Antibody Response of Various Strains of Rubella Virus When Inoculated into Rabbits (35695)

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Several vaccine strains of rubella virus as well as many isolates of wild virus isolated directly from patients are now available. Markers are needed to distinguish the attenuated strains from the wild virus and, if possible, to identify the various vaccine strains. Several markers have been proposed to distinguish the vaccine and wild strains. These have included the interferon production, which is reported to be elevated in vaccine strains, and the rapid development of cytopathic effect in certain tissues, which is reported to occur with vaccine strains (1).

In our studies with infected rabbits, we have found that these animals produce high levels of antibody following inoculation with wild rubella virus. Similar results were reported recently by Oxford and Potter (2) in a comparison of wild and attenuated viruses. They also found that animals inoculated intramuscularly with the Cendehill vaccine strain did not develop antibody. With this sensitive model available, various strains of rubella were tested in an attempt to establish a marker which would distinguish one strain from another on the basis of antibody response.

Materials and Methods. Animals. Eighty-eight 7- to 10-lb New Zealand white rabbits were used. The animals were individually caged in isolation chambers to prevent cross infection.

Virus. Eight strains of rubella virus were tested: (i) Freedman; one passage in primary African green monkey kidney cell culture (P_1 in AGMK). This was a "wild" strain of rubella virus isolated in our laboratory. (ii) KO-2 (P_3 in AGMK) rubella virus. This strain was isolated in Japan by Dr. Reisaku Kono, National Institutes of Health, Tokyo, Japan. (iii) Brown (P_5 in AGMK) rubella virus obtained from Parke, Davis Company, Detroit, Michigan, under Vaccine Development Contract PH 43-66-92. (iv) M-33 (P_{30} in AGMK) rubella virus obtained from Dr. Paul Parkman, Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland. (v) HPV-77 (P_{80} in AGMK) rubella virus, vaccine strain of M-33. (vi) Gilchrist (P_{48} in AGMK) rubella virus. Both the HPV-77 and Gilchrist strains were obtained from Eli Lilly and Company, Indianapolis, Indiana, under Vaccine Development Contract PH 43-66-96. (vii) "Cendehill" rubella virus vaccine (Batch 53-4), was grown and serially passed 53 times in primary rabbit kidney. This virus was obtained from Dr. Richard F. Haff, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania. (viii) RA 27/3 rubella virus, passage 27 in WI-38 cell cultures. This strain obtained from Dr. Stanley A. Plotkin, Wistar Institute, Philadelphia, Pennsylvania.

Two control preparations were each administered to 8 animals. These included AGMK and WI-38 control material. One group of 8 sentinel animals was placed in pairs with the Freedman inoculated animals. These rabbits were monitored for evidence of spread of infection from the inoculated animals by using both virus isolation and antibody detection.

Production of virus pools. The Freedman (P_1), KO-2 (P_3), and Brown (P_5) strains were passed once in primary African green monkey kidney tissue in our laboratory. The cultures were harvested on day 7 following inoculation. Sufficient quantities of other strains were obtained so that they were used without further passage.

All strains, except RA 27/3, were titered in AGMK as described by Sever *et al.* (3). RA 27/3 was titered in RK-13 by Dr. Plotkin as described by Plotkin (4).

All eight strains of virus had titers of $10^{3.2}$/ml or more. Each strain was diluted with Hanks' Media so that the inoculum used in the animal studies had a titer of $10^{3.2}$/ml.

Control tissue culture fluids of AGMK and WI-38 with 2% fetal calf serum plus antibiotics were harvested in the same manner as were the virus pools, and these were used for the control inoculations.

Animal inoculations. Groups of eight rabbits were inoculated in the ear vein with 1 ml of each virus strain. Two groups of eight rabbits also received 1 ml of each of the control materials. Sentinel rabbits were placed with the Freedman inoculated animals on the day the experiments were initiated and these animals were followed for 8 weeks. Throat swabs were obtained from each animal on days 7, 9, and 11, postinoculation. These swab specimens were tested for the presence of virus in AGMK tissue culture using the interference method previously described (3). Serum specimens were obtained from each rabbit before inoculation and at weekly intervals for 5 weeks. Antibody titers were determined by the hemagglutination inhibition (HI) method as previously reported (5).

Results. Six rabbits died before the experiment was completed. The causes of death appeared to be unrelated to the experiment. The animals were from the following groups: two had received Brown strain virus, one had been given M-33, two had received Gilchrist, and one the RA 27/3 virus.

Rubella virus was isolated from throat swabs obtained on days 9 or 11 from all eight animals inoculated with Freedman wild strain. Two isolates of rubella virus were made from two rabbits inoculated with the KO-2 strain of virus. Both of these were from swabs obtained on day 11. Five rabbits inoculated with the low passage Brown strain also had rubella virus detected in throat swabs on days 9 or 11. One specimen taken on day 9 from one rabbit inoculated with M-33 strain had rubella virus. The virus was not found in any of the throat swabs taken from animals which received the four "vaccine" strains (HPV-77, Gilchrist P_{48}, Cendehill, and RA 27/3) nor from the controls or sentinels.

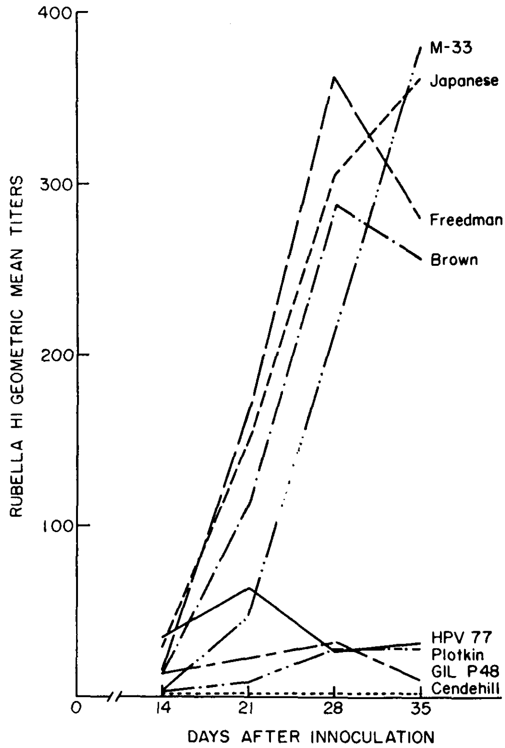

FIG. 1. Antibody titers in rabbits following inoculation with wild and vaccine strains of rubella virus.

The antibody titers found in the rabbits following inoculation are shown in Fig. 1. All of the animals which received low passage virus, including the Freedman's strain, KO-2 strain, and the Brown strain, as well as the animals which received the M-33 strain, rapidly developed antibody within 3 to 4 weeks after inoculation. There was some suggestion of a slight delay in development of antibody with the M-33 strain; however, titers equal to or above those achieved with the other strains were noted by 35 days after inoculation. With each of the candidate vaccine strains, low or no detectable antibody levels were found. HPV-77, the Gilchrist P_{48} and the RA 27/3 strains all showed low persisting titers from 3 to 5 weeks after inoculation. With the Cendehill strain, no antibody was detected in any of the rabbits through 35 days after inoculation.

There was no evidence of infection or antibody response in the control animals nor transmission of infection from the Freedman inoculated animals to the susceptible paired cage mate sentinels.

Discussion. The antibody responses demonstrated in rabbits showed a clear differentiation between the low passage wild strains and vaccine strains of virus. The low passage or wild strains all produced rapid antibody responses which reached high titers. The M-33 strain in this group gave a slight delay in antibody response. The vaccine strains on the other hand all produced only low levels of antibody and with the Cendehill strain there was a complete absence of detectable antibody. Low passage wild virus strains also resulted in shedding of virus from the throats of inoculated animals. This was most pronounced with the Freedman wild strain. There was no transmission of this virus, however, to susceptible cage mates. None of the animals given vaccine strains had detectable virus in the throat.

These observations indicate that the antibody response demonstrated in rabbits can be used to differentiate between the low passage wild virus strains of rubella virus which were tested and the vaccine strains. Furthermore, they permit the identification of the Cendehill strain from the other vaccine strains tested. The presence of virus shedding from the throat may also be used to identify some of the strains since this was most frequent with the very low passage wild virus strains and not present with the vaccine strains studied.

Summary. The inoculation of New Zealand white rabbits with four low passage or wild rubella virus strains and four vaccine strains gave differences in antibody response and virus shedding. These differences can be useful as markers of strain differences and for studies of mechanisms of attenuation. The low passage wild virus strains produced high antibody levels and variable amounts of virus shedding. The vaccine strains resulted in little or no antibody and no virus shedding.

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