum	2.95 x 10^5 (3.8)[a]	2.95 x 10^5 (3.8)
Sendai virus-specific antiserum	6.3 x 10^4 (10)	12.6 (81)[b]

[a]Geometric mean (mean ÷ SD) virus titer.
[b]Significantly ($P < 0.001$) less virus recovered from rats given negative-control (from immune-naive rats) serum intravenously (n = 3 for each group).

collected for histologic examination 5 days after infection. As indicated (Figure 6), passive transfer of anti-Sendai virus IgG resulted in significant reduction in lesion severity in lung airways and parenchyma ($P < 0.05$). The amount of protection was similar to that observed for unfractionated antiserum. Consistent with previous experiments, there was no effect attributable to passive transfer of virus-specific antisera or virus-specific IgG on rhinitis.

Discussion

In the study reported here, we found that the draining lymph nodes of the upper respiratory tract were the initial and major site of antibody production after virus infection. This is similar to results of our earlier studies describing development of antibody responses to another respiratory tract pathogen, *M. pulmonis* (1, 2). Disease in rats caused by Sendai virus and *M. pulmonis* begins in the nasal turbinates and spreads downward into the lower respiratory tract (25). This might explain why URN are the first lymphoid tissues to respond to respiratory tract pathogens. On the basis of the time course of the antibody responses at these different

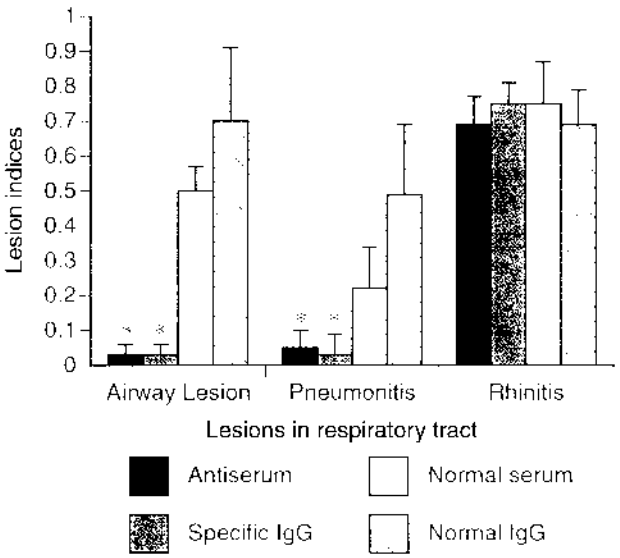


Figure 6. Effect of Sendai-specific IgG on respiratory tract lesions. The LEW rats were given 2 ml of convalescent antiserum, 2 ml of normal rat serum, 2 ml of Sendai virus-specific IgG (3.5 mg), or 2.5 normal rat serum IgG (3.5 mg) intravenously, then were challenge exposed 4 h later. Specific IgG provided the same degree of protection in the lungs as did antiserum. Protection in the nose was not conferred by antiserum or antiserum IgG. The data are expressed as the arithmetic mean ± SD of lesion severity. Statistical significance ($P < 0.05$), compared with control rats (hatched and open bars), is denoted by asterisk.

sites (i.e., URN first, followed by LRN and spleen), it is likely that immune cells (primed B cells or plasma cells) and/or regulatory cells are stimulated in the upper respiratory tract. Lymphocytes from this site seed other lymphoid tissues of the respiratory tract and peripheral tissues via the circulation. In addition, the high numbers of AFC in the URN and LRN, instead of spleen, suggest that humoral immunity is more dependent on regional or localized responses along the respiratory tract.

The corresponding decrease in virus titer and disease association with production of anti-Sendai virus antibody suggests that humoral immunity is involved in clearance of virus and resolution of disease. Lung disease peaked about 5 days after inoculation, and recovery was almost complete by 10 days after inoculation. Virus titer plateaued between 3 and 8 days after inoculation, but clearance of virus by 10 days coincided with resolution of disease. Sendai virus-specific IgM appeared in serum by 5 days after inoculation and did not substantially increase at later time points. In contrast, IgG and IgA responses to Sendai virus increased markedly between 8 and 10 days in nasal passage and lung lavage specimens as well as in serum. Thus, development of local and serum IgG and IgA responses to Sendai virus coincides with recovery from disease and decrease in virus titer. Although this suggests that antibody plays an appreciable role in viral clearance, innate and other adaptive immune mechanisms, such as interferons, inflammation, and cytotoxic T cells (26–28), most likely also are contributing to this process.

Immunoglobulin G was the major antibody class produced during recovery from Sendai virus respiratory tract disease. At 5 days after inoculation, the total antibody response was attributable to approximately equal proportions of IgM, IgA, and IgG AFC, but by 8 days, there was a marked shift toward principally an IgG response in each of the tissues examined. The predominance of Sendai virus-specific IgG AFC coincided with recovery from infection and disease. Furthermore, IgG remained the major class of AFC in rats that recently recovered from disease and were resistant to re-infection, although other innate or adaptive immune mechanisms most likely also play a role in protection. However, we found that passive transfer of virus-specific IgG provided protection from lung disease that was similar to that provided by use of unfractionated antisera. Thus, these results indicate the importance of specific IgG responses in recovery and protection from viral lung disease, a finding consistent with previous studies of respiratory syncytial virus infection in cotton rats (29).

In contrast to lung disease, the upper respiratory tract was not protected from infection or disease after intravenous administration of virus-specific antisera or specific IgG. In similar studies in mice, specific antibody in serum did not diminish Sendai virus replication in nasal turbinates, although it suppressed virus replication and lesions in the lung (13). These results probably reflect inability of antibody in the circulation to readily enter nasal secretions prior to establishment of infection. However, Sendai virus-specific IgG was detected in nasal passage lavage specimens from infected rats, and increases in Sendai virus-specific IgG AFC in the URN corresponded with clearance of virus and recovery from disease. A role for locally synthesized IgG in protection from upper respiratory tract diseases is supported by studies documenting that the nasal application of virus-specific IgG prior to inoculation with virus confers protection from infection (30–32). Also, patients who are specifically immunodeficient in IgG subclasses have a higher incidence of upper and lower respiratory tract infections (33), supporting a role for IgG subclasses in preventing upper respiratory tract infections in humans. However, it must be noted that these patients are able to recover from infections, indicating that mechanisms other than generation of an IgG subclass response are effective in combating disease. Thus, local production of virus-specific IgG is likely an important component in prevention and recovery from viral disease in the upper and lower portions of the respiratory tract.

The results of this study have important implications for development of new vaccination strategies against diseases caused by respiratory tract pathogens. Our results indicate that respiratory tract lymphoid tissues, particularly in the upper portion, are the major sites of antibody production after infection. These responses are present in rats that recently recovered from infection and are resistant to re-infection in the upper and lower portions of the respiratory tract.

Although we documented that antibody in the circulation is effective against lung disease, it does not contribute significantly to resistance against upper respiratory tract infections. A similar result was obtained by use of passive transfer of immune cells (data not shown). However, systemic inoculation is the route used for the currently available influenza vaccine, and this route of vaccination does not

promote immune responses within the upper respiratory tract, despite development of substantial serum antibody responses (34, 35). These data indicate that immune responses in the upper respiratory tract will provide increased resistance to infection. In support, upper respiratory tract infection of mice with influenza virus was prevented by intranasal inoculation with inactive influenza virus (36). In contrast, there was no noticeable protection after systemic inoculation because virus titers were equivalent in nasal turbinates of immune-naive (nonimmunized) mice and mice inoculated subcutaneously. Therefore, generation of local immunity in the upper respiratory tract, using either live or killed virus vaccines, is obviously an area where notable improvement in vaccination against disease caused by respiratory tract pathogens, including influenza and respiratory syncytial virus infection, can be made.

Even though much of the respiratory tract is composed of mucosal tissues, IgG, not IgA, was the major class of antibody produced against infection, and virus-specific IgG also was documented to confer protection. These results suggest that IgG responses are important in host defenses against upper and lower respiratory tract diseases. However, previous studies examining resistance to influenza virus, respiratory syncytial virus, or Sendai virus infections also documented a more substantial role for specific IgA in resistance to viral disease (37–45), including a more effective cross protection between various virus strains than that associated with IgG (44). In support, we found increase of Sendai virus-specific antibody of the IgA class in lavage specimens from the upper respiratory tract as well as from lung. Because of a specialized mechanism for the transport of IgA into mucosal secretions (46), secretory IgA is the major class of antibody in mucosal secretions of the respiratory tract in the absence of disease (47). Furthermore, studies suggest that, during its transport through mucosal epithelium, IgA can inhibit viral replication in infected epithelial cells (46). Although IgG was documented to be the major class of antibody produced in response to respiratory tract viral infection, generation of virus-specific secretory IgA is likely another important mechanism to limit mucosal infections. Thus, in developing new vaccination regimens against respiratory tract disease, induction of circulating IgG responses, combined with local IgA and IgG responses, is likely an effective approach to protect against lower and upper respiratory tract diseases. However, further studies are needed to determine the precise mechanisms through which local IgG participates in recovery from or resistance to upper respiratory tract disease, as well as the role of cell-mediated immunity, such as cytotoxic T cells and other immune mechanisms.

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