Antibody Response of Guinea Pigs to Trivalent Parainfluenza Virus Vaccine Prepared from Embryonated Eggs

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ABSTRACT

DeMeio, Joseph L. (The National Drug Co., Swiftwater, Pa.), and Armand N. Desanctis. Antibody response of guinea pigs to trivalent parainfluenza virus vaccine prepared from embryonated eggs. Appl. Microbiol. 14:558-560. 1966.—A trivalent parainfluenza virus vaccine has been tested in guinea pigs. The parainfluenza 2 virus vaccine component was superior in the magnitude of antibody titers, and in the ability to convert animals serologically after two doses of an undiluted or a 10-fold diluted vaccine. The parainfluenza 1 virus vaccine gave a higher percentage of conversion than parainfluenza 3 virus vaccine after administration of two doses of either undiluted or 10-fold diluted vaccine.

Parainfluenza viruses types 1, 2, and 3 isolated by Chanock and his associates (1, 2) play an important role in the etiology of respiratory infections during infancy. These viruses are associated with croup, bronchopneumonia, bronchiolitis, and moderate to severe bronchitis in children (3). They cause a milder upper respiratory disease in adults (7, 8). In view of the wide geographical distribution of these viruses and their importance as causes of childhood respiratory diseases, effective vaccines against them would be desirable. Vaccine prepared from virus propagated in embryonated hen's eggs might have at least two advantages over one prepared from monkey kidney tissue culture: freedom from the many extraneous agents found in simian tissue culture, and a better antigenic response.

This report describes the preparation of a vaccine containing three types of parainfluenza viruses propagated in embryonated hen's eggs, and the immunological responses obtained when this vaccine was injected in guinea pigs.

MATERIALS AND METHODS

Viruses. Viruses employed were parainfluenza 1 (HAs, strain C39), parainfluenza 2 (CA, strain Greer), and parainfluenza 3 (HAs, strain C243). These agents, received from R. Chanock (1, 2), were isolated by inoculating throat swabs obtained from young children with respiratory illnesses in monkey kidney tissue cultures. The viruses received were adapted to the embryonated hen's egg in our laboratory 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 by the Committee on Standard Serological Procedures in Influenza Studies (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 trivalent vaccine. Parainfluenza 1, 2, and 3 vaccines were prepared from crude allantoic fluid by centrifuging the viruses in a model L Spinco ultracentrifuge at 25,000 rev/min for 1 hr (no. 30 rotor), and reconstituting the pellets to one-tenth the original volume in 0.01 M phosphate-buffered saline (PBS), pH 7.2, to give 1,024 and 512 hemagglutination (HA) units per 0.5 ml, respectively.

Parainfluenza 2 vaccine was processed as above, except that amniotic fluid was used, and the pellet was reconstituted to original volume with PBS to give 512 HA units per 0.5 ml.

All 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 were combined.

Immunization of guinea pigs. 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 discarded. The test guinea pigs were then immunized intraperitoneally with 0.5 ml of the triple vaccine preparation. The trivalent vaccine was tested by inoculating groups of 15 guinea pigs at three concentrations: undiluted, 1:10, and 1:100.
Two weeks after the initial injection, the animals were given a second dose of 0.5 ml of vaccine intraperitoneally. Two weeks after the second injection, the guinea pigs were exsanguinated, and the serum obtained from each animal was measured for HI antibody against each viral component of the vaccine. A fourfold rise in antibody levels was considered significant.

RESULTS

Figure 1 shows the expected regression in the number of serological conversions to each of the viruses upon dilution of the vaccine.

The parainfluenza 2 virus vaccine component appears to be superior to parainfluenza 1 and 3 in all respects, since two doses of the undiluted vaccine converted 93% of the guinea pigs, and two doses of a 10-fold dilution of the vaccine resulted in a conversion of 67% of the animals.

The undiluted parainfluenza 1 virus component of the vaccine showed a conversion rate of 87%, which is superior to the conversion rate of 60% obtained with undiluted parainfluenza 3. However, it should be mentioned that the parainfluenza 1 component had 340 HA units versus 170 HA units contained in the parainfluenza 3 component. Nevertheless, both the parainfluenza 1 component diluted 1:10 (34 HA units) and the undiluted parainfluenza 3 component (170 HA units) had conversions of 60%.

The information in Table 1 indicates the best immunogen in the trivalent vaccine to be parainfluenza 2. The magnitude of reactivity was greatest when sera from immunized animals given two injections of vaccine were tested against parainfluenza 2. In this instance, 11 of 15 animals given undiluted, and 2 of 15 given a 10-fold dilution of the trivalent vaccine had HI antibody titers of 128 or greater when their sera were tested against parainfluenza 2 virus.

On the other hand, none of the sera from animals given undiluted vaccine was able to inhibit hemagglutination at a dilution greater than 1:64, when tested with parainfluenza 1 virus, and sera from only two animals inoculated with undiluted parainfluenza 3 vaccine gave HI responses of 128 or greater.

Since none of the animals inoculated with a 1:100 dilution of the vaccine had a significant serological response, these data were excluded from Table 1.

DISCUSSION

No data are available to serve as a measure for comparison or standard of potency with parainfluenza 1, 2, and 3 grown in the embryonated hen's egg, since parainfluenza virus type 2 was adapted to grow in this host only recently (6). We were obliged to evaluate the preparation on the basis of serological conversions and magnitude of antibody responses in guinea pigs.

Although serological results after immunization of humans and guinea pigs with a parainfluenza vaccine have been reported (9), the vaccine

![Figure 1. Conversion of guinea pigs immunized with 10-fold dilutions of trivalent parainfluenza vaccine.](http://aem.asm.org/attachment/2017/10/16/559-10-16/559-10-16.pdf)
employed in these studies lacked the parainfluenza virus 2 component. The authors noted that parainfluenza virus 3 was superior antigenically to parainfluenza 1; at least two injections of type 1 virus vaccine were necessary to obtain antibody responses. The evidence reported indicates that parainfluenza 1 was a better immunogen. The parainfluenza 1 moiety containing only 34 HA units was able to convert serologically the same number of animals as the parainfluenza 3 moiety containing 170 HA units. Additional factors, however, such as passage level of the propagated virus and differences in the strains employed, cannot be discounted when comparing the immunogenic capacity of these viruses.

It is known that antigenic relationships exist among myxoviruses (5). Therefore, the vaccine described in this paper was prepared in hopes that the combination of three serologically related parainfluenza viruses in a single vaccine would convert all guinea pigs inoculated with two doses of undiluted vaccine. However, these results were not attained. Studies with monovalent vaccines made with parainfluenza viruses having similar hemagglutinating activities might better resolve the question of antigenic superiority among these viruses. Such studies might also give an indication as to the additive effect obtained by sharing of antigens when comparing these vaccines with a polyclonal vaccine.

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LITERATURE CITED