Reaction of Squirrel Monkeys to Intratracheal Inoculation with Influenza/A/New Jersey/76 (Swine) Virus

RICHARD F. BERENDT* AND WILLIAM C. HALL

U. S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21701

Received for publication 24 November 1976

To determine whether a model could be established for laboratory investigations, nine squirrel monkeys were inoculated intratracheally with 10° median egg-infectious doses of influenza virus type A/New Jersey/8/76 (H1N1) (swine influenza virus). They responded with clinically detectable illness including fever, leukopenia, decreased food consumption, increased respiratory rate, occasional coughing, labored breathing, nasal discharge, and lethargy. Convalescence was well advanced by the day 10. All monkeys excreted virus for 7 to 8 days. A scoring procedure (illness score) has been developed for use in studies of vaccine and chemotherapeutic efficacy.

In February 1976, influenza virus bearing swine antigenic determinants (H1N1) was isolated from several recruits at Fort Dix, N.J. (6). Because this virus represented a serotype to which the majority of the American population had no immunity, it was feared that a pandemic might begin during the following fall. Consequently, a decision was made to make vaccine available to all Americans.

Although extensive investigations of the antigenicity and adverse reactions after administration of various swine influenza vaccines have already been carried out (5), no reports of the protection afforded by these vaccines have been published. The principal reason for the lack of protection data is that the virulence and communicability of the recently isolated strain is unknown, and, as a consequence, no experimental inoculation of humans with swine influenza has been attempted in the United States.

A desire to limit the dissemination of the virus has also largely precluded experiments with laboratory animals. Because we have recently published the details of a squirrel monkey model for influenza infections (3) and because we have unique containment facilities for the study of potentially hazardous microorganisms, we were requested by the National Institute of Allergy and Infectious Diseases to investigate the protective efficacy of vaccines and chemotherapeutic agents in primates.

To carry out investigations of prophylaxis, it was first necessary to determine whether the New Jersey strain of influenza virus caused clinical illness in squirrel monkeys and, if so, whether a means of assessing the degree of clinical illness could be developed to compare responses among groups of animals given various treatments. A systemic reaction score has been used by Parkman et al. to evaluate the reaction of human subjects to various vaccine preparations (5) and by Beare and Craig to evaluate the reaction of humans to various test strains of influenza virus (clinical score) (1). The clinical response (illness score) of squirrel monkeys to intratracheal inoculation of virus is presented in this report.

MATERIALS AND METHODS

Virus preparation and assay. A sample of the fifth egg passage of influenza A/New Jersey/8/76 (H1N1) was obtained from the Bureau of Biologics, Food and Drug Administration, diluted 1:1,000 in sterile heart infusion broth (HIB), and inoculated into the allantoic cavity at 10- to 12-day embryo-nated eggs. After 48 h of incubation at 35°C, the allantoic fluid was collected, distributed in 3-ml aliquots, and stored at −70°C. Estimates of the concentrations of virus in terms of egg infectivity were accomplished as previously described (2). The allantoic fluid preparations had a titer of 10^4.2 median egg-infectious doses per ml. Antigenic purity of the viral preparation was confirmed with reference standard materials supplied by the Center for Disease Control. All serological tests were carried out by standard Center for Disease Control procedures (4).

Monkeys. Twenty-one male squirrel monkeys (Saimiri sciureus), obtained from commercial sources, were used in this study. Average weight was 0.7 kg (range, 0.493 to 1.01 kg). Monkeys were housed in wire-bar cages and fed commercial monkey chow supplemented with fruit until the time of the experiment. Water was provided ad libitum. During experiments food was limited to six commercial biscuits daily.

Intratracheal inoculation. The procedure for intratracheal inoculation of virus has been described previously (3). Briefly, it involves inoculation...
through a plastic catheter that has been passed over the epiglottis and into the trachea of a lightly anesthetized monkey.

**Bacterial and viral isolation.** Established methods were used to detect bacteremia (2, 3). Virus was isolated from the oropharynx by swabbing the back of the throat and the tonsil area with a swab moistened with HIB. The contents of the swab were washed into 1.0 ml of HIB containing 50 μg of gentamicin, 100 U of penicillin, and 100 μg of streptomycin per ml. The suspensions were incubated at room temperature for 60 min to kill bacteria and were then inoculated into embryonated eggs (six eggs per sample). After 48 h of incubation at 35°C, followed by overnight refrigeration, the presence of virus was detected by routine hemagglutination procedures.

**Clinical and laboratory determinations.** Rectal temperature, hematocrit, total and differential leukocyte counts, respiratory rate, pharyngeal virus isolation, food and water consumption, body weight, nasal discharge, coughing and sneezing, labored breathing, and activity were recorded daily. Blood for serum hemagglutination inhibition antibody determinations was obtained before virus inoculation and at 7, 14, and 28 days after infection.

**RESULTS**

**Preliminary observations.** Nine monkeys were inoculated intratracheally with 10⁷ median egg-infectious doses of virus. Figure 1 presents the deviation from baseline values (baseline values are the mean of three daily observations on each of the nine monkeys) of five of the response parameters. Frequency of occurrence of three more parameters is also illustrated in the same figure. Activity and dyspnea are omitted from the figure because lethargy and labored breathing were seen daily in every monkey. Hematocrit values are not included because of variation due to daily bleeding. Al-

---

**Fig. 1.** Change in selected clinical parameters after intratracheal inoculation of the New Jersey strain of influenza virus. Vertical bars represent the standard error of the mean.
though sick monkeys seemed to drink less water than healthy ones, it was impossible to measure water consumption accurately because these monkeys would only drink from bowls and lost water by splashing.

Monkeys became ill within 24 h after virus inoculation; fever was most prominent at this time and then slowly subsided. Most of the other parameters seemed to reach a maximum (minimum for leukocyte count) in 2 to 5 days and then slowly subsided. All values had returned to normal within 10 days (not shown); virus shedding also ceased by this time. At no time were bacteria isolated from the blood. At 10 days the monkeys had also begun to gain weight. The geometric mean of the reciprocal of the serum hemagglutination inhibition titers was <10, 1.9, 33.1, and 127.0 at -5, 7, 14, and 28 days after inoculation, respectively.

Pathology. In a separate experiment, an untreated monkey receiving influenza virus was killed and necropsied 6 days after virus inoculation. Pulmonary changes were similar to those previously described for squirrel monkeys infected with the Aichi/2/68 strain of type A influenza virus (3). Microscopically, the bronchial and bronchiolar epithelium was moderately altered by proliferative changes accompanied by focal erosions and areas of deciliation. There was focal ulceration, loss of cilia, and moderate proliferative change. In some areas, the proliferative epithelium extended along alveolar septa and partially occluded alveolar spaces. There was mild infiltration of the pulmonary interstitium around affected bronchioles by macrophages and a few neutrophils. Proteinaceous fluid and a few inflammatory cells partially filled alveoli around some of the more severely affected bronchioles.

Illness score. An illness score was devised to score the response of monkeys over the first 7 days after infection. After that, variation in rate of recovery from the illness precluded the use of a scoring system. The scoring system was arbitrary and was designed to weight most heavily those parameters that could be objectively measured. In the case of virus shedding, additional weight was given if relatively large amounts of virus were recovered (>67% of inoculated eggs contained virus). The score calculated for the nine monkeys described above is given in Table 1. The theoretical minimum possible score would be zero; a critically ill monkey would score approximately 77 (assuming a 20% weight loss and maximum values for the other parameters). Hence, the mean score of 47.6 represents only moderate illness, although all monkeys were infected.

<table>
<thead>
<tr>
<th>Parameter and scoring procedure</th>
<th>Mean score for 9 infected monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Virus shedding: 1 for each day that virus was detected, plus 1 additional for each day that ≥67% of eggs were positive</td>
<td>8.75 (1.25)*</td>
</tr>
<tr>
<td>2. Temp: 2 for each day that rectal temperature was ≥1°F (≥0.6°C) above baseline value</td>
<td>8.0 (1.31)</td>
</tr>
<tr>
<td>3. Body wt: 1 for each percent loss of body weight at 7 days</td>
<td>9.9 (1.94)</td>
</tr>
<tr>
<td>4. Leukocyte concn: 1 for each day that total leukocyte count was ≤1,500 below baseline value</td>
<td>4.4 (0.84)</td>
</tr>
<tr>
<td>5. Respiratory rate: 1 for each day that rate was ≥40% above baseline value</td>
<td>3.5 (0.78)</td>
</tr>
<tr>
<td>6. Appetite: 1 for each day that biscuit consumption fell below 67% baseline value</td>
<td>5.9 (0.58)</td>
</tr>
<tr>
<td>7. Nasal discharge: 2 if nasal discharge was observed for ≥5 days</td>
<td>2.0 (0.0)</td>
</tr>
<tr>
<td>8. Coughing/sneezing: 2 if coughing was observed for ≥2 days</td>
<td>1.25 (0.37)</td>
</tr>
<tr>
<td>9. Dyspnea: 2 if labored breathing was observed for ≥2 days</td>
<td>2.0 (0.0)</td>
</tr>
<tr>
<td>10. Activity: 2 if activity was reduced for ≥2 days</td>
<td>2.0 (0.0)</td>
</tr>
<tr>
<td>Overall mean illness score</td>
<td>47.6 (2.89)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are standard error of the mean.

**DISCUSSION**

The New Jersey strain of influenza virus causes a mild disease in squirrel monkeys after intratracheal inoculation. The disease is characterized by fever, leukopenia, anorexia, increased respiratory rate, occasional coughing, slight nasal discharge, and proliferation of the virus. The animals were lethargic and demonstrated labored breathing for about 7 days. Convalescence was well under way by the 10th day. Signs of disease were very similar to those previously seen in experiments with influenza A/Aichi/2/68 (H3N2) (3). Virus isolations have not been done routinely in experiments with the Aichi strain, but the illness score for four monkeys inoculated with doses equivalent to those used with the New Jersey strain was 34.0. The score for the nine monkeys discussed in these experiments (subtracting the score attributed to virus shedding) was 29.3. These differences were not significantly different. Although insufficient monkeys have been necropsied to provide a reliable comparison, the lesions indicate that the disease process in lung tissue is about the same as that seen with the Aichi strain.

Our observations of a mild disease in monkeys is consistent with the observations of
Beare and Craig in human volunteers inoculated with the New Jersey strain of virus (1). These investigators noted mild reactions, although all subjects were infected and all excreted virus.

The illness score that we have developed has provided a useful tool for comparing the effect of various treatments. In particular, analysis of the contribution of each parameter to the total index has enabled us to determine the identity of those parameters that are most affected. The scoring system has been used in vaccine experiments that will be published at a later date.

ACKNOWLEDGMENTS

We are pleased to acknowledge the technical assistance of Mark Schneider and Robert Magruder. We thank Francis Ennis, Bureau of Biologics, Food and Drug Administration, for supplying virus and Walter Dowdle, Center for Disease Control, for reference antigens for this study.

LITERATURE CITED