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Mini-Review

The role of RSV neutralizing antibodies in the treatment and prevention of respiratory syncytial virus infection in high-risk children

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Introduction

Respiratory syncytial virus is the most important respiratory pathogen of infants and young children world-wide and produces annual epidemics of bronchiolitis and pneumonia. (Brandt et al., 1973; Chanock et al., 1965; Glezen et al., 1973; Kim et al., 1973; Parrott et al., 1974; Channock et al., 1961; Sims et al., 1976; Glezen et al., 1977; Coates et al., 1964; Glezen et al., 1971; Loda et al., 1968; Macasact et al., 1968; Maletzky et al., 1971; Parrott et al., 1971; Selwyn et al., 1990). Several groups of children are at particular risk for serious RSV lower respiratory tract infection (LRI). These include infants less than 6 months of age (Bruhn et al., 1977 (a); Glezen et al., 1981) and those with underlying pulmonary (Groothuis et al., 1988; Abman et al., 1988; McIntosh et al., 1973) or cardiac disease (McDonald et al., 1982) and immunodeficiency states (Hall et al., 1986; Chandwani et al., 1990). In 1954 respiratory syncytial virus was first isolated from chimpanzees with upper respiratory tract infections and was named the chimpanzee coryza agent (CCA) (Morris et al., 1956). Chanock and co-workers subsequently isolated a CCA-like virus from young infants with lower respiratory tract infections (Chanock et al., 1957; Chanock et al., 1968). They renamed it respiratory syncytial virus (RSV) for two of its properties; its recovery from children with lower respiratory tract infections, and its ability to form syncytia in cells in tissue culture.

During the early period of categorizing the clinical and laboratory features of RSV, the role of serum antibodies in the protection of the lower airway was also being defined. Several observers felt that neutralizing antibodies did not prevent re-infection and suggested that they played little part in protection of the lower airway (Beem et al., 1967; Glezen et al., 1986). Despite questions

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regarding the role of antibody response and protection, researchers worked to develop a vaccine against RSV. In 1966, the National Institute of Allergy and Infectious Diseases (NIAID) organized trials with a formalin inactivated vaccine (lot 100). Chin and co-workers studied 191 children who received injections of RSV vaccine (Chin et al., 1969). Immune response, as measured by complement fixation (CF) antibody, was good in older children; 82/120 had 4-fold or greater titer rises. Response was less satisfactory in younger children. Tragically, young vaccinated children (< 1 year) who developed natural RSV illness in the following year had more serious illness when compared with hospitalized non vaccinees. Two deaths occurred in the group followed by Kim and co-workers (Kim et al., 1969). On post mortem, both children also had evidence of secondary bacterial infection. This finding of greater RSV illness severity in vaccinees was corroborated by Kapikian and Fulginiti (Kapikian et al., 1969; Fulginiti et al., 1969).

The role of neutralizing antibody in vaccinated infants remained unclear. Kim observed that serum neutralizing antibody rises occurred just prior to and during the acute phase of RSV illness in all infants who became ill (Kim et al., 1969), however Kapikian observed a neutralizing antibody rise in only 47% of vaccinees (Kapikian et al., 1969). In the mid 1980's the link between neutralizing antibody and exaggerated RSV illness was re-examined. Murphy and co-workers measured antibody titers with enzyme-linked immuno absorbent assay (ELISA) to the RSV fusion (F) and attachment (G) glycoproteins in sera from infants originally immunized with lot 100 RSV vaccine (Murphy et al., 1986). Twenty-one young infants (2–6 months of age) developed good antibody responses to F protein but had poor responses to G glycoprotein. In contrast, 15 older children (7–40 months of age) developed good antibody responses to both F and G protein. All children studied had poor neutralizing antibody responses. Treatment of RSV with formalin appeared to have altered the epitopes of the F and/or G glycoproteins that stimulate neutralizing antibodies, with the result that the immune response consisted largely of non-neutralizing antibodies. Murphy suggested that these non-neutralizing antibodies correlated with the exaggerated lower respiratory tract disease experienced by young vaccinated infants. More recent work in mice suggested that an altered T lymphocyte response, characterized by an inadequate CD8+ cytolytic response and an enhanced CD4+ response was also partly responsible for enhanced pulmonary response in young vaccinees. (Connors et al., 1992 (a); Connors et al., 1992 (b)). This work appeared to exonerate RSV neutralizing antibodies from playing a role in exaggerated pulmonary responses. In the last decade a body of animal and human data has been developed that strongly suggests that neutralizing antibodies are beneficial in protecting the lower airway from serious RSV infection.

Immunotherapy

In 1978 Prince and co-workers first described the cotton rat model for RSV infection (Prince et al., 1978). Infant and adult cotton rats develop RSV replication in both the upper and lower respiratory tracts following intranasal inoculation. This model is semi-permissive to RSV replication, develops characteristic histological changes in the nasal turbinates, trachea and lungs, and is able to mount neutralizing antibody response to RSV infection. Cotton rats also develop complete resistance to subsequent RSV infection for up to 18 months after being inoculated with live RSV virus and this resistance correlates well with serum neutralizing antibody levels (Prince et al., 1983). When cotton rats are immunized with formalin-inactivated RSV vaccine, and subsequently challenged with RSV, they develop pulmonary disease which is histologically similar to that which was observed in vaccinated children dying of RSV pneumonia (Prince et al., 1986). An important weakness in the model is that while cotton rats develop characteristic pathological changes in the lungs, they do not develop clinical disease. Despite this, the cotton rat has proved to be an excellent model for experiments defining the role of RSV neutralizing antibody. Prince and co-workers began studying the impact of human intravenous gammaglobulin (IGIV) on the prevention of RSV respiratory tract infection in this model (Prince et al., 1985 (a); Prince et al., 1985 (b); Prince et al., 1987). Human convalescent antiserum (Sandoglobulin) with an RSV neutralizing antibody titer of 1:2800 in a 5% solution was administered intraperitoneally to infant cotton rats (0.5 ml/10 g body weight) 24 h after an intranasal challenge with 10^4 pfu of RSV. The level of virus reduction in pulmonary and nasal tissue was directly proportional to the RSV neutralizing antibody titer in serum. Reduction of virus was always greater in the lung than in the nose (Prince et al., 1985 (a)) and the serum neutralizing antibody titer necessary for eradication of virus from the lower respiratory tract was approx. 1:600. This work suggested that commercial IGIV could not produce serum levels that would have a significant impact on treatment of RSV lower respiratory tract disease. No evidence of the enhanced pulmonary pathology that had occurred with the formalin inactivated vaccine (Prince et al., 1985 (b)) was observed on rechallenge with RSV. IGIV was also administered intratracheally and proved to be 180-fold more potent in reducing RSV replication in the lung (Prince et al., 1987). Finally, experiments with several different monoclonal antibodies to RSV F and G glycoproteins also demonstrated efficacy in reducing viral titers in the cotton rat lung (Walsh et al., 1984).

The demonstration that IGIV and monoclonal antibody is effective in reducing viral replication is important because there is currently no completely effective treatment for serious RSV illness in humans. Ribavirin, a synthetic nucleoside, is the only antiviral drug approved by the Food and Drug Administration for the treatment of RSV lower respiratory tract infection (Hall et al., 1983). Several studies have demonstrated efficacy, particularly when instituted early in the course of illness, and when given to ventilated infants

(Groothuis et al., 1990 (b); Outwater et al., 1988). However, safety and cost concerns have lead investigators to look for alternative or adjunctive treatment measures (Wald et al., 1988). Animal experiments suggest that a combination of immune globulin and ribavirin may have a synergistic effect (Gruber et al., 1987) in the treatment of serious RSV disease.

Epidemiologic observations in humans also suggest that RSV neutralizing antibody levels play a role in amelioration of RSV disease. Infants with higher maternal antibody levels develop RSV infections later and have less severe illness (Glezen et al., 1981; Jacobs et al., 1971; Bruhn et al., 1977 (b); Lampricht et al., 1976; Ogilvie et al., 1981; Ward et al., 1983). In contrast, preterm infants with low maternal antibody levels develop more severe RSV illness than do their full term counter-parts, even in the absence of chronic lung disease (Ogilvie et al., 1981; Ward et al., 1983; Hall et al., 1979). In a IGIV therapy study conducted by Hemming and co-workers, 35 previously healthy infants and children received either 2 gm/kg of 5% IGIV (Sandoglobulin), (RSV neutralizing titer 1:5000), or a comparable amount of albumin placebo (Hemming et al., 1987). Infants receiving IGIV experienced significant reductions in nasal shedding of RSV ($P<0.05$) and improvement in oxygen saturation ($P<0.05$). However, no difference in disease severity was observed as defined by number of hospital days. This study therefore demonstrated IGIV infusions to be safe, but clinical efficacy was not conclusively demonstrated.

Currently, multicenter trials involving high-risk and normal infants hospitalized with moderate to severe RSV lower respiratory tract infection are in progress. These studies are placebo-controlled and blinded; children receive either 1.5 gm/kg of an RSV-enriched immune globulin (RSVIG) or a comparable volume of intravenous albumin placebo. The results of these trials may answer the question of whether there exists a role for RSVIG in the treatment of serious RSV lower respiratory tract illness.

Immunoprophylaxis

Experiments in the cotton rat and other animal models have demonstrated IGIV to effectively prevent RSV lower tract disease. In contrast to the exceedingly high neutralizing antibody levels needed for therapy, prophylaxis with RSV neutralizing antibody titers greater than 1:350 were associated with complete protection of the cotton rat lung (Prince et al., 1983).

Epidemiologic data in humans also suggests that immunoprophylaxis against RSV infection in young children should be effacious. In 1971, Jacobs and co-workers observed that RSV infection in full term infants during the first month of life was less severe than that observed in older infants where maternal antibody titers were lower (Wald et al., 1988). Parrott and co-workers observed attenuation of RSV infection of full term infants during the first month of life (Parrott et al., 1973). They retrospectively correlated neutralizing antibody with

disease severity and found protective levels in infants to be 1:300–1:400: levels similar to those required for protection in the cotton rat lung (Prince et al., 1985 (a)). Additional investigators have reported an inverse correlation between RSV illness severity and RSV neutralizing antibody titers in infants and children (Gruber et al., 1987; Jacobs et al., 1971; Bruhn et al., 1977; Lampricht et al., 1976).

These data combined with the development of several commercial intravenous gamma globulin preparations provided the opportunity to test the hypothesis that IGIV, given in a dosage adequate to produce titers of RSV neutralizing antibodies $\geqslant$ 1:350, might ameliorate or prevent severe RSV lower respiratory tract disease in high-risk children. Hemming and co-workers tested commercial preparations of IGIV and found that while several lots contained sufficient amount of RSV antibody there was a significant lot to lot variability (Hemming et al., 1987).

In 1988 NIAID approved funding for a multicenter trial to test safety and feasibility of IGIV infusions to prevent serious RSV lower respiratory tract infection in high-risk infants. These patients included premature infants less than 6 months old at study onset, children with bronchopulmonary dysplasia (chronic lung disease of prematurity), and children with congenital heart disease, particularly those with pulmonary hypertension. These three groups experience significant morbidity from RSV illness (Groothuis et al., 1988; Abman et al., 1988; McIntosh et al., 1973) even with repeated infections (Groothuis et al., 1990 (a)). In a Phase I open trial performed by Groothuis and co-workers, a commercial IGIV preparation (Gamimune-N 5% solution), with a titer of 1:1100 of RSV neutralizing antibody, was infused at three separate doses (500 mg/kg, 600 mg/kg, 750 mg/kg) ($n=23$) at three different centers (Groothuis et al., 1991). IGIV was infused monthly (3 to 5 infusions) throughout the respiratory season. Complications were few, with intravenous cannulation being the greatest problem. Twelve children developed RSV infections over two respiratory seasons. Ten illnesses were mild and one moderately ill child required hospitalization. One ventilator-dependent child died of progressive RSV illness despite receiving two infusions of 500 mg/kg of IGIV. However, even at 750 mg/kg (the highest dose) a mean peak titer of only 1:100 was achieved. This was one-third the level proven to protect the lower airway in animal and human studies. A subsequent study performed by Meissner and co-workers also utilized Gamimune-N with an anti-RSV titer of 1:950 in a 5% solution (Meissner et al., 1993). Children with bronchopulmonary dysplasia or severe congenital heart disease received either IGIV at 500 mg/kg in monthly infusions ($n=25$) or no IGIV ($n=24$). No adverse reactions occurred during the study period. Twelve culture proven RSV illnesses occurred; six in each group. There was a trend toward less severe RSV illness as measured by hospital days (8.8 $\pm$ 5 days (treated) vs. 12.8 $\pm$ 7.6 days (controls)); this did not reach statistical significance. Again, peak neutralizing antibody titers were low ($<$ 1:100).

Concern about the low RSV neutralizing antibody titers produced by

commercial IGIV preparations as well as the lot to lot variability prompted the development of a respiratory syncytial virus-enriched immune globulin (RSVIG). Preparation of RSVIG is identical to that of commercial IGIV except that plasma is obtained from donors specifically selected for their high serum RSV neutralizing antibody titers (Siber et al., 1992). RSVIG was utilized for the subsequent 3 year Prophylaxis trial (Groothuis et al., 1993). In this blinded, randomized trial, 249 children, who were preterm (<6 months), had bronchopulmonary dysplasia or congenital heart disease, were enrolled over a 3 year period (1989–1992). One group received a high dose of 750 mg/kg/dose (15 ml/kg/dose) of RSVIG, a second group received a low dose of 150 mg/kg/dose (3 ml/kg/dose) of RSVIG and a control group received no RSVIG. There was no difference in overall RSV incidence between groups. However, children receiving high dose RSVIG had a reduction in both the incidence (64%) and severity (72%) of RSV lower respiratory tract disease. High dose recipients had significantly fewer instances of moderate to severe RSV lower tract infection ($P=0.01$). There also was a significant reduction in hospitalizations (63%, $P=0.02$), hospital days (63%, $P=0.02$), and ICU days (97%, $P=0.05$). Less ribavirin use was also observed in high dose infants ($P=0.05$) than in control children. In contrast, low dose RSVIG had little impact on these outcome measures. RSVIG had few adverse effects (19 out of 580, 3%); these consisted of mild reversible fluid overload, fever, and mildly decreased oxygen saturations. Six deaths occurred; 3 in the low dose group and 3 in the high dose group ($P=0.15$). No death was found to be attributed to either RSVIG use or RSV illness. RSVIG treated children who developed RSV illness in a subsequent season had no more severe disease than did control children.

Summary

Studies assessing the use of RSV immune globulin in the treatment of RSV illness are not yet completed. However, a large multicenter trial demonstrated that prophylaxis with RSV immune globulin was safe and efficacious in prevention of serious RSV disease in high-risk infants. Refinements in the practical application of RSVIG are needed, as intravenous lines are difficult to place and maintain in these fragile infants. With the development of concentrated polyclonal and/or effective monoclonal antibody preparations, it may be possible to immunize intramuscularly (Tempest et al., 1993; Barbas et al., 1992). The efficacy of RSV-specific monoclonal antibodies must still be defined, and the appropriate viral epitopes targeted. While these issues still need to be addressed, it is exciting to have finally produced a safe and effective way to prevent severe RSV disease in high-risk young children.

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