The Role of Neutralizing Antibodies in Protection of American Indian Infants Against Respiratory Syncytial Virus Disease

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Background: Navajo and White Mountain Apache infants have respiratory syncytial virus (RSV) hospitalization rates 2–5 times that of the general U.S. infant population. To evaluate whether these high rates can be attributable to low concentrations of maternally derived RSV neutralizing antibodies, we conducted a case–control study.

Methods: Study subjects enrolled in a prospective, hospital-based surveillance study of RSV disease and a group randomized clinical trial of a 7-valent pneumococcal conjugate vaccine. Cord blood specimens were assayed for neutralizing RSV antibody titers. Infants hospitalized with a respiratory illness had a nasal aspirate obtained to determine whether RSV was present. Infants with an RSV respiratory hospitalization were matched by date of birth and geographic location to infants who did not have an RSV hospitalization before 6 months of age.

Results: For every 1 $\log_2$ increase in titer of cord blood RSV neutralizing antibodies there was a 30% reduced risk of hospitalization with RSV (OR = 0.69, $P = 0.003$). However, among infants hospitalized with RSV, there was no association between cord blood RSV neutralizing antibody and the severity of the RSV illness.

Conclusions: These findings indicate that American Indian infants with high concentrations of maternally derived RSV neutralizing antibodies are protected from RSV hospitalization before 6 months of age. However, these antibodies do not modify the severity of illness once disease has occurred. The basis for elevated rates of RSV disease among American Indian infants cannot be attributed to a failure of maternal RSV neutralizing antibodies to confer protection.

Key Words: respiratory syncytial virus, American Indians, neutralizing antibody, infants

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Respiratory syncytial virus (RSV) disease is a significant cause of morbidity and mortality among young infants worldwide. In the United States, RSV bronchiolitis is the leading cause for hospitalization of infants.[1] A majority of RSV hospitalizations and RSV-associated mortality occurs in infants less than 6 months of age.[1–4] RSV causes a disproportionate amount of morbidity and mortality among American Indian and Alaska Native populations, with hospitalization rates 3–5 times higher than in the general U.S. population.[2,5–8] The etiology of this excess morbidity from RSV disease is not well understood. Although risk factors, such as wood-burning stoves and household crowding, have been associated with a higher risk of respiratory infections and RSV disease in native populations, they do not fully explain the significantly higher rates of disease.[9,10] It is also clear that it is not attributed to a high prevalence of underlying medical conditions, because the high rates of disease are occurring predominantly among an otherwise healthy full-term infant population.

Navajo and White Mountain Apache (WMA) infants have specifically been found to be at high risk of RSV hospitalization. Hospitalization rates among Navajo and WMA infants have been reported to be 2–5 times greater than that of the general U.S. population.[6,11] Navajo children <1 year of age had a rate of 78.1 per 1000 children, and the WMA infants had a rate of 164.3 per 1000 children.[11] Of the hospitalized infants, 82% were 6 months of life or younger and only 14% had a medical condition recognized as a risk factor for severe RSV disease.

The role of maternally-derived RSV-specific neutralizing antibody in the protection against hospitalization for RSV disease and/or severe RSV disease in infants has been explored in several studies. However, although some studies found a protective effect of RSV-specific neutralizing antibody against RSV disease, others were only able to show protection from severe disease.[12–15] To our knowledge, no studies have been conducted among Navajo and WMA infants to investigate the relation between maternally derived RSV neutralizing antibody and the occurrence and severity of RSV disease. Because the exploration of the increased risk of RSV disease in this population is not known, we hypothesized that low titers of maternal antibody, low rates of transplacental transfer of RSV-specific antibody, failure of maternally-derived antibodies to protect the infant, or an increased rate of decline of maternally derived antibodies could play a role in the increased risk of RSV disease in this

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population. The goal of this study was to address these hypotheses by assessing the relationship between RSV neutralizing antibody in cord blood and the occurrence and severity of RSV hospitalization among Navajo and WMA infants less than 6 months of age.

MATERIALS AND METHODS

Study Site. This study was conducted among Navajo and WMA infants residing on the Navajo Nation or the WMA reservations, both located in the Southwestern United States. Healthcare on these 2 reservations is administered by the Indian Health Service. The Navajo Area is comprised of 8 Indian Health Service units, 6 of which operate hospitals. The WMA reservation has a single service unit with 1 hospital in Whiteriver, AZ. Three service units on the Navajo reservation (Chinle, Gallup, and Fort Defiance) and the 1 service unit (Whiteriver) on the WMA reservation participated in this study.

Recruitment. We conducted a case–control study among subjects who were enrolled in either a prospective, population-level, hospital-based surveillance study to determine the rate of RSV-associated acute lower respiratory tract infections among Navajo and WMA children younger than 2 years or a group randomized trial to assess the efficacy of a 7-valent pneumococcal conjugate vaccine[11,16] Some subjects were enrolled in both of these studies. These studies were conducted concurrently from 1997–2000. Regardless of study enrollment, all activities pertaining to data collection and this analysis were identical. Mother–infant pairs were recruited immediately after delivery or when an infant was admitted to the hospital with a respiratory illness. Recruitment took place either on the obstetrical ward, on the pediatric ward, or via a home visit. Written informed consent was obtained for all mother–infant pairs before enrollment into the study. This study was approved by the Phoenix area Institutional Review Board (IRB), Navajo Nation IRB, WMA Tribal Council, and Johns Hopkins IRB.

Clinical Data. Cord blood and maternal venous blood (up to 7 days postpartum) samples were collected year round from all women delivering at the participating facilities. Surveillance for hospitalizations associated with respiratory illnesses in children less than 2 years of age was conducted at these Indian Health Service hospitals and nearby private facilities from October 1 through March 31 of each year to coincide with the RSV season. Surveillance was conducted for respiratory hospitalizations with a diagnosis of RSV, bronchiolitis (cause not otherwise specified), pneumonia, hypoxia, respiratory distress, or reactive airway disease. Whenever a subject had a respiratory illness admission, a nasopharyngeal (NP) aspirate was obtained to determine the RSV status of the subject upon admission. NP aspirates were collected within 72 hours of the admission date.

Laboratory Methods. NP aspirates were tested within 2 hours of collection using commercial RSV-specific enzyme immunoassay kits. NP aspirates collected between October 1997 and March 1999 were tested using the Abbot EIA Test Pack (Abbott, Oak Park, IL). This kit was no longer commercially available beginning in the fall of 1999; therefore, for the 1999–2000 season the Directogen kit (Becton-Dickinson, Franklin Lakes, NJ) was used.

Cord and postpartum bloods were centrifuged for 5 minutes at 3000 rpm and aliquots of sera were then frozen at −70°C. Serum specimens were later shipped on dry ice to the laboratory at Johns Hopkins University for RSV antibody testing. RSV neutralizing antibody was measured in the cord and postpartum blood samples by complement-enhanced 60% plaque reduction neutralization assay.[17] Briefly, serum was diluted 1:10 and was heat inactivated at 56° for 30 minutes. Four-fold serial dilutions of the sera were incubated with approximately 50–100 plaque forming units of RSV virus. The presence of virus plaque was determined by plaque assay on Hep-2 cell monolayers in 24 well plates using an immunostain method. Neutralizing antibody titer was defined as the reciprocal of the test serum dilution at which the number of input virus infectivity expressed as plaque forming units was reduced by 60% in comparison with the number of plaques observed with negative control serum. Infant and maternal sera were tested at different times.

Assessment of Disease Severity. For each subject who had a RSV positive NP aspirate, a review of the medical record was conducted. Information collected included age, gestational age, birth weight, gender, area of residence, severity of disease, and underlying chronic conditions. To standardize our assessment of severity, we used a previously published index of severity of RSV disease in hospitalized children, in which 1 point each was assigned for apnea, pH <7.35, P_{CO2}, >45, SAO_2 $<87\%$, and length of stay >5 days, and 2 points were assigned for mechanical ventilation. Apnea had to be a recorded event in the hospital or emergency room record. Arterial blood gases were not a required element of the scale and were only included when documented in the medical record. Points were summed for each subject, with a total potential score of 7 points. Severe disease was defined as a total score of ≥ 3 points.[18]

Statistical Analysis. RSV disease was categorized as severe (score ≥ 3) or nonsevere (score <3). Logistic regression was used to examine the association between RSV neutralizing antibodies in the cord blood and severity of RSV hospitalization. Only subjects who were hospitalized with an RSV associated infection before 6 months of age were included in the analysis as cases.

To explore the association between risk of RSV hospitalization and cord blood, a matched case control design was used. Cases were defined as any Navajo or WMA infant who was hospitalized for RSV infection before 6 months of age. Controls were defined as Navajo or WMA infants without a RSV associated-hospitalization before 6 months of age. A cord blood specimen had to be available for cases and controls to be included in the analysis. A 1:2 case to control ratio was used. Cases were matched to controls based on service unit of birth and date of birth (± 15 days). Univariate and multivariate conditional logistic regression was used to evaluate differences in RSV neutralizing antibody titers in cord blood between cases and controls which were reported as odds ratios. Potential confounders such as gender, gestational age, birth weight, and breast-feeding status were evaluated in the model. Ninety-five

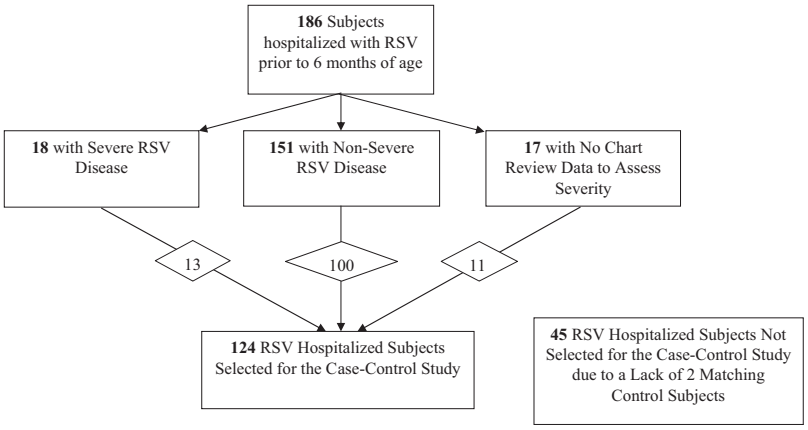

FIGURE 1. Flow chart of study subjects for each analysis.

percent confidence intervals that did not include 1.0 were considered statistically significant.

To assess transplacental transfer of RSV neutralizing antibody, we examined the relationships between titers of antibody in maternal serum and in corresponding cord blood specimens. For each pair, we calculated the ratio of cord blood antibody titer and maternal antibody titer. For the entire set of specimens, we also calculated geometric mean titer (GMT) of RSV neutralizing antibodies in maternal postpartum and cord blood samples. Placental transfer of RSV neutralizing antibodies was defined as the geometric mean of the ratio of cord blood titers and postpartum titers.

A flowchart of the study subjects and which analysis they were used for is shown in Figure 1. Stata 8.1 (Stata Corp, College Station, TX) software program was used for the statistical analysis.

RESULTS

Rate of Transplacental Transfer of RSV Neutralizing Antibody. Fifty-five mother–infant pairs had their postpartum and cord blood sera assayed for RSV neutralizing antibodies for the transplacental transfer analysis. A plot of each postpartum and cord blood RSV neutralizing antibody titers for mother–infant pairs are shown in Figure 2. The GMT of RSV neutralizing antibodies in the maternal postpartum sera was 469.5 (95% CI: 382.3–576.6), and the GMT in the cord blood sera was 558.8 (95% CI: 418.2–746.7). The transplacental transfer rate of these antibodies was therefore 1.19. For individual mother–infant pairs, the range of rates of transplacental transfer was 0.08–8.40.

Association Between the Titer of RSV Neutralizing Antibody in Cord Blood and Severity of RSV Disease. A total of 169 subjects who were admitted with an acute lower respiratory tract infection caused by RSV infection before 6 months of age and with a cord blood specimen available for RSV neutralizing antibody testing were included in the analysis. Of those 169 subjects, 18 (10.7%) had severe RSV disease. The mean age at hospitalization was statistically similar between the severe and nonsevere cases, 55.3 and 77.6 days, respectively ($P = 0.07$). Sex, mean gestational age, and proportion born premature were all statistically similar between the severe and nonsevere cases (data not shown). The severe cases were significantly more likely to have apnea compared with the nonsevere cases (38.9% versus 3.3%, respectively, $P < 0.0001$). The GMT of RSV neutralizing antibodies among subjects with severe RSV disease versus those with nonsevere RSV disease was 386.1 and 385.6, respectively. There was no association between severity of RSV disease and the cord blood RSV neutralizing antibody titer or between the likelihood of having severe RSV disease and the likelihood of having a cord blood titer greater than 1:500 (Table 1).

Risk of Hospitalization and Cord Blood RSV Neutralizing Antibody Titers. To investigate the association between RSV-specific neutralizing antibodies in cord blood and risk of hospitalization with an RSV associated acute lower respiratory tract infection, we conducted a case–control analysis. We identified 124 cases that were matched to 248 controls. The cases and controls were well matched by gender, birth hospital, birth weight, age, and gestational age (Table 2).

The GMTs of cord blood RSV neutralizing antibody were statistically different between cases and controls. The

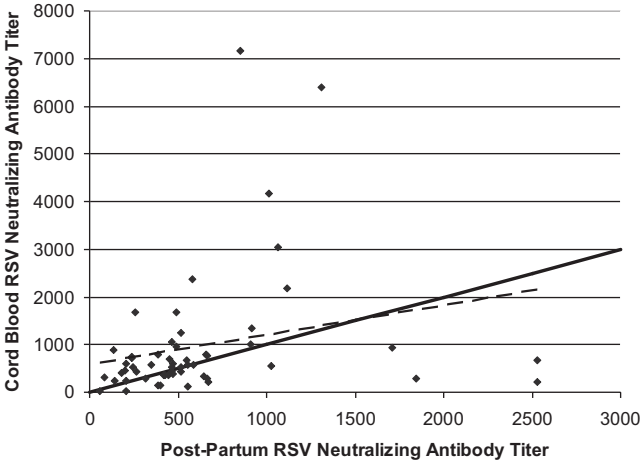

FIGURE 2. Comparison of post-partum and cord blood RSV neutralizing antibody titers for the 55 mother–infant pairs. The solid line represents the line of identity, whereas the dashed line is the line of best fit for the data.

TABLE 1. RSV-Specific Neutralizing Antibodies in Cord Blood of Infants Less than 6 Months of Age by Severity of RSV Disease

	Disease Severity of RSV Cases	
	Severe (N = 18)	Non-Severe (N = 151)
RSV neutralizing antibody titers		
Minimum	33	15
Maximum	4230	4988
GMT (95% CI)	386.1 (225.0–662.5)	385.6 (336.8–441.5)
Odds ratio (*P* value)	1.005 (0.996)	1.000
Cord blood titer ≥500		
N (%)	7 (39%)	57 (38%)
Odds ratio (*P* value)	1.000 (0.925)	1.000

TABLE 2. Demographics of RSV Cases and Matched Controls

Characteristic	Cases (N = 124) % or Mean	Controls (N = 248) % or Mean
Gender		
Males	49.4%	50.0%
Site		
Chinle	31.5%	31.5%
Fort Defiance	18.6%	18.6%
Gallup	31.5%	31.5%
Whiteriver	18.6%	18.6%
Breast fed		
Yes	5.6%	6.5%
No	26.6%	26.2%
Missing	67.7%	67.3%
Birth weight* (grams)	3310 (n = 122)	3347 (n = 219)
Gestational age* (weeks)	39.1 (n = 122)	39.2 (n = 203)

*Difference in birth weight and gestation age between cases and controls not statistically significantly different, conditional logistic regression *P* value = 0.81 and 0.52, respectively.

GMT of cases was 392.1 (95% CI: 336.9–456.4) compared with 538.0 (95% CI: 470.9–609.2) among controls. When the proportion of subjects with RSV neutralizing antibody titers ≥500 was evaluated, fewer cases than controls had such a value (OR = 0.61, *P* = 0.034) (Table 3). We used conditional logistic regression to further explore the relationship between cord blood RSV titers and protection against hospitalization. We found that for every $\log_2$ increase in titer of cord blood RSV-specific neutralizing antibody, subjects were over 30% less likely to be hospitalized with an RSV infection before 6 months of age (OR = 0.69, *P* = 0.003; Table 3). We did not find any association between gestational age, birth weight, or breast-feeding status and the risk of RSV hospitalization (data not shown).

TABLE 3. RSV-Specific Neutralizing Antibody Titers in Cord Blood of RSV Cases and Matched Controls

	Cases (N = 124)	Controls (N = 248)
RSV neutralizing antibody titers		
Minimum	22	16
Maximum	4230	14797
GMT (95% CI)	392.1 (336.9–456.4)	538.0 (470.9–609.2)
Odds ratio (*P* value)	0.69 (0.003)	1.00
Cord blood titer ≥500		
N (%)	51 (41.1)	130 (52.4)
Odds ratio (*P* value)	0.61 (0.034)	1.00

DISCUSSION

This study investigated the role of cord blood RSV neutralizing antibody in protecting Navajo and WMA infants less than 6 months of age from hospitalization with severe and nonsevere RSV infection. This population has been shown to be at high risk for RSV hospitalization, but no studies have specifically addressed the role of maternal antibodies in the protection of such infants from RSV hospitalization.[11]

We found a similar GMT for RSV neutralizing antibody in the maternal postpartum serum and a slightly higher transplacental transfer rate of RSV neutralizing antibodies among our population of infants compared with previous reports. A study by Suara et al[19] reported the efficiency of transplacental transfer RSV neutralizing antibodies to be 0.99 and 1.02 for RSV/A virus and 0.99 and 1.03 for RSV/B virus in The Gambia and Houston, respectively. The transplacental transfer rate for our subjects was slightly higher at 1.19. In addition, maternal postpartum RSV neutralizing antibody titers in our study were similar to those reported previously.[19] These findings lead us to reject 2 of our original hypotheses: low titers of maternal antibody are not present and low transplacental transfer rates are not occurring in this population. Therefore, these factors do not seem to be contributing to the high risk of RSV disease among these young infants.

When looking only at infants who were hospitalized with RSV infections, we were unable to see a protective association between the magnitude of cord blood RSV neutralization antibodies and the severity of illness (OR = 1.01, *P* = 0.996). This lack of association contrasts with findings in other populations.[14,15] The study by Lamprecht et al[14] found that among infants with RSV pneumonia but not RSV bronchiolitis, there was a significant inverse relationship between severity of illness and the level of maternal antibody. Glezen et al[15] examined infant sera obtained shortly before hospitalization for RSV infection and found that infants with low neutralizing antibody titers just before illness had more severe disease than infants with high titers and that severe illness did not occur in infants with titers of 1:16 or greater.

There are several possible reasons why we did not find a protective effect of cord blood RSV neutralizing antibodies with severity of RSV hospitalization. The amount of RSV neutralizing antibody in the cord blood may not accurately reflect the level present just before infection; Titers of RSV neutralizing antibody could have decreased from the time of birth. Another possible reason is that our definition of severity differed from the severity scales used in previous studies. All of our cases were hospitalized cases, and we assessed severity among these hospitalized cases. It may be that cord blood titers predict severity when a broader range of disease severity (ie, inpatient and outpatient episodes) is considered. We were unable to duplicate the scales used in these previous studies, because we did not collect the same clinical data that was used in those investigations. In addition, we only had 18

severe inpatient cases which could have limited the power of the study to detect a difference in the 2 groups. However, this is unlikely because the GMTs were very similar for the 2 groups. Finally, it has become increasingly clear that RSV disease is mediated by a complex interplay between viral replication, the immune response, and airway size.[20] Neutralizing antibody can only influence the level of RSV replication, and it may be that once the virus has reached the lungs (as is the case in most infants hospitalized with RSV infection), factors other than the level of viral replication ultimately determine disease severity.

With our case–control analysis, we were able to show that cord blood RSV neutralizing antibodies were protective against hospitalization with RSV, regardless of severity. We found a 30% reduction in the odds of being hospitalized with RSV for every 1 $\log_2$ increase in cord blood RSV-specific neutralizing antibody (OR = 0.69, $P = 0.003$). These results were similar to findings in other studies of the general U.S. population. Ogilvie et al[12] investigated infection with RSV and found significantly higher maternal RSV-specific IgG titers among mothers whose babies were not hospitalized with RSV compared with mothers of babies who were hospitalized with RSV during the first 6 months of life. A more recent study by Piedra et al[13], showed that for every 1 $\log_2$ increase in neutralizing antibody titer to RSV/A and RSV/B there was a significant increase in odds of not having an RSV-associated hospitalization (OR = 1.22 and 1.25 for RSV/A and RSV/B, respectively). However, a study by Bulkow et al[9] among Alaska Native infants was unable to find any association between cord blood RSV neutralizing antibody and RSV hospitalization. This conflicting finding may be because the Bulkow study had a large percentage of premature infants; the infants in their study had GMTs lower than those found in our study and perhaps these titers did not reach a sufficient threshold for protection. In addition, this population of Alaska Native infants has a much higher risk of RSV than our population and this intense burden of disease may outweigh the protection maternally derived antibody gives infants in lower risk populations.

This study has some limitations. Cases had to be enrolled in the prospective, population based study, where as controls came from both the prospective population based study and from a concurrent randomized clinical trial. If controls selected from the randomized clinical trial had a different risk of RSV disease than other controls or cases, then biases may have been introduced. However, we found that an equal percentage of cases and controls were dually enrolled in both studies, which should minimize any selection bias that may have occurred. The RSV antigen detection assays used in the study had a reported sensitivity and specificity of 91% and 83%, respectively for the Abbott RSV ELISA assay and 93–97% and 90–99%, respectively, for the Directigen RSV ELISA assay. Therefore, the potential exists for misclassification of cases and controls. However, because the sensitivity and specificity of the 2 assays are relatively high, it is unlikely that this occurred with great frequency. Finally, the Abbott RSV ELISA and Directigen tests do not distinguish between RSV A and RSV B infections, and we only tested our sera for titers of neutralizing antibody to RSV A.

Because RSV A and RSV B cocirculate each year, it is likely that some hospitalizations were the result of RSV B infections, and we could not determine the impact of neutralizing antibody on prevention of those infections.

This study demonstrates that high rates of RSV hospitalization occur among infants in this population in the setting of protective effects of maternally acquired antibody. Future efforts at disease prevention in these populations and populations with similar disease risk epidemiology should focus on sustaining the protective RSV antibody titers throughout infancy through passive or active immunization strategies.

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