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Intranasal Monoclonal Immunoglobulin A against Respiratory Syncytial Virus Protects against Upper and Lower Respiratory Tract Infections in Mice

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The role of secretory antibody in protection against respiratory syncytial virus (RSV) infection was examined by using monoclonal immunoglobulin A (IgA) antibody for intranasal passive immunization of mice. Eight anti-RSV IgA hybridomas were produced by fusing myeloma cells with lung lymphocytes from RSV-immunized mice. Five IgA antibodies recognized RSV strains of both the A and the B subgroups, and two of these neutralized virus in a plaque reduction assay. Monoclonal IgA antibody HNK20, which bound to F glycoprotein, was most effective, reducing plaques by 50% at a concentration of 0.1 μg/ml for both subgroup A and subgroup B strains. HNK20 also neutralized all of eight clinical isolates of RSV tested. When delivered intranasally to mice 24 h prior to RSV challenge, HNK20 reduced virus titers in the lungs by nearly 100-fold. Maximal protection occurred at a dose of 0.5 mg/kg of body weight. Significant protection against lung infection was seen when the interval between antibody treatment and challenge was as long as 72 h. HNK20 also decreased virus titers in the nose approximately 10-fold when given 1 h, but not 24 h, before challenge. When mice were treated with HNK20 intranasally 3 days after challenge, viral titers were reduced in the lungs but not the nose. The results indicate that topical application of relatively small amounts of monoclonal IgA can protect against both upper and lower respiratory tract infections caused by RSV.

Primary infection with respiratory syncytial virus (RSV) occurs during the first 2 years of life, causing self-limited disease of the upper or lower respiratory tract. In some cases, infection of the lower respiratory tract causes severe bronchiolitis or pneumonia (14). Those at highest risk for lower respiratory tract disease include infants and children with bronchopulmonary dysplasia, congenital heart disease, and immunodeficiency disorders.

Since efforts to develop a safe and effective vaccine against RSV have not yet succeeded, passive immunization with polyclonal or monoclonal antibodies has been explored as an alternative strategy for prophylaxis. The evidence suggests that specific circulating antibody, if present at a high concentration, can protect against lower respiratory tract infection and disease. For example, infants are resistant to infection during their first 6 weeks of life, when maternal antibody levels are still high (6), and animals are protected against lung infection by parenteral administration of anti-RSV polyclonal antibody or monoclonal antibody (MAb) prior to RSV challenge (20, 24, 27).

For protection of the upper airways, secretory antibody may be more important than circulating antibody. In adult volunteers challenged with RSV, the neutralizing antibody titers in nasal secretions were found to correlate with decreased virus shedding and protection against disease (17, 28). A decrease in virus shedding also correlates with the appearance of anti-RSV secretory immunoglobulin A (IgA) in the nasal secretions of infants, although not all neutralizing activity was found to be antibody mediated (15). A role for secretory antibody in protection against RSV is further suggested by experiments in which passive antibody has been applied topically to the respiratory mucosa. These studies, in mice, cotton rats, and owl monkeys, showed that intranasal delivery of human IgG, MAbs, or human monoclonal Fab before or after challenge reduces virus titers in the respiratory tract (2, 5, 9, 18, 19, 25). In the case of human IgG, the efficacy of intranasally applied antibody is much higher than that of parenterally applied antibody (19).

Most studies of topically applied antibody against RSV have examined protection against lower respiratory tract infection. Since naturally acquired RSV infection is initiated in the upper airways, passive administration of antibody to the upper respiratory tract could prevent the spread of infection to the lower respiratory tract, reducing the risk of disease, and could also decrease virus shedding, reducing the risk of virus transmission. In this report, we describe the generation of IgA and IgG MAbs against RSV and show that an IgA MAb applied intranasally protects against upper and lower respiratory tract infections.

MATERIALS AND METHODS

Virus strains. RSV strain A2 (subgroup A) was obtained from the American Type Culture Collection (ATCC; Rockville, Md.). Strain 18537 (subgroup B) was provided by J. L. Patterson, Children's Hospital, Boston, Mass. RSV clinical isolates were provided by T. Chonmaitree and P. L. Ogra, University of Texas Medical Branch at Galveston. Virus was propagated in monolayers of Vero or HEp-2 cells (ATCC) grown in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). For production of virus

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stocks, subconfluent monolayers were infected with 0.1 PFU of RSV per cell. After 3 to 4 days, when syncytia were extensive, cells were scraped into the growth medium and were disrupted by sonication at 4°C. The virus suspension was clarified (2,000 × *g*, 10 min, 4°C), and the supernatant was stored at −80°C.

Plaque and neutralization assays. Virus was quantitated by plaque assay in Vero cell monolayers grown in 24-well polystyrene tissue culture plates. Serial 10-fold dilutions of virus were adsorbed to the cells for 1 h at 37°C, and then the cells were overlaid with 1 ml of MEM–10% FBS containing 0.75% agarose. After 4 days, a second 1-ml overlay containing 0.01% neutral red was added. Plaques were counted 6 to 8 h later.

For plaque reduction neutralization assays, RSV strains A2 or 18537 were incubated with serial 10-fold dilutions of MAb for 1 h at 37°C prior to being added to the cells. The neutralization endpoint was defined as the lowest concentration of antibody reducing plaque numbers by ≥50%. For neutralization of clinical isolates, the percent reduction of plaques at a single concentration of antibody (10 μg/ml) was tested.

Hybridoma production and ELISA. Two groups of BALB/c mice (Charles River Laboratories, Wilmington, Mass.) were immunized for anti-RSV hybridoma production. Group 1 received live virus intranasally on days 0, 14, and 35, RSV-infected Vero cells plus 10 μg of cholera toxin (Calbiochem, La Jolla, Calif.) in 2% $NaHCO_3$ intragastrically on day 63, and both live virus intranasally and virus-infected cells intragastrically on day 80. Intranasal immunizations in a volume of 50 μl were given to mice under isoflurane anesthesia, allowing fluid aspiration into the lungs. Group 2 received live virus intranasally on days 0, 9, 40, and 72. Four days after the final immunization in each group, mice were killed, and lung and Peyer's patch tissues (group 1) or lung tissues alone (group 2) were collected. Cells were isolated by collagenase treatment and disruption of tissue and were fused with P3X63Ag8U.1 myeloma cells (ATCC) as described previously (29). P3X63 Ag8U.1 myeloma cells and newly generated hybridomas were grown in RPMI medium supplemented with 10% FBS. After identification and cloning, the hybridomas were adapted to and grown in Serum Free and Protein Free Hybridoma Medium (Sigma Chemical Co., St. Louis, Mo.) without any supplements. Hybridoma 2D6, which produces murine IgA against *Vibrio cholerae* lipopolysaccharide (30), was grown in the same protein-free medium.

Hybridomas were screened for anti-RSV MAb production by enzyme-linked immunosorbent assay (ELISA) by using polystyrene plates coated with goat anti-RSV antiserum (Biodesign International, Kennebunkport, Maine) and then RSV suspension. After incubation with the hybridoma supernatant, bound antibody was detected with peroxidase-conjugated rabbit anti-mouse Ig or anti-mouse IgA (Zymed Laboratories, South San Francisco, Calif.). The wells were washed between steps with phosphate-buffered saline (PBS) containing 0.05% Tween 20. After the addition of 0.3 mg of 2,2′-azinobis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS; Sigma) per ml with 0.03% H_2O_2, the A_{405} was read in a V_{max} plate reader (Molecular Devices, Menlo Park, Calif.). The RSV specificity of the MAbs was confirmed by showing a lack of binding to lysates of uninfected cells. For quantitation of IgA MAb in mouse tissues, a standard curve of purified HNK20 IgA was constructed.

ELISA screening for reactivity to RSV glycoproteins expressed in vaccinia virus constructs was carried out as described above by using virus-infected cells as antigen. Microtiter plates seeded with 2×10^4 Vero cells per well were infected with vaccinia virus expressing RSV F, RSV G (provided by B. R. Murphy, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases), or hepatitis B virus surface antigen (ATCC) overnight and were fixed with 2% formalin. After the addition of ABTS solution and development, 50-μl samples from each well were transferred to a second 96-well plate for absorbance reading.

MAb production. Cell culture medium from hybridomas grown in protein-free medium was concentrated in a stirred cell by using a 100,000-molecular-weight cutoff membrane (type YM100; Amicon, Inc., Beverly, Mass.). The IgA concentration of the resulting material was determined by ELISA by using a standard curve constructed with purified IgA MAb 2D6 of known concentration. IgA 2D6 was purified from protein-free medium as described previously (11). ELISAs for quantitating MAbs were run as described above, with the exception that the plates were coated with rabbit anti-mouse Ig (Zymed) rather that anti-RSV antibody and RSV. IgG MAbs were quantitated by scanning densitometry of Coomassie blue-stained bands of polyacrylamide gels.

Immunoblotting and immunoprecipitation. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis analysis of IgA was performed without reducing agents to maintain the multimeric structure of the protein. Proteins were separated on 5 to 15% gradient gels and were transferred to nitrocellulose, and IgA was detected with alkaline phosphatase-conjugated goat antibody against mouse alpha chain (Zymed, San Francisco, Calif.).

For immunoprecipitation, RSV-infected Vero cells were labeled for 4 h at 37°C in methionine-deficient medium containing 200 μCi of [^{35}S]methionine (DuPont NEN, Boston, Mass.). Cells were washed with 5 ml of PBS and were lysed in buffer containing 1% Triton X-100, 0.5% deoxycholate, and 0.1% SDS. The lysate was mixed with MAbs, and immune complexes were precipitated with anti-mouse Ig-coupled polyacrylamide beads (Bio-Rad Laboratories, Hercules, Calif.). Control anti-F glycoprotein MAb 133/1H was obtained from Chemicon International, Temecula, Calif. The precipitated proteins were run on a 12.5% polyacrylamide gel, and radiolabeled bands were detected by fluorography.

Immunofluorescence. Vero cell monolayers grown in plastic eight-chambered microscope slides (Nunc, Naperville, Ill.) were inoculated with clinical isolates or RSV strain A2, incubated at 37°C for 4 days, and fixed with 100% methanol for 10 min at −20°C. Control monolayers were not infected. Wells were treated with HNK20 or control MAb 2D6 and then rhodamine-conjugated goat anti-mouse Ig (Cappel Research Products, Durham, N.C.). Fluorescence was scored by using a Nikon Labophot-2A microscope.

Mouse challenge assay. Challenge assays were carried out in 6- to 12-week-old female BALB/c mice in groups of three or four mice each. For virus inoculation, mice were anesthetized by isoflurane inhalation, and 25 μl of virus stock containing 10^5 to 10^6 PFU of RSV was pipetted onto the end of the nose, allowing it to be inhaled. MAbs were diluted as necessary with PBS and were delivered in the same manner either before or after virus inoculation. Four days after challenge, mice were killed by cervical dislocation. Lungs and nasal turbinates were removed, placed into centrifuge tubes containing 3 ml of cold MEM–10% FBS, and sonicated on ice for 30 s. Tissue suspensions were clarified for 10 min (2,000 × *g*, 10 min, 4°C), and supernatants either were tested immediately by plaque assay or were frozen at −80°C for subsequent testing. Average tissue weights of 0.16 g for lungs and 0.03 g for nasal turbinates were used to calculate the number of PFU per gram of tissue. The significance of differences between groups was determined by

TABLE 1. In vitro and in vivo effects of anti-RSV MAbs

MAb	Isotype	Neutralizing concn. (μg/ml)[a]	Decrease in virus titer in lung (log_{10})[b]
HNK16	IgG	>100	0.1 ± 0.09
HNK18	IgG	>100	0.8 ± 0.17[c]
HNK21	IgG	>100	0.4 ± 0.04[c]
HNK11	IgA	>100	0.0 ± 0.13
HNK19	IgA	>100	0.2 ± 0.14
HNK20	IgA	0.1	1.7 ± 0.43[c]
HNK22	IgA	>100	0.1 ± 0.14
HNK24	IgA	10	0.4 ± 0.10

[a] Lowest concentration of antibody required for 50% reduction of RSV plaques.

[b] Values are means and standard deviations of reductions in the number of PFU per gram of lung tissue in mice treated intranasally with 20 μg of MAb 24 h prior to RSV challenge. RSV titers in lung tissue were determined 4 days after challenge. Groups contained three to four mice each.

[c] Significantly different from virus titer in control mice ($P < 0.05$).

a two-tailed *t* test by using Statview II software (Abacus Concepts, Inc., Berkeley, Calif.).

RESULTS

Generation and selection of MAbs. Hybridomas secreting anti-RSV MAbs were generated in two separate fusions by using mucosa-associated lymphoid tissue from mice immunized by the intranasal route or the combined intranasal and intragastric routes. Fusion of cells derived from dissociated lung yielded a panel of 24 anti-RSV hybridomas, 16 of which produced IgG and 8 of which produced IgA. None of a similar number of hybridomas generated by fusion of Peyer's patch cells produced antibody against RSV. Culture fluids of anti-RSV hybridomas were initially screened by ELISA to select those that cross-reacted with RSV subgroups A and B. Five IgA MAbs and three IgG MAbs with strong levels of binding to strains of both subgroups were chosen for testing of biological activity against strain A2 by plaque reduction assay. Two IgA MAbs, HNK20 and HNK24, neutralized RSV (Table 1). HNK20 was the most effective, reducing plaque numbers by 50% at a concentration of 0.1 μg/ml. HNK24 required 10 μg/ml to reduce plaques by 50%. None of the three IgG MAbs neutralized RSV.

All eight anti-RSV MAbs were screened for their abilities to protect against pulmonary RSV infection in mice. Mice were treated intranasally with 20 μg of anti-RSV MAb or nonspecific control MAb 2D6, an IgA against *V. cholerae* lipopolysaccharide (30). Twenty-four hours later, mice were challenged intranasally with RSV. Lungs were removed and homogenized 4 days after challenge, and the number of PFU per gram of tissue was determined. Treatment with one IgA MAb, HNK20, and two IgG MAbs, HNK18 and HNK21, caused a significant reduction ($P < 0.05$) in the virus titer in the lungs compared with the titer in the lungs of mice treated with control MAb (Table 1). HNK20 was the most effective, reducing virus levels in the lungs by nearly 100-fold. HNK18 and HNK21 were protective, despite the fact that they had no activity in the neutralization assay. In contrast, HNK24 neutralized virus in vitro, but it did not cause a significant reduction in the virus titer in the lungs in vivo. HNK20 was chosen for further study on the basis of its in vitro and in vivo activities.

HNK20 characterization in vitro. The specificity of HNK20 was examined by immunoprecipitation of lysates of radiolabeled RSV-infected Vero cells. HNK20 precipitated two proteins corresponding in mobility to the F_1 and F_2 subunits of the F (fusion) glycoprotein (Fig. 1). These bands comigrated with the bands precipitated by 133/1H, a MAb previously shown to bind to F glycoprotein (1). Further evidence for the F glycoprotein specificity of HNK20 was provided by ELISA, showing binding to recombinant vaccinia virus expressing RSV F glycoprotein, but not to RSV G glycoprotein or hepatitis B virus surface antigen recombinants (data not shown).

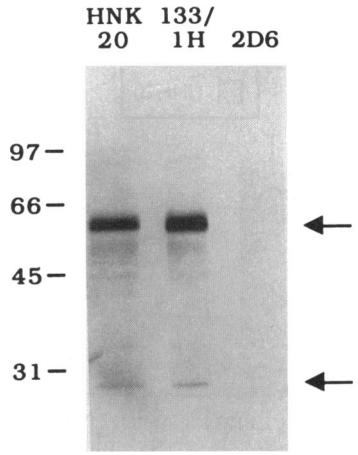

FIG. 1. HNK20 binds to F glycoprotein. Lysates of ^{35}S-labeled, RSV-infected Vero cells were mixed with the indicated MAbs and then with anti-mouse Ig coupled to polyacrylamide beads. Immunoprecipitated proteins were separated on an SDS–12.5% polyacrylamide gel and autoradiographed. Control MAbs were 133/1H, anti-F glycoprotein MAb (1), and 2D6, anti-*V. cholerae* lipopolysaccharide MAb (30). The positions of the F_1 and F_2 subunits of F glycoprotein are indicated with arrows at the right. Molecular masses (in kilodaltons) are shown at the left.

The polymeric structure of HNK20 was examined by running concentrated antibody on a 5 to 15% gradient acrylamide gel under nonreducing conditions and staining for IgA bands in an immunoblot (Fig. 2). The antibody was found to be produced in monomeric, dimeric, and higher polymeric forms. The major species was dimeric.

The neutralizing activity of HNK20 against RSV subgroup B

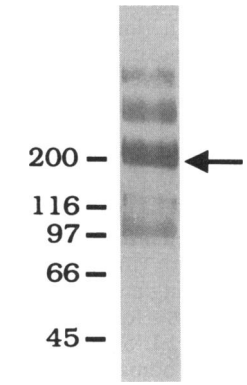

FIG. 2. HNK20 is produced in monomeric and polymeric forms. Concentrated HNK20 culture supernatant was run on an SDS–5 to 15% polyacrylamide gel without reduction and was immunoblotted with mouse IgA-specific reagents. Bands are present at the positions corresponding to IgA monomers, dimers, and higher polymers. The dimeric band is indicated by the arrow at the right. Molecular masses (in kilodaltons) are shown at the left.

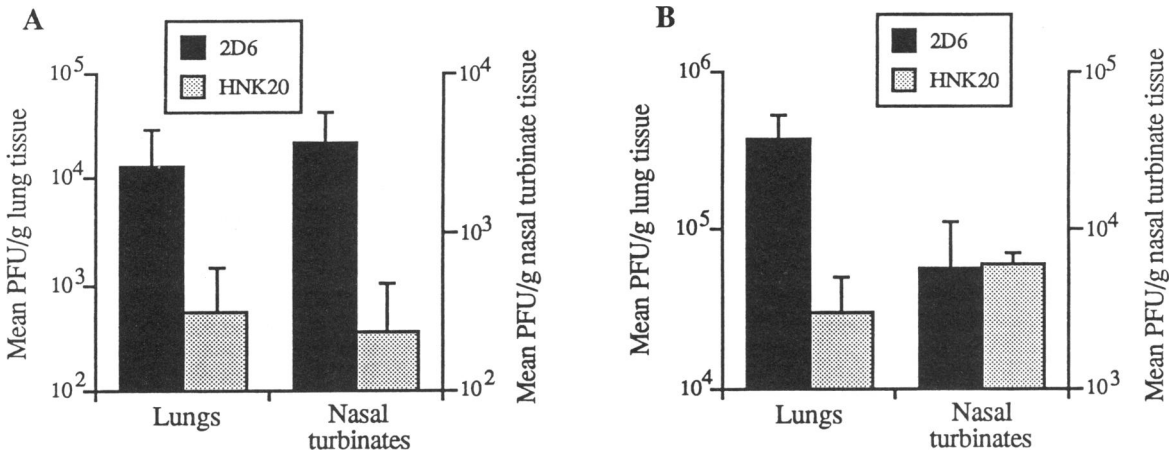

FIG. 3. Protection against lung and nasal turbinate infection by intranasal HNK20. HNK20 or nonspecific control IgA 2D6 was delivered in 20-μg doses to mice either 1 h (A) or 24 h (B) before RSV challenge. RSV titers in lung and nasal turbinate tissues were determined 4 days after challenge. Error bars indicate standard deviations for four to five mice per group. Virus titers in both lung and nasal tissues were reduced when MAb was delivered 1 h before challenge. Delivery of MAb 24 h before challenge reduced the virus titer in the lungs only.

strain 18537 was similar to that of subgroup A strain A2, with greater than 50% reduction in plaque numbers when HNK20 was used at 0.1 μg/ml. The ability of HNK20 to bind and neutralize a series of clinical isolates was also tested. Immunofluorescence microscopy showed that HNK20 bound to Vero cells infected with all of eight RSV clinical isolates (five subgroup A and three subgroup B) tested. Neutralization of the isolates by HNK20 was tested in a plaque reduction assay. When virus isolates were treated with 10 μg of HNK20 per ml, plaque numbers were reduced by 78.9 to 96.2%, a range comparable to that seen with strain A2.

In vivo activity. When HNK20 was applied intranasally 24 h before RSV challenge, the titers of virus in nasal turbinate tissues 4 days later were not reduced (Fig. 3). However, when the interval between intranasal antibody treatment and challenge was shortened to 1 h, virus levels in the nasal tissues of HNK20-treated mice were significantly lower ($P = 0.01$) than in those of mice treated with control MAb 2D6 (Fig. 3). The reduction in virus levels in nasal tissue was similar to that seen in the lungs, and when a range of HNK20 doses was delivered 1 h before challenge, the decrease in virus titer correlated with the dose (Fig. 4). A similar dose-response was seen for a reduction in virus titer in the lungs, although a lower dose of HNK20 was required to effect maximal protection (Fig. 4). The dose causing a maximal effect in the lungs was 10 μg per mouse, equivalent to approximately 0.5 mg/kg of body weight.

To determine whether the in vivo activity of HNK20 might have resulted from in vitro neutralization of virus by residual MAb in tissues, the amount of MAb in homogenates of respiratory tract tissues from HNK20-treated mice was examined. Four mice were treated intranasally with 10 μg of HNK20 but were not challenged with virus. Four days after treatment, the mice were killed and lung and nasal turbinate tissues were processed as described above for determination of virus titer. When run in a quantitative ELISA for mouse IgA against RSV antigen, nasal turbinate homogenates had no detectable HNK20 antibody (less than 7 ng/ml). In lung homogenates, an average of 95 ng of HNK20 per ml was detected. This level of antibody might be expected to cause some neutralization of RSV. As a further test, therefore, tissue homogenates were analyzed in a neutalization assay. No neutralizing activity could be detected in lung or nasal turbinate homogenates from HNK20-treated mice.

To determine the duration of activity in the lungs, mice were treated intranasally with 10 μg of HNK20 at intervals of 1, 2, or 3 days prior to RSV challenge. With a 1-day interval, the virus titer in the lungs was reduced by 1.9 $\log_{10}$ ($P = 0.02$), while with the 2- and 3-day intervals there was a reduction of 0.9 $\log_{10}$ ($P = 0.03$) in each group. HNK20 could not be detected by ELISA in serum 1 to 4 days after intranasal treatment with 10 μg of HNK20, indicating that the long-term effects of HNK20 did not result from uptake of antibody into the circulation.

The therapeutic efficacy of intranasal treatment was tested by infecting mice with RSV and treating them 3 days later with 1 or 10 μg of HNK20. Respiratory tract tissue was harvested on the following day and titrated. The virus titer in the lungs was reduced by treatment with 10 μg of HNK20 ($P = 0.04$), but there was no effect on the virus titer in nasal tissue (Fig. 5).

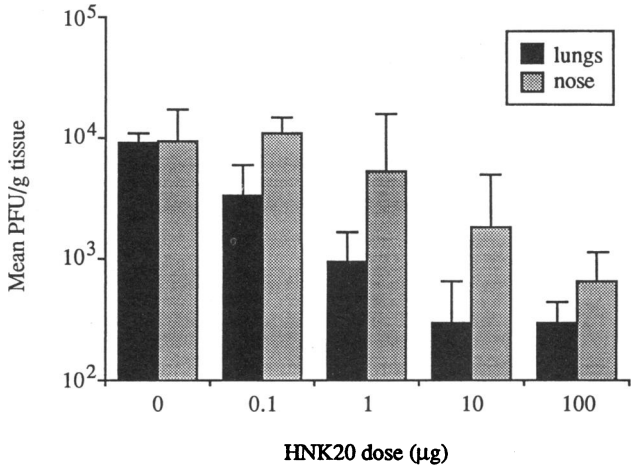

FIG. 4. Reduction of virus titers in lung and nasal tissues by intranasal treatment with graded doses of HNK20. Mice were treated intranasally with the indicated amount of HNK20 antibody 1 h before RSV challenge. Control mice were not treated before challenge. RSV titers in lung and nasal tissues were determined 4 days after challenge. Error bars indicate standard deviations for three to four mice per group.

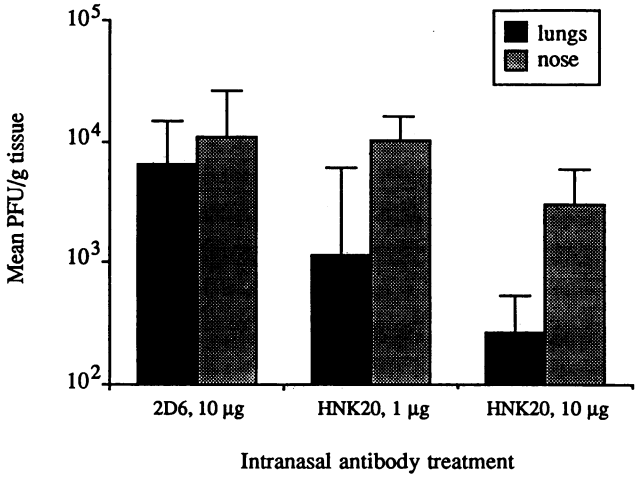

FIG. 5. Intranasal HNK20 treatment after RSV infection reduces virus titers in lung tissue but not nasal tissue. Mice were infected with RSV and were treated intranasally with 1 or 10 μg of HNK20 or 10 μg of nonspecific IgA (2D6) 3 days later. RSV titers in lung and nasal turbinate tissues were determined 4 days after infection. Error bars indicate standard deviations for four mice per group.

Parenteral treatment with HNK20 was not effective. Intraperitoneal injection of 200 μg of HNK20 24 h before or 3 days after RSV challenge had no effect on virus titers in either the lungs or nasal turbinate tissue (data not shown). Intraperitoneal treatment with 100 μg of HNK20 at 2, 4, or 6 h before challenge also had no effect on virus titers (data not shown).

DISCUSSION

A role for circulating antibody in protection against RSV infection has been demonstrated repeatedly by passive immunization with MAbs and polyclonal antibodies. Human polyclonal IgG antibody delivered parenterally protects against lung infection in cotton rats and owl monkeys (8, 20) and is beneficial to human infants when it is used to treat infections or when given prophylactically (7, 9, 10). Murine IgG MAbs against RSV F and G glycoproteins and a reshaped human MAb against F glycoprotein also protect against lung infections in animals when they are given parenterally (2, 24, 25, 27).

In the upper respiratory tract, where secretory antibody is thought to be the most important mediator of immunity (15, 17, 28), parenteral antibody treatment is less effective (20, 27). IgA, the major antibody isotype of respiratory tract secretions (3), is thought to prevent the interaction of pathogens with the respiratory tract epithelium by blocking attachment sites and by cross-linking and agglutinating microorganisms, facilitating their clearance by mucociliary transport (16). Secretory IgA is polymeric, promoting efficient cross-linking of antigens, and is associated with secretory component, giving it greater resistance to proteolytic enzymes (4). In mice, protection against viral infection of the respiratory tract can be examined by passive immunization with IgA MAbs. For example, topical application of IgA MAb against Sendai virus was shown to protect mice against lower respiratory tract infection (13). In another study, mice were injected intravenously with IgA MAb against influenza virus, allowing the secretion of IgA onto mucosal surfaces (21). When challenged with virus, the mice were protected against infection of the lungs and nose.

For the present study, we produced MAbs against RSV for use in passive mucosal protection experiments. Mucosally derived lymphocytes were used for hybridoma production in order to maximize the generation of IgA MAbs and to derive MAbs that better represent the specificities of the mucosal immune response. Of the five IgA MAbs tested, only two neutralized virus in vitro, suggesting that much of the secretory antibody response against RSV may be nonneutralizing. Two IgG MAbs were somewhat protective in vivo, even though they showed no neutralizing activity in vitro. The mechanism of protection is not known, but virus particles may have been cross-linked by antibody, preventing penetration of the mucous layer overlying the epithelium. Other mechanisms could also be involved, because nonneutralizing MAbs against other viruses have been shown to be protective in vivo when delivered parenterally (26).

In the present study, we concentrated on HNK20, a neutralizing IgA MAb against F glycoprotein, and examined passive protection against infection of upper and lower respiratory tract tissues with RSV. Delivered intranasally prior to virus challenge, HNK20 reduced virus titers in lung tissue. Low doses (0.05 to 0.5 mg/kg) were required, and mice were protected against lung infection when antibody was applied as long as 3 days before RSV challenge. The mechanism by which antibody remains active in the lungs for so long is not known, but our observations are consistent with the prolonged duration of protection observed in cotton rats treated intranasally with human IgG (19). The mechanism did not appear to involve the uptake of HNK20 into the circulation because no HNK20 could be detected in the serum of treated mice and parenteral delivery of HNK20 was not protective. More likely, the antibody was resident in the lungs, because we were able to show the presence of HNK20 in lung homogenates 4 days after intranasal treatment. Upper airway protection by HNK20 was of a shorter duration. HNK20 was effective when given 1 h before challenge, a surprising observation given the fact that nasal mucus is cleared rapidly into the nasopharynx. In future experiments, we will attempt to extend the duration of upper respiratory tract protection by using formulations that lengthen the residence time of antibody in nasal tissue.

Passive immunization with polyclonal antibody is being developed for protection of high-risk infants against disease due to RSV infection (7, 23). Antibody could be delivered parenterally or topically, but in either case, it must presumably reach the respiratory tract mucosa to block infection. RSV replicates primarily in the epithelium (14), and it has been shown that Sendai virus, another paramyxovirus, buds from the apical surface of polarized epithelial cells in culture (22). This suggests that extracellular spread of infection occurs at the luminal surface of the epithelium. Perhaps as a consequence of this, human IgG is much more efficacious in cotton rats when it is applied directly to the mucosal surface than when it is delivered parenterally (19, 20). Murine and reshaped human MAbs, as well as a human monoclonal Fab, are also highly effective against lung infection when delivered intranasally (2, 5, 25). Our results suggest that passive intranasal immunization with anti-RSV MAbs may be an effective treatment for the prevention of upper and lower respiratory tract RSV infections in humans. By limiting upper airway infection, such treatment might prevent the spread of infection to the lower respiratory tract. A reduction in the numbers of individuals with upper respiratory tract infection might also limit the transmission of virus to uninfected individuals.

Prevention of human infection will require neutralization of RSV subgroups A and B. HNK20, like a number of other MAbs against the F glycoprotein, neutralized virus of both subgroups, suggesting that this MAb alone could be used for

human treatment. This is supported by our observation that HNK20 neutralized all of a panel of eight RSV clinical isolates. Intranasal treatment 3 days after RSV challenge also reduced the virus titer in the lungs, suggesting that topical treatment of infected individuals might abrogate lung infection after the initiation of disease. We did not, however, see an effect of therapeutic IgA treatment on virus titers in the upper respiratory tract after the infection was established. This is in agreement with the studies of others showing that therapeutic intranasal administration of anti-RSV antibody by drops or aerosol has little or no effect on virus replication in the nose (9, 18, 19).

The relative advantages of IgA and IgG for passive mucosal protection have not been resolved. A recent study comparing IgG and IgA MAbs as well as monomeric and polymeric IgA against Sendai virus suggests that there is no significant advantage of polymeric IgA in protection against lower respiratory tract infection (12). In future studies, we will compare IgA and IgG MAbs bearing identical antigen-binding domains for their abilities to protect against RSV infection of the upper and lower respiratory tracts.

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