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Protection from Respiratory Syncytial Virus Infection in Cotton Rats by Passive Transfer of Monoclonal Antibodies

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The effects of passive administration of neutralizing monoclonal antibodies (MAB) to respiratory syncytial virus glycoproteins (GP90 and VP70) was evaluated in cotton rats challenged with respiratory syncytial virus. Animals injected with MAB to VP70 had lower mean viral titers in lung tissues than did controls ($\log_{10}$ 2.5 versus 5.4 PFU/g; $P < 0.001$), as did cotton rats given MAB to GP90 ($\log_{10}$ 2.1 versus 5.0 PFU/g; $P < 0.001$). Fifty percent of animals given either MAB had no detectable virus in lung tissues, whereas virus was detected in the lungs of all controls. Virus growth in nasal turbinates was decreased but not eliminated in recipients of either MAB.

Respiratory syncytial virus (RSV), an enveloped RNA virus related to the paramyxoviruses, is the major cause of severe lower respiratory tract infection in infants and young children (10). Unlike many viral infections, immunity to RSV infection is incomplete. Although both cell-mediated and antibody-mediated immunity have been studied, the effect of circulating antibody on the clinical course of infection with RSV has not been clearly established (4, 6, 10). The most severe infections frequently occur in the first few months of life, a time at which maternal antibody is often present (10). In addition, neither killed nor live attenuated RSV vaccines have been successful in the prevention of infection, despite the induction of anti-RSV antibody (1, 5).

The RSV genome codes for at least seven proteins, of which four have been given structural assignments (11). The structural proteins identified include 90,000- and 70,000-dalton envelope glycoproteins (GP90 and VP70), a 26,000-dalton matrix protein, and a 44,000-dalton nucleocapsid protein (11). Recently, we have shown that both GP90 and VP70 interact with neutralizing monoclonal antibodies and that VP70 is the fusion protein (13). The fusion protein probably functions to fuse the viral and cellular membranes, resulting in viral entry into host cells, and also causes cell-cell fusion, permitting the spread of virus to adjacent cells, producing the characteristic syncytium formation seen in tissue culture. Although the role of GP90 has not been determined, it may mediate attachment of the virus to host cells analogous to the hemagglutinin of parainfluenza viruses (2). Therefore, similar to paramyxoviruses, antibody directed to either envelope glycoprotein of RSV may be capable of in vitro neutralization of virus. However, the ability of antibody against individual glycoproteins to confer in vivo protection from RSV infection has not been demonstrated.

To define the role of circulating antibody in RSV infection, we examined the ability of passively transferred monoclonal antibody directed to GP90 and to VP70 to protect cotton rats from challenge with RSV. The cotton rat is a well-established model of RSV infection (12). Although neither illness nor death results and pathological changes are minimal, the growth of RSV in both upper and lower respiratory tissues is well characterized. After intranasal inoculation, virus uniformly replicates, reaching a peak titer of 10^5 PFU/g of tissue in the lungs and nasal turbinates by day 4. Virus titers then fall steadily, becoming negative by day 10, simultaneous with the appearance of neutralizing serum antibody.

Three-week-old outbred cotton rats in groups of five to six were injected by the intraperitoneal route with 0.6 to 0.8 ml of mouse ascitic fluid containing monoclonal antibody to either GP90 or VP70 (16 and 3 mg of gamma globulin per ml, respectively). Control animals received either saline or monoclonal antibody to RSV nucleocapsid protein (NP44). The production and characterization of these monoclonal antibodies have been described in detail previously (13) and are shown in Table 1. Three hours after injection with a monoclonal antibody, penthrane-anesthetized cotton rats were infected by intranasal inoculation of 10^4 PFU of RSV (Long strain). Four days later, animals were sacrificed by cervical dislocation, and the nasal turbinates and lungs were removed under sterile conditions. The tissues were homogenized in minimal essential medium, fast-frozen, and stored at −70°C until titers were determined. RSV titers were performed by inoculating serial 10-fold dilutions of homogenized tissues onto HEp-2 cell monolayers in 24-well plates. The monolayers were incubated under agarose for 4 days, fixed with glutaraldehyde, and stained. Typical syncytial plaques were counted, and the RSV titer was expressed as PFU per gram of tissue. Blood samples, obtained by orbital puncture before infection and at the time of sacrifice, were assayed for the monoclonal antibody by an indirect fluorescent antibody test (13). Briefly, 1:25 dilutions of cotton rat sera were incubated on RSV-infected HEp-2 cells, followed by fluorescein isothiocyanate-labeled goat antimouse antibody.

None of the experimental animals demonstrated signs of illness or died after infection. However, cotton rats given monoclonal antibody to VP70 had statistically lower mean lung titers of virus ($\log_{10}$ 2.5 PFU/g) than those given monoclonal antibody to the nucleocapsid protein ($\log_{10}$ 5.4 PFU/g; $P < 0.001$; two tailed t test) or control animals ($\log_{10}$ 5.5 PFU/g) (Fig. 1). Similar results were also obtained in a repeat experiment, although the percent decrease in viral titers was not as great ($\log_{10}$ 3.5 versus 4.9 PFU/g; $P < 0.01$). In the two experiments, 6 of 12 animals given monoclonal antibody to VP70 had no detectable virus in lung tissues, whereas all control animals had detectable virus (chi-square = 5.7; $P < 0.005$). The effect on nasal turbinate titers was not always statistically significant, although viral titers were generally decreased. In one experiment no decrease was

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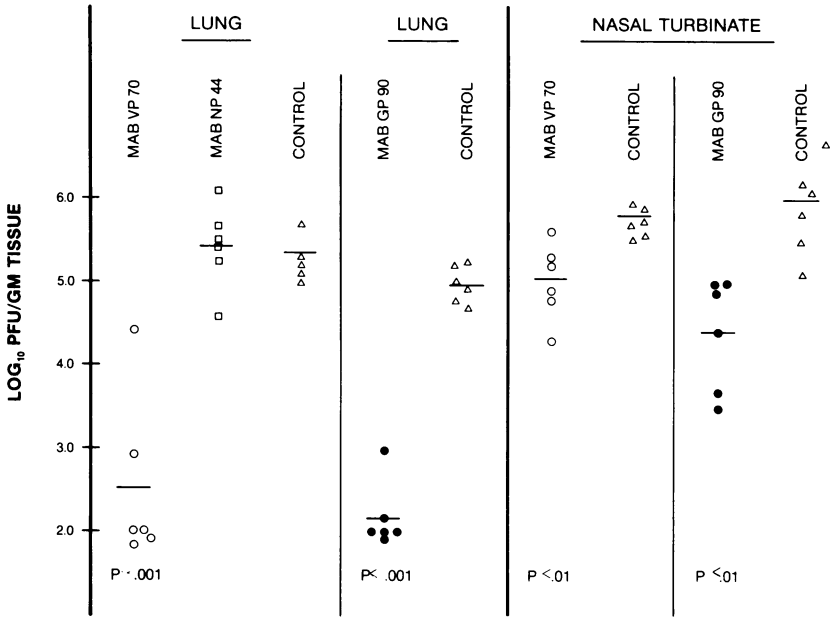

FIG. 1. RSV titer in cotton rat lungs and nasal turbinates at time of sacrifice. Results of representative experiments for each monoclonal antibody are shown. Animals were injected with monoclonal antibody and then infected with RSV. After 4 days, nasal turbinates and lungs were assayed for virus. Symbols: ○, monoclonal antibody to VP70; □, monoclonal antibody to NP44; ●, monoclonal antibody to GP90; △, control. For statistical analysis, all animals with no detectable virus were assigned the lowest value detectable by this assay (ca. 10^2 PFU/g). The *P* value was determined by the Student *t* test. MAB, Monoclonal antibody.

noted, whereas in the second a 60% decrease in titer ($\log_{10}$ 5.1 versus 5.7 PFU/g) was noted (Fig. 1).

Cotton rats injected with monoclonal antibody to GP90 also had significantly lower lung titers compared to control animals in two experiments ($\log_{10}$ 2.4 versus 4.1 and 2.1 versus 5.0) (Fig. 1). Seven of eleven infected animals which received monoclonal antibody to GP90 had no detectable virus in the lungs. All control animals had detectable virus ($P < 0.005$). Monoclonal antibody to GP90 also resulted in a 95% decrease in nasal turbinate titers compared to controls ($\log_{10}$ 4.7 versus 6.0; $P < 0.01$). However, all 11 treated animals had detectable virus in nasal tissues, as did all the control animals. Except for a single animal injected with monoclonal antibody to VP70, all animals had monoclonal antibodies in the serum at a titer greater than 1:25 by indirect immunofluorescence at the time of sacrifice. Serum neutralizing titers greater than 1:25 were present in all animals given monoclonal antibody to VP70, whereas no neutralization was seen in any control serum.

Emergence of mutant influenza virus has been reported when virus is grown in the presence of neutralizing monoclonal antibodies (3). To exclude the presence of non-syncytial-forming mutant virus in the animals with undetectable virus, viral titers were also assayed by immunofluorescent staining of inoculated HEp-2 cells using monoclonal antibody to the nucleocapsid protein and fluorescein-labeled goat antimouse antibody. No evidence of altered virus was detected in any animals injected with either monoclonal antibody.

In these experiments passive immunization with circulating monoclonal antibody to either of the two envelope glycoproteins of RSV reduced or prevented entirely the growth of virus in the lungs of experimental animals. That less effect was found on viral replication in nasal tissues is not surprising since immunity to nasal infection with many viruses, including RSV, is probably dependent on nasal secretory antibody (8), whereas immunity to lung infection may be more dependent on circulating antibody. The ability of circulating antibody to prevent lung infection in cotton rats is consistent with the findings of two clinical studies in which the severity of pulmonary infection in infants was inversely related to the level of circulating maternally derived neutralizing antibody (4, 6).

That none of the animals injected with monoclonal antibody showed evidence of more severe RSV infection is of interest in view of the observation of increased severity of illness with both RSV and measles infection in individuals who had previously received Formalin-inactivated vaccines (5). It is known that Formalin treatment of measles virus selectively denatures the fusion protein, reducing its antigenicity (9). It has been demonstrated for measles and postulated for RSV that Formalin-inactivated vaccines induce an antibody response that is restricted to only some of the viral proteins and that this results in altered immunological reac-

TABLE 1. Production and characterization of monoclonal antibodies

Monoclone designation	Immunoglobulin subclass[a]	RSV protein specificity[b] (mol wt)	Virus neutralization	
			Without complement	With complement
D14	IgG2a	NP44 (44,000)	<1:4	<1:4
L4	IgG2b	VP70 (70,000)	1:512	1:2,048
L7	IgG2a	GP90 (90,000)	<1:4	1:2,048

[a] IgG, Immunoglobulin G.

[b] Determined by immunoprecipitation of radiolabeled RSV proteins.

tivity to subsequent viral infection (7). Based on this observation, Merz et al. have suggested that an effective paramyxovirus vaccine must induce antibody to both envelope glycoproteins (7). The data presented, although not refuting this, suggest that circulating neutralizing antibody to either of the two envelope proteins of RSV may be beneficial by limiting viral growth to the upper respiratory tract.

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