Effect of the Route of Inoculation on Antibody Formation in Guinea Pigs Immunized with Parainfluenza Virus Vaccines

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The efficiency of the intramuscular route of immunization of guinea pigs was compared with the intraperitoneal route when trivalent parainfluenza virus vaccine was employed as immunogen. When the routes of immunization were compared by effective dose 50, the intramuscular route was more effective. Likewise, a statistical evaluation of conversion rates and of titers revealed significantly higher values for the intramuscular than for the intraperitoneal route to all three components in two of the lots of vaccine tested.

To assay antigen content of parainfluenza virus vaccines, Jensen et al. (8), after comparing responses to various doses and injection routes, adopted a dose of 0.5 ml given intraperitoneally to guinea pigs. During early work with aqueous trivalent parainfluenza virus vaccine (5), we, too, followed the above immunizing procedure. Later, trivalent parainfluenza virus vaccine was added to alum-precipitated diphtheria and tetanus toxoids and pertussis vaccine combined. In this instance, the intramuscular route was used since the alum was to provide a reservoir from which the antigens could be slowly released. Upon testing sera obtained from guinea pigs immunized with the above two vaccines, we noted appreciable antibody rises more frequently with animals inoculated by the intramuscular route than with those receiving intraperitoneal injections.

To verify our preliminary results, we tested three lots of aqueous trivalent parainfluenza virus vaccines by employing the two different routes of immunization. This report presents data indicating that, when the vaccine is administered intramuscularly rather than intraperitoneally, greater values that are statistically significant are demonstrable to all three components of parainfluenza virus vaccine.

**MATERIALS AND METHODS**

*Viruses.* Viruses employed were parainfluenza 1 (HA$_2$, strain C39), parainfluenza 2 (CA, strain Greer), and parainfluenza 3 (HA$_3$, strain C243). These agents, received from R. Chanock (2, 3), were isolated by inoculating throat swabs obtained from young children with respiratory illnesses into monkey kidney tissue cultures. The viruses received were adapted to the embryonated hen's egg after repeated passage in our laboratory; this was accomplished by inoculating 7-day-old embryos amniotically and pooling the amniotic fluids collected after 5 days of incubation at 37 C.

*Serology.* Hemagglutination-inhibition (HI) tests were performed as described previously (4), except that 0.5 ml of a 0.4% suspension of guinea pig erythrocytes was added to each tube before incubating the red blood cells and virus mixture at 37 C for 60 min.

*Preparation of vaccines.* Parainfluenza 1, 2, and 3 vaccines were prepared from crude amniotic fluid by centrifuging the viruses in a Sharples centrifuge at 62,000 × g with a flow rate of 50 ml per min and by resuspending the sediment to 33% of the original volume in 0.01 M phosphate-buffered saline (PBS), pH 7.2. All of the above vaccines were inactivated individually by treatment with 1:2,000 Formalin under constant agitation at 37 C for 20 hr. After inactivation, equal amounts of the monovalent vaccines were combined to give the final trivalent vaccine. Vaccine lots 6279 and 6364 had been stored for 18 months at 4 C when tested, whereas lot 6654 was tested within 3 months after manufacture and storage at 4 C.

*Potency test.* Hartley strain guinea pigs, weighing between 350 and 400 g each, were prebled and tested for antibodies to each of the three parainfluenza viruses. Animals with serum antibody titers of 1:8 or greater against any of the three parainfluenza viruses were not used. The test guinea pigs were then immunized intramuscularly or intraperitoneally with 0.5 ml of the vaccine preparations. The vaccines were tested by inoculating groups of 15 guinea pigs at two concentrations, undiluted and diluted 1:10. After 2 weeks, the animals were given a second dose of 0.5 ml of vaccine by the initial route. Two weeks after the second injection, the guinea pigs were exsanguinated, and the serum obtained from each animal was meas-
TABLE 1. Comparison of ED50 for three lots of aqueous trivalent parainfluenza virus vaccine administered intraperitoneally and intramuscularly

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Route of administration</th>
<th>ED50 of guinea pigs to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parainfluenza 1</td>
</tr>
<tr>
<td>Lot 6279</td>
<td>Intraperitoneal</td>
<td>&lt;1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td>2.2</td>
</tr>
<tr>
<td>Lot 6364</td>
<td>Intraperitoneal</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td>1.0</td>
</tr>
<tr>
<td>Lot 6654</td>
<td>Intraperitoneal</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td>5.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Less than 50% of the guinea pigs were converted serologically when immunized with undiluted vaccine.

<sup>b</sup> No guinea pigs converted serologically.

The results obtained with the three vaccines were subjected to statistical analyses; the conversion rates were compared and a ranking analysis was applied to the titers of preparations administered intraperitoneally or intramuscularly, undiluted and diluted 1:10.

Lots 6279 and 6654 gave comparable results in conversion rates (Table 2). Undiluted parainfluenza 1, parainfluenza 2 diluted 1:10, and undiluted parainfluenza 3 showed significantly higher conversion rates by the intramuscular route for both of these lots. In addition, lot 6654 showed a significant difference (P < 0.01) for parainfluenza 3 diluted 1:10. The third lot (lot 6364) showed a significant difference with undiluted parainfluenza 3.

When titers were compared by means of a ranking test (Table 2), lots 6279 and 6654 gave similar results. In these two lots, all differences were statistically significant except for parainfluenza 1 diluted 1:10 and undiluted parainfluenza 2. When conversion rates and median titers were compared (Table 2), lot 6364 gave identical results.

DISCUSSION

The superior antibody response obtained with the intramuscular route of inoculation of trivalent parainfluenza vaccine was surprising in view of some previously published information. In discussing the production of antibodies in experimental animals, Carpenter (1) preferred the intravenous and intraperitoneal routes since they gave "excellent antiseraums," whereas intramuscular injections yielded only fair titers. Raffel (9) stated that in many instances the route of injection is probably of minor importance in determining the amount and characteristics of antibody produced, although he did note some exceptions.

On the other hand, Freund and Bonanto (6) found that the effectiveness of an immunizing agent was dependent on its size and the route of inoculation; i.e., large particles such as bacteria were more effective when given intravenously or intraperitoneally, whereas smaller particles such as viruses were most effective when inoculated into the tissues. The reason for the relative inefficacy...
of intraperitoneally injected viral antigens is their rapid elimination from the body (6, 9).

C. G. Aulisio and J. A. Morris have indicated (personal communication) the need for a standard potency test for vaccines, since procedures employed in three different laboratories to measure the antigenic potency of adenovirus vaccines prepared for use in the United States varied with the route of inoculation or dosage. These investigators have concluded that different assay procedures give different results, and have emphasized not only the necessity for a standard reference vaccine but also for data to correlate the vaccine potency assays in animals with efficacy in man. Since the parainfluenza virus vaccines are clinically administered to man by the intramuscular route, the intramuscular administration of the vaccine to animals would be expected to be more meaningful in eventual clinical terms.

Although all components of lots 6279 and 6654 revealed significant differences between the routes of inoculation (Table 2), two components of lot 6364 failed in this respect. The lack of a statistically significant difference between the intramuscular and intraperitoneal routes of inoculation for the parainfluenza 1 moiety may have been due to a loss of potency for this component of the vaccine. Although the parainfluenza 2 component showed a greater $E_{90}$ value for the intramuscular over the intraperitoneal route of inoculation, no significant difference was noted (Table 2). In this instance, the lack of statistical difference cannot be ascribed to a loss in potency.

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LITERATURE CITED