Immunoprophylaxis of Group B Respiratory Syncytial Virus Infection in Cotton Rats

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Two antigenic groups of respiratory syncytial virus (RSV) have been identified: A (RSV/A) and B (RSV/B). Topical administration of human IgG screened for high titers of antibody to RSV/A (RSVIg) is protective against RSV/A infection in the cotton rat model. The study attempted to determine if topical RSVIg would also be protective against RSV/B. Cotton rats were pretreated intranasally with RSVIg or with monospecific antiserum obtained from animals previously infected with RSV/A or RSV/B (day 0), challenged intranasally with RSV/A or RSV/B (day 1), and sacrificed for virus titration (day 5). Cotton rat antiserum to RSV/B protected against RSV/A and RSV/B, while antiserum to RSV/A protected only against RSV/A. RSVIg, although prepared on the basis of activity against RSV/A, was also protective against RSV/B.

Over 30 years ago [1], the existence of antigenic groups of respiratory syncytial virus (RSV) was suggested by the observation that monospecific antiserum raised in ferrets largely neutralized most, but not all, heterologous strains. One strain in particular, designated 18537 (and later shown to be the prototype of group B strains), showed very little antigenic relationship to most other strains as measured by an in vitro neutralization assay. Several years elapsed before the more sensitive technology of monoclonal antibodies allowed more precise characterization of strain differences. RSV strains are now routinely typed using a panel of monoclonal antibodies and assigned to antigenic groups (A and B) and subgroups (e.g., A1–A4).

Recent clinical studies have shown that a polyclonal human IgG preparation enriched for RSV activity through donor selection (RSVIg) is highly effective in preventing severe RSV disease in high-risk infants [2, 3]. RSVIg is enriched for RSV activity by screening potential plasma donors using an automated microneutralization assay [4] that uses a group A virus, due to the general dominance of group A strains in annual RSV epidemics [5]. The ability of such a preparation to protect against group B viruses might be questioned in view of the studies by Coates et al. [1]. Although their work used antisera raised in ferrets, no in vivo studies were done because, at the time, there was no animal model permissive for pulmonary RSV infection. Thus, we revisited their observations and used the cotton rat to assess the efficacy of RSVIg and of monospecific cotton rat antisera in preventing group B pulmonary infection.

Materials and Methods

Outbred cotton rats (Sigmodon fulviventer) were obtained from a colony maintained at Virion Systems. Two prototype strains of RSV were used: Long, a group A strain (RSV/A; American Type Culture Collection, Rockville, MD), and 18537, a group B strain (RSV/B; Dept. of Pediatrics, Uniformed Services University of the Health Sciences). Stocks of these viruses were grown in HEp-2 cells and contained 10^7 and 10^6 pfu/mL, respectively. Serum was obtained from donor cotton rats 3 weeks after intranasal inoculation with RSV/A or RSV/B or no inoculation (control). RSVIg is a 5% solution of purified human IgG with high neutralizing antibody titer to RSV/A (RespiGam; MedImmune, Gaithersburg, MD).

Cotton rats were anesthetized by methoxyflurane inhalation for experimental handling. Animals were pretreated by intranasal instillation of 0.1 mL of either RSVIg or cotton rat immune or nonimmune serum (day 0) [6]. Twenty-four hours later they were challenged intranasally with 0.1 mL of virus suspension (day 1). Four days after challenge (the time of peak virus titers in control animals), the animals were sacrificed by carbon dioxide intoxication, and lungs were obtained for virus titration [7] (day 5). Virus titers were determined by plaque assay and expressed as plaque-forming units per gram of tissue [7]. Neutralizing antibody titers of RSVIg and monospecific antisera were determined by 60% plaque reduction assay [7].

Geometric means of virus titers of pretreated groups were compared with those of control groups using the 2-tailed Student’s t test.

Results

Control cotton rat serum had no neutralizing activity against RSV/A or RSV/B. RSV/A antiserum showed high neutralizing
Figure 1. Topical immunoprophylaxis of RSV/A and RSV/B infection with cotton rat antiserum. Donor cotton rats were inoculated with RSV/A or RSV/B or left uninfected; serum was obtained 3 weeks later. Sera were pooled within groups. Recipient cotton rats were divided into groups of 7 or 8, pretreated with cotton rat serum, challenged with RSV/A (10^6 pfu) or RSV/B (10^5 pfu), and sacrificed 4 days later for virus titration. Data are geometric mean titers (log_{10})/SE. * Virus titers significantly lower than those of controls.

Figure 2. Topical immunoprophylaxis of RSV/B infection with high-titer human IgG (RSV Ig) at various challenge doses. Cotton rats were divided into groups of 8, pretreated with RSV Ig or left untreated, challenged with differing dilutions of RSV/B, and sacrificed 4 days later for virus titration. Data are geometric mean titers (log_{10}) + SE. * Virus titers significantly lower than those of controls.

Discussion

The clinical and epidemiologic significance of RSV groups remains unclear. Strain 18537 (group B) was isolated from a patient in 1962, just 6 years after the prototypical Long strain (group A), suggesting that at least two antigenic groups have cocirculated indefinitely. There appears to be no selective advantage for either group, as nearly every RSV epidemic involves simultaneous circulation of both groups, and neither has shown significant antigenic evolution over the past four decades. Indeed, the ability of RSV Ig obtained from plasma donors and thus preferentially reflecting antigenic characteristics of contemporaneous strains to neutralize prototypical A and B groups suggests that antigenic evolution is not important for the survival of RSV, an assertion further supported by animal studies showing that priming with Long strain confers solid immunity against contemporaneous group A virus [9].
In contrast, influenza viruses have continual antigenic drift and periodic antigenic shift, resulting in contemporary strains scarcely related to earlier ones. The capacity of influenza virus to mutate quickly appears to be driven by a virus-host interaction that induces lasting immunity to homologous virus. That is, infection by 1 strain of influenza virus induces long-term resistance to reinfection and apparently the homologous strain induces life-long protection against clinical illness. Influenza is a highly contagious disease that affects most of the population during an epidemic. Therefore, the strategy by which influenza ‘‘stays in business’’ is to mutate continually. The more antigenically diverse mutants then have a selective advantage in reinfecting a population with ‘‘herd immunity’’ to the parent strains of virus. The mutability of influenza is enhanced by a multisegmented genome that is highly prone to reassortment with other strains.

RSV, on the other hand, contains a single segment of RNA that does not facilitate major antigenic evolution. Like influenza, RSV is highly contagious and infects most of the population during each epidemic. Unlike influenza, however, it does not elicit long-term immunity. Indeed, studies of adult volunteers have shown that immunity to RSV frequently lasts only a few months. By the next annual RSV epidemic, most of the population is susceptible to reinfection by the same strain of virus. While the mechanism driving this viral survival strategy is not yet defined, it appears to rely upon the virus’s ability to evade or down-regulate a portion of the host immune response that mediates long-term immunity. If 1 strain of RSV can repeatedly reinfect the same population each year, there is no selective advantage to mutant strains of the virus. The cocirculation each year of groups A and B as well as subgroups of each is consistent with such a hypothesis, as is the antigenic similarity between contemporaneous and prototypical RSV strains, whose clinical isolation is separated by four decades.

From the standpoint of passive prophylaxis of RSV, such a scenario suggests that a polyclonal IgG product like RSVIg will continue to be effective against a virus with inherent antigenic stability. In contrast, IgG prophylaxis would likely be marginally effective (or in the case of antigenic shift, ineffective) in the face of a continually mutating virus such as influenza.

The slightly higher in vitro activity of RSVIg against group A strains of RSV could be due to any of three factors. First, the use of a group A virus to screen plasma donors, while assuring high anti-A activity, would not guarantee equivalent anti-B activity. Second, the general prevalence of group A in annual epidemics might result in underrepresentation of anti-B IgG in the population of plasma donors. Third, it would be possible that group B viruses did not elicit as effective an antibody response as group A. Our data (shown in figure 1) suggest that this third hypothesis is not correct: Cotton rat antiserum to group B was more effective against homologous virus than was antiserum to group A. The first two hypotheses remain plausible, however, and we are not currently able to determine whether one is more influential than the other. The ability of RSVIg to protect against group B virus, in light of the observation that homotypic antiserum (cotton rat) against group A virus does not afford heterotypic protection, suggests that the anti-B activity of RSVIg derives from the broad repertoire of anti-B antibodies that one would anticipate in a preparation of IgG reflecting the antigenic experience of thousands of plasma donors.

The fact that the current studies in the cotton rat, which has been a highly accurate model of passive RSV immunoprophylaxis, showed RSVIg to be effective against group B and group A infections suggests that RSVIg has broad protective capability.

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References