Pilot Study of Recombinant Interferon Alpha-2a for Treatment of Infants with Bronchiolitis Induced by Respiratory Syncytial Virus

J. PORTNOY,* R. HICKS, F. PACHECO, AND L. OLSON

Sections of Allergy/Immunology and Infectious Diseases, The Children’s Mercy Hospital, and The University of Missouri-Kansas City School of Medicine, Kansas City, Missouri 64108

Received 3 August 1987/Accepted 8 January 1988

Eleven children with bronchiolitis induced by respiratory syncytial virus received 10,000 to 70,000 U of recombinant interferon alpha-2a per kg of body weight per day. None developed signs of toxicity, and all but one developed an antiviral state following treatment. Interferon alpha-2a appears to be safe for infants with bronchiolitis. Its efficacy for the treatment of this condition remains to be determined.

Bronchiolitis is an infectious syndrome of lower respiratory tract obstruction that occurs during the first 2 years of life. In many localities it is the most frequent cause of hospitalization of infants (11, 12). Efforts to develop a vaccine have been unsuccessful thus far, so specific chemotherapy would be an advantageous development.

The antiviral properties of interferon (IFN) have been described in many studies (2, 6, 7, 15). IFN has its main antiviral effect by inducing a state of resistance to virus replication in host cells. This antiviral state (AVS) can be measured in vitro (9). Commonly reported side effects of IFN include pyrexia, malaise, nausea, and fatigue as well as dose- and treatment-duration-dependent leukopenia, granulocytopenia, and thrombocytopenia (1, 14). In addition, exogenous recombinant IFN-α may induce neutralizing antibodies in some patients (16; K. E. Mogensen, P. Daubas, I. Gresser, D. Sereni, and B. Varet, Letter, Lancet 1:1227–1228, 1981). This has caused concern because such antibodies can block the effects of IFN (4). There have been no reports of circulating immune complexes containing IFN in humans.

To study the potential usefulness of exogenous recombinant IFN-α for the treatment of bronchiolitis in infants, a clinical trial was performed to assess side effects and toxicities. This research was approved by the institutional review board at the University of Missouri-Kansas City medical school, and informed consent was obtained from the parents or guardians of all patients.

Children (ages of 2 to 8 months) with signs and symptoms of bronchiolitis which included wheezing, tachypnea, and respiratory distress, were enrolled as subjects for this study. Patients with cardiopulmonary disease were excluded. Since nasal secretions from such patients are routinely tested for respiratory syncytial virus antigen by enzyme-linked immunosorbent assay (Ortho Diagnostics, Inc., Raritan, N.J.), the study patients were selected sequentially from those who tested positive. Respiratory syncytial virus cultures were not routinely performed with samples from these patients.

All patients received supportive therapy such as intravenous hydration, oxygen, and bronchodilators. Ribavirin was not administered to any of these patients. Patients received daily intramuscular injections of IFN-α (Roferon; Roche Pharmaceuticals, Nutley, N.J.) until discharged from the hospital or to a maximum of five doses. The first two patients received 10,000 U/kg (body weight) per day. This dose was increased for every two patients until 70,000 U/kg per day was reached (Table 1). The higher dose has been given safely to children (S. Levin, personal communication). Assessments of wheezing, retractions, air movement, use of accessory muscles, and cyanosis were made daily by one of the investigators.

Samples of blood were obtained daily to monitor the complete blood count, platelet count, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, lactate dehydrogenase, alkaline phosphatase, creatinine, and IFN system assays as described below. Nasal secretion samples were obtained by bulb suction and placed in 0.5 ml of Eagle minimal essential medium with 10% newborn calf serum. Samples were refrigerated until assayed.

IFN concentrations were measured by a method described previously (3, 13). Dilutions of a reference IFN preparation (recombinant IFN-α which had been previously compared with a standard National Institutes of Health IFN preparation containing 5,000 U/ml) and 100 µl of serum or nasal secretions were added to 96-well tissue culture plates. IFN-sensitive cells (WISH, ATCC CCL-25) were then added at a concentration of 10^3 cells per well. After 24 h in Eagle minimal essential medium with 10% newborn calf serum at 37°C in 6% CO₂, the cells were infected with 100 µl of 10^5 50% tissue culture infective doses of vesicular stomatitis virus (ATCC VR-158). Cell death was quantitated 48 h later by neutral red dye exclusion from nonviable cells at 540 nm with a Titertek Multiscan (Flow Laboratories, McLean, Va.). Samples to be measured were compared with the known laboratory reference with a regression curve, and IFN concentrations were expressed in units per milliliter. This assay can detect 4 U of IFN activity per ml.

The AVS assay depends on the fact that IFN-stimulated cells resist viral replication whereas normal cells do not (8). Mononuclear cells from study patients and from uninfected controls were isolated on Ficoll gradients, washed with phosphate-buffered saline, and suspended in RPMI 1640 medium with 10% calf serum. Duplicate 100-µl samples containing 10^5 cells were added to 96-well tissue culture plates along with 100 µl of 10^3 50% tissue culture infective doses of vesicular stomatitis virus. Virus concentrations in supernatants from each well were measured 48 h later by dilution on WISH cell monolayers. Cell death was quantitated by neutral red exclusion. Since control cells permitted the vesicular stomatitis virus to replicate, their supernatant virus titers increased compared with those of supernatants from virus-resistant cells. The logs of the supernatant dilu-

* Corresponding author.
tions giving 50% cell death of cells from control and study patients were then compared. Lower ratios represented reduced virus replication in the patient cells and thus a greater degree of AVS.

For detection of IFN antibodies, microtiter wells (Immulon 2; Dynatech Industries, Inc., McLean, Va.) were coated with IFN (30,000 U/ml) in a bicarbonate buffer. Serum samples diluted in phosphate-buffered saline were added to the wells, and then 1:500 alkaline phosphatase-conjugated goat anti-human immunoglobulin G (Sigma Chemical Co., St. Louis, Mo.) was added. The optical density at 405 nm was quantitated after the addition of p-nitrophenyl phosphate (Sigma). Antibody was defined as present if the optical density at 405 nm was twice the background reading. Rabbit anti-IFN antibody (100 neutralizing units per ml) supplied by Roche Pharmaceuticals was the positive control.

Ages and weights of 11 treated children are shown in Table 1. Four children were available for follow-up examinations 2 to 3 months later. All patients improved after 3 to 5 days of treatment. This is consistent with the usual pattern of improvement in respiratory syncytial virus infections. Most patients were discharged within 3 days. No adverse effects of IFN, including fever, were noted in any of the patients. There were no significant changes in complete blood count, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, lactate dehydrogenase, alkaline phosphatase, or creatinine during treatment or in the four follow-up samples.

IFN was not detected in nasal secretions before or during treatment, which is consistent with previous reports (5, 10). Most patients initially had detectable serum IFN which persisted during treatment. There was no correlation between the dose of IFN given and concentrations in serum. It is possible that the doses used were inadequate to alter the concentrations in serum. Four follow-up serum samples did not have detectable IFN.

IFN antibodies were not detected in any of the patients including the four examined 2 to 3 months after the study. The main reason for concern about IFN antibodies is that they might neutralize exogenous IFN (4). Once the patient is well and no longer requires IFN, the presence of antibodies is less important.

Despite the initial presence of endogenous IFN, mononuclear cells were not in an AVS (Fig. 1). During the first 2 days of treatment, the mononuclear cells became virus resistant (P < .05 by one-way analysis of variance), as did those from patients who received 10,000 U of IFN per kg. This resistance decreased on day 3. Since this study did not include an untreated control group, it is not possible to be certain that the changes in AVS were due to exogenous IFN. Whether the development of this AVS will be helpful in reducing bronchiolitis morbidity is unknown.

In this uncontrolled study we have shown that as little as 10,000 U of exogenous IFN-α per kg per day may induce a significant in vitro response (AVS) in patient mononuclear cells and that toxicity is not found with doses as high as 70,000 U/kg per day. In addition, antibody to IFN did not develop during treatment or after 2 to 3 months in patients available for follow-up examinations.

This study was supported in part by grants from the William Randolph Hearst Foundation and the Helen and Herman R. Sutherland Charitable Lead Trust.

LITERATURE CITED


---

**TABLE 1. Ages and weights of and IFN doses for the 11 patients in the study**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (mo)</th>
<th>Weight (kg)</th>
<th>IFN dose* (U/kg per day)</th>
<th>No. of doses</th>
<th>Initial oxygen supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>5.5</td>
<td>10,000</td>
<td>2</td>
<td>40% Hood</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>6.8</td>
<td>10,000</td>
<td>3</td>
<td>40% Hood</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>6.3</td>
<td>13,000</td>
<td>2</td>
<td>40% Hood</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>7.4</td>
<td>13,000</td>
<td>4</td>
<td>60% Respirator</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>4.0</td>
<td>22,000</td>
<td>3</td>
<td>30% Hood</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>6.3</td>
<td>22,000</td>
<td>3</td>
<td>30% Hood</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>6.8</td>
<td>33,000</td>
<td>3</td>
<td>30% Hood</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>5.6</td>
<td>33,000</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>7.2</td>
<td>50,000</td>
<td>3</td>
<td>30% Hood</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>6.7</td>
<td>50,000</td>
<td>3</td>
<td>40% Hood</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>2.3</td>
<td>70,000</td>
<td>4</td>
<td>30% Hood</td>
</tr>
</tbody>
</table>

Mean ± SD

| 4.9 ± 2.2 | 5.9 ± 1.5 | 3.2 ± 0.9 |

* Doses were given at the time of enrollment in the study and daily in the morning (e.g., a patient who received three doses was treated for 3 days).