Transplacental Transmission of Rubella Virus Infection in Rabbits

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Received for publication 28 July 1970

Rabbits were inseminated artificially and inoculated 1 week later with rubella virus. The attenuated vaccine strain HPV-77 or strain 67-1127 which had been through 12 cell culture passages was used. Half of the rabbits in each group had been inoculated 1 month prior to insemination with the corresponding strain. Evidence of infection induced by either strain was obtained by immunofluorescence in lung, spleen, and placenta. Rubella virus antigen was also found in the lung of a 2-month-old rabbit of a dam inoculated with strain 67-1127. A high rate of stillbirths and neonatal deaths and poor weight gain occurred in litters of the 67-1127 group.

Transplacental transmission of rubella virus infection has been studied experimentally in several animal species (1-3, 5-8). In rabbits, congenital infection has been found to occur with virus strains of various passage levels in cell cultures (2, 3, 5). This report presents the results of an initial study of the capacity of the attenuated vaccine strain HPV-77 and of a strain of several cell culture passages to induce congenital infection in rabbits.

MATERIALS AND METHODS

The virus strains used were HPV-77 from live-virus vaccine lot no. 12 (received from Paul D. Parkman, National Institutes of Health) and 67-1127 isolated from a nasal swab of an infant with rubella syndrome. HPV-77 was passed four times in LLC-MK2 cells in this laboratory, and 67-1127 was isolated and passed twice in primary African green monkey kidney (AGMK) cell cultures and nine times, subsequently, in LLC-MK2 cells. No evidence of agents other than rubella virus in the 67-1127 strain was found by tests in primary cell cultures of cynomolgus and rhesus monkey kidney and human amnion, WI-38 diploid human lung cells, embryonated eggs, and suckling and adult mice. Supernatants of suspensions of LLC-MK2-infected cells and fluid, frozen and thawed rapidly three times, were injected intravenously in 1.0-ml amounts containing 10^9 TCID50. Uninoculated control cells and fluid were prepared and injected similarly.

Fifty-one adult female rabbits of a Flemish giant-chinchilla cross were inseminated artificially. Nineteen were inoculated with the vaccine strain HPV-77, 20 with strain 67-1127, and 12 with control material. Each group was subdivided; one subgroup was injected 1 month before and 7 days after insemination in an attempt to obtain data on whether the immune response to the first injection might alter the effect of the second. The other subgroup was injected 7 days after insemination only. Eight rabbits of each virus group and three controls were sacrificed during gestation.

For virus isolation, three to six tubes of AGMK cell cultures were inoculated with each specimen. Throat and blood cultures from does at intervals after virus injection, tissue suspensions from does sacrificed during gestation, and products of conception were tested. Three blind passages were made. Immunofluorescence tests (IF) were made with impression films of tissue sections stained with rabbit antirubella gamma G globulin conjugated with fluorescein-isothiocyanate. For histological examinations, sections of specimens of various organs from does and fetuses were fixed in Bouin's fluid.

Antibody response was determined by hemagglutination-inhibition (HI) tests by the microtiter technique. The sera titered were serial specimens from adult rabbits up to 6 or 8 months postinoculation and from all surviving young of virus-inoculated dams at 1, 2, and 3 months postbirth. Certain sera from young of virus-inoculated dams were tested for antibody associated with immunoglobulin M by treatment of the sera with 2-mercaptoethanol (2-ME; reference 4). Tests for delayed dermal hypersensitivity were made by intracutaneous injections of 0.1-ml amounts of extracts of rubella virus suspensions treated with NaOH glycine buffer with or without subsequent ether extraction. Litters were observed for gross abnormalities and weight gain.

RESULTS

Pregnancy rates varied in different groups from 55 to 100%. These variations and the small number of litters in some groups precluded comparisons between the two test virus strains and also between groups that were injected 1 month prior
to insemination and those that were not. A high rate, 77.4%, of still births and neonatal deaths occurred in litters produced by dams inoculated with strain 67-1127. The combined rate for HPV-77 and control groups was 29%. (Table 1).

Evidence of virus infection in rabbits inoculated with either test strain was found by IF in lung, spleen, and placenta, but virus isolation attempts were unsuccessful even in repeated tests of tissues showing IF. Specific IF was seen in the impression films of lungs from 7 of 16 rabbits sacrificed 1 or 3 weeks after virus inoculation. Of the seven rabbits with viral antigen in the lungs, spleens of four of five tested and placentas of two sacrificed 1 week after virus injection also showed specific IF. IF was not seen in fetal tissues but was present in the lung of a 2-month-old rabbit of the 67-1127 group that died during a bleeding from the heart. Tissues of control rabbits were negative.

In sacrificed rabbits, histologic examination revealed focal interstitial pneumonitis in six of eight inoculated with strain 67-1127 and in two of nine rabbits given HPV-77. One of three control rabbits also showed focal pneumonitis.

No gross abnormalities were seen in the young. The mean weight gain of litters from inoculated does was compared with that of control litters of equal or larger size. The weight gain from 2 to 4 weeks of age was less than in controls in all three surviving litters of strain 67-1127 groups and in one of the four litters of the vaccine virus group. The differences in the mean gain were from 25 to 100 g. At 3 months of age, the mean weights of four of the seven litters of virus-inoculated groups were less than those of comparable controls: two of strain 67-1127 groups weighed less by 250 and 400 g; two of the vaccine virus group, by 100 and 130 g.

Antibody response in does, based on the geometric mean of HI titers of sera, was 4- to 10-fold higher to strain 67-1127 than to the vaccine strain during the first 5 weeks postinoculation. Maximum titers were reached in 5 to 8 weeks at which time the differences were less; the titers of 67-1127 and vaccine virus groups were 1,760 and 780, respectively, in rabbits given one dose of virus, and 3,675 and 2,470 in rabbits given two doses. After 3 months, antibody levels declined gradually in all groups; after 6 or 8 months, the titers were between 510 and 690. Antibody levels of fetal blood at 25 to 28 days of gestation were similar to those of the respective does. In sera of 1-month-old young of virus-inoculated dams, HI titers were from <10 to 320; at 2 months of age, antibody was detected in the members of only one of the surviving litters; at 3 months, no antibody was found. Treatment of sera with titers of 160 or 320 with 2-ME failed to demonstrate immunoglobulin M-associated antibody. Antibody response to an injection of virus in seven of the young at 5 months was similar over a 4-week period to that of the previous primary response of the mother.

Delayed dermal hypersensitivity reactions were demonstrated in seven of nine does 10 to 12 weeks after injection of either test virus. Control antigen elicited no reactions, and none appeared in control rabbits.

**DISCUSSION**

The observations reported are indicative of transplacental rubella infection in rabbits and are
in general accord with those of Kono et al. (3) and London et al. (5). Evidence of infection in does inoculated with a large dose of either the vaccine strain HPV-77 or a field strain 67-1127 was found by IF, most often in lungs but also in spleens and placentas. Our failure to isolate virus from the rabbits and products of conception may have been the result of attenuation of strain 67-1127 which had a history of 12 passages in cell cultures or to the use of tissue suspensions for isolation attempts rather than explants, the latter found more successful by Kono et al. (3). The possibility of the production of incomplete non-infectious virus demonstrable only by IF must also be considered. Both test viruses induced delayed dermal hypersensitivity.

The findings of rubella virus antigen by IF in the lung of a 2-month-old rabbit of a dam inoculated with strain 67-1127 is indicative of congenital infection. The high rate (77%) of stillbirths and neonatal deaths in litters of strain 67-1127 groups is suggestive, especially so when compared with the combined rate (29%) in HPV-77 and control groups. The failure of some litters of virus-inoculated dams, especially of 67-1127 groups, to gain weight as readily as controls is also suggestive. No malformations in fetuses or progeny were seen on gross examination. Rubella antibody was detected in offspring at 1 or 2 months of age but not at 3 months. No conclusions can be drawn from the failure to find persisting antibody; many of the young of the 67-1127 group died early.

ACKNOWLEDGMENTS

The authors acknowledge with pleasure the assistance of Minerva Bruno and the excellent technical assistance of Glenda Armstrong and Charlotte Eastwood.

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