Safety and Immunogenicity of a Purified F Protein Respiratory Syncytial Virus (PFP-2) Vaccine in Seropositive Children with Bronchopulmonary Dysplasia

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Respiratory syncytial virus (RSV) causes serious respiratory illness in preterm children with bronchopulmonary dysplasia. In a prospective randomized placebo-controlled trial, 21 children received one dose of PFP-2 (purified fusion [F] protein) vaccine or influenza vaccine (placebo). Children were followed for adverse reactions and RSV illness over two respiratory seasons. Sera were obtained for determination of IgG titers to RSV F protein and neutralizing antibody titers before and 1, 6, and 12 months after vaccination. Adverse reactions were few. Four-fold F protein rises occurred in 9 of 10 PFP-2 and 0 of 11 placebo recipients. Six PFP-2 recipients had low prevaccination neutralizing antibody titers (<1:450); all had 4-fold rises. By 12 months, F protein and neutralizing antibody titers in all 21 children were similar. RSV illness occurred in 6 of 11 placebo versus 1 of 10 PFP-2 recipients (P = .06); 1 placebo child required hospitalization. PFP-2 vaccine appears safe and immunogenic and may protect children with bronchopulmonary dysplasia against serious RSV disease on reinfection.

Methods

Study population. Children >12 months of age with bronchopulmonary dysplasia were the subjects of this study. Bronchopulmonary dysplasia was defined as chronic lung disease occurring as a result of premature delivery and ventilatory support and an ongoing oxygen requirement of at least 1 month’s duration [4]. All children had had culture- or serologically proven RSV infections in a previous respiratory season. All had received influenza vaccination in the previous year. All study subjects were followed as outpatients in the Neonatal High Risk Follow Up Program at Children’s Hospital, Denver.

Study design. This trial was initiated in October and November of 1991. Children were randomized to receive intramuscular injections of either 0.5 mL of RSV PFP-2 vaccine (50 μg) or 0.5 mL of trivalent influenza vaccine (both from Wyeth Lederle Vaccines and Pediatrics) in a double-blind placebo-controlled fashion. A single lot of either PFP-2 or the appropriate influenza vaccine was used for all study children in a single season. All study children also received an unblinded dose of trivalent influenza vaccine 4–6 weeks after study vaccine was given. The administration of two 0.5-mL doses of influenza vaccine, received by the placebo group, has been demonstrated to be safe and to provide additional protection in this population [13]. Families were provided a questionnaire to record all potential adverse reactions for 3 days after vaccination. They were asked to notify the study coordinator and to bring the child in to be examined if any unusual reaction occurred. Systemic reactions reported included irritability, drowsiness, abnormal cry, vomiting, seizures, and urticaria. Local reactions included erythema, induration, and pain or tenderness at the injection site. Sera were obtained immediately before vaccination and at 1, 6, and 12 months after vaccination. These sera were analyzed for IgG titers to F glycoproteins and for neutralizing antibody titers against the RSV A2 strain.

Each cohort was followed through two RSV respiratory seasons. Weekly telephone calls were made by a blinded study nurse. Children with symptoms of cough, coryza, breathing difficulty, or fever...
were evaluated within 24 h by a physician. A nasal wash specimen was obtained on all ill visits for the detection of RSV by rapid antigen and culture. If a child was RSV-positive, the severity of the illness was assessed by a previously developed respiratory score [13]. This score rates severity of RSV respiratory disease on a 1–5 scale: 0 = no symptoms, 1 = upper respiratory tract infection, 2 = mild lower respiratory tract infection, 3 = moderate lower respiratory infection, 4 = serious lower respiratory infection, 5 = lower respiratory infection requiring assisted ventilation [13].

**Vaccine, virus identification, and serologic assays.** The PFP-2 vaccine, developed and produced by Wyeth-Lederle Vaccines and Pediatrics, is >99.9% F glycoprotein of the A2 strain of RSV. PFP-2 contains <0.1% of the G glycoprotein. The F protein was purified by ion-exchange chromatography and formulated on 0.5-mg doses of aluminum hydroxide. One lot of PFP-2 vaccine was used throughout each study season. Rapid identification of RSV in nasopharyngeal secretions was made by EIA (Abbott, Abbott Park, IL), and RSV was cultured by standard methods [11]. Sera were assayed for IgG antibodies to the F glycoprotein by standardized methods [13]. Neutralizing antibody against RSV strain A2 was measured by use of a microneutralization assay also described previously [12].

**Results**

Twenty-one children participated in this study. Ten children received PFP-2 and 11 received the trivalent influenza vaccine as placebo. Mean age at study entry (32.2 vs. 30.0 months), sex, race, previous RSV hospitalizations, day care exposure, passive smoke exposure, and number of siblings in the home were comparable between groups.

**Safety.** Adverse reactions were few. Systemic acute reactions included irritability (2 infants in the PFP-2 group, 2 in the placebo group) and drowsiness (1 infant in the PFP-2 group). Local reactions included pain and tenderness (1 patient in each group) and redness (1 patient in the PFP-2 group). No child developed evidence of enhanced illness on RSV reinfection during a follow-up respiratory season. The number and severity of RSV infections that occurred in the 21 study subjects during the subsequent respiratory season were tabulated. Six placebo recipients developed clinical RSV infection compared with only 1 PFP-2 recipient \( (P = .06, \text{ Fisher’s two-tailed test}) \). The placebo group comprised 4 patients with upper respiratory tract infections (respiratory score, 1), 1 child with mild lower respiratory infection (respiratory score, 2), and 1 child who developed moderate lower respiratory infection (respiratory score, 3) and required hospitalization. The single PFP-2 recipient, who was RSV-positive, presented with mild lower respiratory infection (respiratory score, 2).

**Immunogenicity.** Table 1 summarizes the F glycoprotein–specific and neutralizing antibody titers in all 21 study patients. Four-fold immune responses to F protein antibody were observed in 9 of 10 PFP-2 recipients and 0 of 11 placebo recipients. Four-fold RSV neutralizing antibody responses occurred primarily in 6 PFP-2 recipients with low prevaccination RSV titers (<1:450). At 1 and 6 months after vaccination, PFP-2 recipients had significantly higher F protein and neutralizing antibody titers than did placebo recipients.

**Discussion**

Children with chronic lung disease are at high risk for severe recurrent RSV illness. Preterm infants with bronchopulmonary dysplasia constitute one of the largest groups of such populations [4, 5]. Investigators have recently assessed the safety and immunogenicity of purified F protein vaccines in a variety of seropositive infant populations. Trials using a PFP-1 vaccine (containing 95% F protein and 5% G glycoprotein) have found this vaccine to be safe and immunogenic in healthy seropositive children [8, 9, 11]. A more highly purified and homogeneous F protein vaccine has recently been developed by use of ion-exchange chromatography. This PFP-2 vaccine, containing >99.9% F glycoprotein, has been demonstrated to be safe and immunogenic in seropositive children with cystic fibrosis [10].

The present trial demonstrates the safety and immunogenicity of the PFP-2 vaccine in a seropositive population of infants with bronchopulmonary dysplasia prospectively observed over two respiratory seasons. Immediate adverse vaccine reactions were minimal, and enhanced RSV illness in a subsequent season was not observed. Four-fold F protein antibody titer rises occurred in all PFP-2 recipients. The highest RSV neutralizing antibody responses were observed primarily in those PFP-2 vaccine recipients with low prevaccination titers (<1:450). This finding is comparable to observations in elderly people [14]. The finding that suboptimal RSV neutralizing antibody titers can be boosted has important clinical relevance, since high RSV neutralizing antibody titers (>1:400) are required to protect the lower airway from RSV replication and infection [12, 13]. Several placebo recipients also developed elevated RSV neutralizing antibody titers. This suggests that some antibody rise could have occurred following natural infection. Only 1 PFP-2 vaccine recipient developed clinical RSV illness, however, compared with 6 placebo recipients (10% vs. .55%). While the population studied in this trial was small, the trend toward efficacy is encouraging. These findings should encourage the implementation of larger studies to further assess safety and efficacy with PFP-2 vaccine in populations who are at risk for severe recurrent RSV illness.

Children with higher RSV neutralizing antibody titers before PFP-2 vaccination maintained protective titers throughout the study. Persistence of antibody was observed in some placebo recipients, suggesting that intercurrent RSV infection may have naturally boosted some children. Only 10% (1/10) of PFP-2 recipients developed RSV illness, compared with 55% (6/11) of placebo recipients. The sample size is too small to assess significance; however, these findings should encourage [4, 5] larger studies with PFP-2 vaccine in populations of children who are at risk for severe recurrent RSV illness.
Table 1. RSV F protein and neutralizing antibody responses in 21 children with bronchopulmonary dysplasia.

<table>
<thead>
<tr>
<th>Titer, time point</th>
<th>PFP-2</th>
<th>Placebo</th>
<th>P*</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Log2 titer</td>
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<tr>
<td>F protein antibody</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Before vaccination</td>
<td>10</td>
<td>15.0 ± 2.0</td>
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<td>1 month after vaccination</td>
<td>10</td>
<td>20.0 ± 1.1</td>
<td>11</td>
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<td>6 months after vaccination</td>
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<tr>
<td>Neutralizing antibody</td>
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<tr>
<td>6 months after vaccination</td>
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NOTE. Data are mean ± SD.
* 2-tailed Student’s t test.

Acknowledgments

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References
