Mechanism of Antibody-Mediated Viral Clearance in Immunotherapy of Respiratory Syncytial Virus Infection of Cotton Rats

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Antibody-mediated clearance of respiratory syncytial virus from cotton rat pulmonary tissues occurs in the absence of complement and in the absence of the Fc portion of the immunoglobulin G molecule, suggesting that complement-independent, cell-independent neutralization is the major mechanism of clearance.

The role of serum antibody to respiratory syncytial virus (RSV) in resistance and pathogenesis of disease has long been a subject of discussion and controversy. Early studies suggested that maternally transmitted antibody was not protective and that vaccine-induced antibody might actually be harmful (2). However, subsequent observations in both human subjects and experimental animals have shown that the amount of serum antibody to RSV correlates well with resistance to severe RSV disease. In addition, RSV antibody administered passively can have significant prophylactic and therapeutic benefits in cotton rats and owl monkeys (4, 7a, 8). Trials of therapeutically administered immunoglobulin G (IgG) in infants and children hospitalized with RSV disease showed that antibody recipients experienced enhanced clearance of virus from the upper respiratory tract and improved clinical response compared with placebo-treated control patients (5). The potential for large-scale clinical use of passively administered IgG in RSV immunoprophylaxis and immunotherapy underscores the need to understand the mechanisms by which passively transferred antibodies mediate an antiviral effect in vivo. Four possible mechanisms exist by which antibody can exert an antiviral activity: (i) antibody-dependent cellular cytotoxicity (ADCC), requiring the interaction of lymphocytes with the Fc portion of the IgG molecule; (ii) complement-dependent viral neutralization, involving the classical pathway of complement activation; (iii) complement-dependent lysis of infected cells, involving F(ab')2 and the alternative pathway of complement activation; and (iv) complement-independent, cell-independent viral neutralization (3). Using the cotton rat model of RSV infection, we studied the effects of Fc and complement depletion on the efficacy of topical immunotherapy to determine which of these four mechanisms accounts for the observed in vivo effects.

Cotton rats (Sigmodon hispidus) were obtained from the Veterinary Resources Branch, Division of Research Services, National Institutes of Health. Purified human IgG (Sandoglobulin, lot 2.370.069.0; Sandoz, Inc., East Hanover, N.J.) was treated by pepsin digestion, and F(ab')2 fragments were purified by sequential passage over Sephadex G-75 and protein A columns (6). Fc contamination, determined by competitive inhibition enzyme immunoassay, was less than 0.2%. Since approximately half the IgG molecule was removed by pepsin digestion, a 5% (wt/vol) solution of F(ab')2 and a 10% solution of IgG were used for direct comparison. The in vitro neutralization titers, determined by plaque reduction assay with a 60% endpoint (9), was 1:752 for a 5% solution of F(ab')2, compared with 1:1058 for a 10% solution of untreated IgG. [This observation is consistent with earlier studies which showed that both Fab and F(ab')2 fragments can neutralize a variety of animal viruses in vitro (7).]

The basic design of the study was to infect cotton rats intranasally with 108.0 PFU of RSV (strain A2) on day 0, treat anesthetized animals with topical IgG or F(ab')2 by direct instillation into the respiratory tracts on day 3, and sacrifice the animals on day 4; days 3 and 4 represent the time of peak viral titer in the lungs. Concurrently infected animals which were not treated with IgG or F(ab')2 served as the basis for determining the extent of viral clearance effected by topical immunotherapy. Initially, F(ab')2 was administered intraperitoneally but was quickly degraded so that neutralizing activity could not be measured in the serum 24 h later. All subsequent experiments therefore employed topical administration of IgG or F(ab')2.

ADCC requires the interaction of cell-associated viral antigen with the F(ab')2 portion of the IgG molecule bound by its Fc portion to lymphocytes. Enzymatic removal of Fc from the IgG molecule thus blocks ADCC. By comparing the efficacy of F(ab')2 and whole-molecule IgG in clearing RSV from pulmonary tissues, it is possible to determine the relative importance of ADCC in antibody-mediated viral clearance (Table 1). When used at an equimolar concentration, IgG (group 2) and F(ab')2 (group 5) were equally effective, each bringing about a 100-fold reduction in pulmonary virus. The possibility that the small amount of Fc remaining in the F(ab')2 preparation (<0.2%) contributed to viral clearance was examined by using 10-fold (group 3) and 100-fold (group 4) dilutions of IgG. The IgG administered to group 4 contained a 10-fold-higher amount of Fc than that found in the F(ab')2 preparation but had no effect on viral titer, indicating that the antiviral effect seen in group 5 was due exclusively to F(ab')2.

The role of complement was studied by comparing the
efficacy of antibody in clearing virus from complement-depleted and -sufficient animals (Table 1). Cobra venom factor (Naja naja; Cordis Laboratories, Inc., Miami, Fla.) was administered intracardially, at a dose of 200 U per kg of body weight, to cotton rats challenged 2 days earlier with 10³.3 PFU of RSV. These animals (group 7) were treated with topically administered IgG 24 h after the administration of cobra venom factor (day 3). On day 4 the animals were sacrificed, and viral titers in lung homogenates were determined (9). Serum 50% hemolytic complement titers at the time of IgG administration (guinea pig C3 test kit, catalog no. 737-303; Cordis) were greater than 1:5,600 in complement-sufficient animals, whereas a 1:50 dilution of serum from cobra venom factor-treated animals did not contain detectable complement activity. Complement-depleted animals treated with IgG (group 7) showed a greater than 100-fold decrease in virus titer compared with the untreated, complement-sufficient (group 1) and untreated, complement-depleted (group 6) animals. Complement-sufficient animals treated with IgG (group 2) showed a level of viral clearance similar to that seen in group 7, indicating that antibody-mediated clearance of RSV is not dependent on complement. Additionally, complement-depleted cotton rats were treated with F(ab′)₂ (group 8) and were found to experience a degree of viral clearance similar to that in groups 2, 5, and 7.

Previous studies of cotton rats and owl monkeys showed that the therapeutic effect of antibody was permanent, i.e., there was no rebound of viral titer after immunotherapy (8). The possibility that the reduction in viral titer was an in vitro artifact caused by the interaction of virus and antibody after tissue homogenization was ruled out by cross-homogenization experiments with the cotton rat (7a, 8). In these experiments, infected lungs which had been treated in vivo with antibody were homogenized jointly with infected, untreated lungs. The failure of the antibody-treated lung tissue to depress viral titer in untreated tissue suggested that the effect of antibody was due to in vivo neutralization and was not an artifact of neutralization in vitro. Additional evidence of in vivo neutralization came from experiments with owl monkeys (4), in which reduction in viral titer was observed in antibody-treated animals whose titers were determined by bronchial lavage rather than homogenization of pulmonary tissues. Since RSV is cell associated and can spread through
cellular fusion, it is not easily understood how intracellular virus is cleared. However, recent studies of Aleutian mink disease virus have shown that passively administered antibodies restrict the intracellular level of viral replication, though the mechanism is not known (1). In the context of these observations, it would appear that of the four major mechanisms of antibody-mediated RSV clearance (i.e., ADCC, complement-dependent neutralization, complement-dependent cell lysis, and complement-independent, cell-independent neutralization), complement-independent, cell-independent neutralization is of greatest importance during topical immunotherapy of RSV. While these experiments do not rule out the possibility that the other mechanisms also participate in clearance of virus from treated animals, significant clearance of virus in the absence of complement, the Fc portion of the IgG molecule, or both suggests that their relative contribution is less than that of direct neutralization of virus.

Antibody-mediated immunotherapy has yet to be demonstrated with other respiratory viruses; it should therefore not be inferred from these data that direct neutralization would be an effector mechanism in the clearance of other viruses or that ADCC and complement-independent mechanisms would not necessarily be important. The susceptibility of RSV to this mode of therapy might be the result of its site-restricted replication in vivo, in which only mucosal surfaces (primarily small airways) are involved.

**LITERATURE CITED**


