Effect of Passive Antibody on Parainfluenza Virus Type 3
Pneumonia in Hamsters

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Received for publication 9 February 1976

Both parainfluenza virus type 3 and respiratory syncytial virus may produce life-threatening pneumonia or bronchiolitis in infants less than 6 months old. Almost all infants in this age group possess passively acquired maternal antibodies to both viruses. It has been suggested that maternal antibodies may actually participate in the pathogenesis of these diseases in early infancy. This investigation examined the effect of moderate levels of passive antibody on the development of pneumonia in hamsters infected intranasally with parainfluenza virus type 3. The pneumonitis produced in this model was not enhanced by the presence of moderate levels of serum antibody to this virus. Furthermore, reinfection after an initial "sensitizing" infection under the cover of passive antibody did not result in a more severe pneumonitis. These studies do not support either of the two hypotheses that have been advanced to explain the pathogenesis of infections with respiratory syncytial virus in early infancy.

Parainfluenza type 3 (para-3) virus is second only to respiratory syncytial (RS) virus as a cause of pneumonia and bronchiolitis in infants (2, 9, 10, 13). Life-threatening illnesses caused by these viruses frequently occur during the first 6 months of life when virtually all infants possess passively acquired maternal antibodies to both viruses. It would appear that low or moderate levels of circulating antibodies do not afford protection against these viruses; furthermore, it has been suggested by some investigators that maternal antibodies may participate in the pathogenesis of severe lower-respiratory disease occurring in the first few months of life (3, 7).

To study the effect of passive antibody upon infection with para-3, we adapted the hamster pneumonia model described by Buthala and Soret (1). Preliminary studies confirmed their observations of pulmonary histopathology and its relationship to virus replication in the lungs and to the production of neutralizing antibodies. This report will describe the effect of passive antibody or primary infection and reinfection.

MATERIALS AND METHODS

Hamsters. Specific-pathogen-free animals, weighing 70 to 100 g each, were obtained from Sprague-Dawley, Madison, Wis. They were kept in isolated, locked animal quarters, and all personnel entering their quarters were instructed to wear masks. All animals were screened for neutralizing antibodies to para-3 virus prior to infection and all were antibody free.

Virology. The HA 1 strain of para-3 virus, obtained from R. M. Chanock, had multiple passages in HeLa cell cultures. A virus pool was grown in HeLa cells, frozen and thawed, and then centrifuged to sediment cellular debris. The supernatant was dispersed in 1.0-ml aliquots and snap-frozen at −70 C. The resulting plane titer was 35,000 plaque-forming units per ml, and the infectivity titer in a plaque microtiter was 105 mean tissue culture infective doses per 0.025 ml. An uninfected HeLa cell culture was prepared exactly as described above to serve as a control inoculum. Virus quantitation was determined by counting plaques produced on HeLa cell monolayers. After outgrowth of the HeLa cells in plastic plates of 35-mm diameter, the cell sheets were washed with phosphate-buffered saline, and the inoculum was applied in a 0.1-ml volume. The plates were placed in a humidified incubator with 5% CO2 for a 1-h absorption period. The cell sheet then was overlaid with Eagle basal medium containing 10% fetal calf serum and 0.75% methyl cellulose. After reincubation for 72 h, the medium was aspirated, the cell sheet was fixed with 10% formalin and stained with hematoxylin and eosin, and the plaques were counted. Neutralizing antibodies were measured in microtiter with the end point determined by direct observation of viral cytopathology on an inverted microscope.

Procedures. The hamsters were anesthetized by intraperitoneal injection of 8 mg of sodium pentobarbital before bleeding, infection, or sacrifice. They were infected by intranasal inoculation of 700 plaque-forming units of virus in a volume of 0.2 ml.
Neutralizing antibodies were measured on sera obtained pre- and postinfection by cardiac puncture. At the time of sacrifice, the lungs were removed aseptically, and individual lobes were either fixed in 10% formalin for histological sections, snap-frozen for fluorescent-antibody studies, or homogenized in a 10% suspension for virus quantitation. Fluorescent-antibody staining of viral antigen was successfully accomplished utilizing high-titered, directly conjugated monkey serum kindly provided by Chien Liu of the Kansas University Medical Center (12). Histological changes in lung sections were graded on a 1 to 4 scale for each of the following categories: intraluminal exudate, and peribronchial, perivascular, and interstitial infiltrates.

RESULTS

Basic model. Hamsters infected with para-3 virus showed no observable evidence of illness prior to sacrifice, but histologically there was consistent production of pneumonia. Lung lesions consisted of scattered endobronchial exudates composed of equal numbers of mononuclear and polymorphonuclear cells, peribronchial and perivascular round cell infiltrates, and small discrete areas of interstitial pneumonia (Fig. 1a through c). Viral antigen was localized in the respiratory epithelium by fluorescent-tagged antibody (Fig. 1d).

The sequence of the development of virus replication, histological changes, and serum antibodies is shown in Fig. 2. Each point was determined by averaging the results of studies of 11 animals. Maximal quantities of virus were recovered on days 3 and 5 after inoculation; virus was not detectable on day 7. The histopathology peaked on day 5. Microscopic changes noted 6 h after inoculation consisted mainly of endobronchial mononuclear exudates. In animals sacrificed at 3 and 5 days, peribronchial and interstitial infiltrates predominated, and the endobronchial exudate was comprised of both polymorphonuclear and mononuclear phagocytes. Serum-neutralizing antibodies were not detected until day 7, when about one-half of the animals had measurable, but low, titers. By day 10, all animals were antibody positive.

Reinfection. Resistance to reinfection was studied by challenging animals with the same virus inoculum 30 days after the primary infection. Negligible infiltrates were seen in animals sacrificed 3 or 5 days after reinfection, and

![Fig. 1.](http://iai.asm.org/)

(a) Photomicrograph of bronchiole with peribronchiolar lymphocytic infiltration and scant intraluminal exudate in lung of hamster infected with para-3 virus. (b) Higher magnification of intraluminal exudate showing both mononuclear and polymorphonuclear phagocytes. (c) Perivascular round-cell infiltration and scattered areas of interstitial infiltration. (d) para-3 virus antigen stained by direct immunofluorescence in respiratory epithelial cells of hamster bronchus.
of antibody serum received serum antibody with fixation.

para-3 virus

One virus titers only, infected

Three 1:5,120.

tonely with

FIG. 2. Sequence of detection of lung virus, serum-neutralizing antibodies, and lung histopathology in hamsters infected intranasally with para-3 virus. Each point was determined by averaging the results of studies of 11 animals. pfu, Plaque-forming units.

Fig. 3. Lung histopathology scores of hamsters infected intranasally with para-3 virus and sacrificed 3 or 5 days after primary infection or reinfection.

virus was not recovered from the lung (Fig. 3).

Infection after passive immunization. Pooled human gamma globulin (HGG) was utilized as a source of high-titered antibody to para-3 virus. Hamsters were injected intraperitoneally with 0.5 ml of HGG (Cutter) that had para-3 virus neutralization titers of 1:1,280 to 1:5,120. These animals regularly had serum antibody titers of about 1:16, 24 h after their injection. Three groups of hamsters were bled serially over a 28-day period to determine antibody titers. One group of 10 hamsters received HGG only, one group of 5 was infected with para-3 virus only, and one group of 10 animals was infected 24 h after HGG administration. Animals infected with para-3 virus after HGG injection experienced the same gradual decay of serum antibody that was seen in animals that received HGG only (Fig. 4); in contrast, those with virus infection only had a brisk rise in serum antibody titers after day 10.

Reinfection after primary infection with passive antibody. To examine the consequences of primary infection in the presence of passive antibody, and of subsequent reinfection, a series of three experiments was performed. In each experiment, 30 animals were divided into four groups: group 1 received HGG, was infected 24 h later, and was sacrificed at 5 days; group 2 received HGG, was infected 24 h later, was reinjected on day 30, and was sacrificed on day 35; groups 3 and 4 did not receive HGG but were infected and sacrificed or reinjected and sacrificed on the same schedule. Figure 5 summarizes the results of these experiments. With primary infection, animals treated with HGG had less pulmonary infiltration than did animals without passive antibody; however, the relationship was reversed upon reinfection when animals initially infected in the presence of passive antibody developed more infiltration than did those that received no HGG. It should be noted that the amount of infiltration after reinfection with HGG was still less than that after primary infection without HGG. Virus titers in lung suspension of animals with passive antibodies were slightly lower than those without. Five days after reinfection, virus was not detectable in lung suspensions of either group. (In a separate experiment, small quantities of virus were found in lung suspensions of animals sacrificed on day 2 after reinfection, but only in those treated with HGG at the time of their initial infection.) Antibody titers measured in these experiments were similar to the pattern demonstrated for infection with and without passive antibody in Fig. 4.

Comparison of patterns of pulmonary histology in all groups of animals revealed little difference except that animals given passive antibody tended to have less perivascular infiltration during the first infection and more interstitial infiltration upon reinfection (Fig. 6).

DISCUSSION

Craighead et al. (4) first reported experimental infection of hamsters with para-3 virus as evidenced by virus recovery and antibody rise

Fig. 4. Serum-neutralizing antibody titers of hamsters passively immunized with pooled human immunoglobulin (Ig) before infection with para-3 (P3) (n = 10) compared with titers of appropriate control hamsters (n = 15).
but observed no histopathological changes in the lungs. Liu and his associates (12) localized the viral antigen to the cytoplasm of the respiratory epithelium of hamsters by immunofluorescence. Buthala and Soret (1) were the first to detect histological changes in the lungs of infected animals; our observations of the sequence of development of pneumonia and antibody response are similar to those of Buthala and Soret.

Hamsters with passive homotypic antibody failed to develop an active serum antibody response to infection with para-3, but animals of the same age without passive antibody consistently developed high levels of antibody. This observation is similar to studies of infection of newborn infants with oral poliovirus vaccine, which showed that the development of an active antibody response was inversely related to the maternal antibody level (11). It therefore seems likely that the failure of many infants less than 6 months old to develop an antibody response to infections with para-3 virus (or RS virus) results from suppression of the active antibody response by maternal antibody. This does not support the concept of "immunological immaturity" that has been suggested by other investigators for RS virus infections (6, 14).

Important questions remain concerning the interaction of passive antibody with other parameters of the immune response and host defense mechanisms. In the hamster pneumonia model, animals with passive antibody had consistently less para-3 virus recovered from lung tissue and consistently less pulmonary infiltrate than did animals infected without passive antibody. These differences were not considered to be significant but do indicate that further studies should be performed to determine whether higher levels of passive antibody will provide significant protection against infection with para-3 virus. Studies of a mouse pneumonia model have demonstrated that passive antibody will protect against influenza virus infection (5), and observations of natural para-3 virus infections in calves have shown that they are protected for about 10 weeks by passive antibody from maternal colostrum (15).

The pathogenesis of lung damage in viral infections of infants less than 6 months old with homotypic maternal antibody remains obscure. It has been postulated that one of two immunopathological mechanisms may be involved in the production of bronchiolitis by RS virus in-
fection. The first hypothesis, proposed by Chanock and his associates (3), suggests that the severity of bronchiolitis is enhanced by a destructive process initiated by complexing of maternal antibody with viral antigen on the respiratory epithelium. The second theory, advanced by Gardner et al. (7), proposes that bronchiolitis is an anaphylactic reaction to the second infection with RS virus; this theory would require that a mild or inapparent sensitizing infection occur during the first weeks of life under the cover of maternal antibody. Since a practical animal model has not been developed for RS virus diseases, the present investigations were performed with the para-3 virus hamster model because para-3 virus produces a similar disease picture in young infants (8, 10, 13). In these experiments, passive antibody did not enhance the production of pneumonitis produced by para-3 virus unless upon primary infection or reinfection. Therefore, these studies do not support either of the hypotheses proposed for RS virus pathogenesis.

Although passive antibody has a dampening effect on the active serum antibody response, it is not known if it also may affect surface antibody production or cell-mediated immunity. In this model, human immune globulin was utilized as the source of passive immunity, and preliminary observations with homologous antibodies have shown similar effects, but it must be verified for all measurable parameters. Experimental data must be correlated with careful clinical and epidemiological studies of natural infections in infants so that rational decisions can be made about the use of vaccines in the vulnerable infant population. It is difficult to consider immunophrophylaxis in this age as long as there is any possibility of the participation of immunopathological processes in the pathogenesis of natural infections.

ACKNOWLEDGMENTS

The technical assistance of Julia Bell Graham is gratefully acknowledged. Constructive review of the manuscript was kindly performed by Floyd W. Denny. This investigation was supported by Public Health Service grant HL15111 from the National Heart and Lung Institute.

LITERATURE CITED


