Genetic Stability in Humans of the Rabbit Immunogenic Marker of Cendehill Rubella Vaccine Virus

By

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Summary

Laboratory studies were undertaken to differentiate between rubella virus isolates of 'wild' and vaccine origin. Groups of 4 to 5 rabbits (total of 114) were inoculated subcutaneously and intradermally with two consecutive doses of one of seventeen rubella virus strains at a 4-week interval. Sera were collected from each rabbit biweekly for a period of 8 weeks and once 14 weeks after the first inoculation, and were tested for rubella virus hemagglutination-inhibiting (HI) antibodies. It was found that rabbits inoculated with the Cendehill vaccine virus showed no antibody response to two doses of virus, while one dose of each of two 'wild' virus strains elicited high antibody titers in all but one rabbit inoculated. These antibody titers increased following the second virus dose and remained at a high level for 14 weeks. In contrast, rabbits inoculated with 8 virus strains isolated from Cendehill vaccinees, exhibited no immune response to the first virus dose and showed transient, low antibody titers to the second dose. This moderate reversion of the rabbit immunogenic marker of the Cendehill virus in the human host, was not sufficient to render the 'one dose—30 day' test unsuitable for the characterization of rubella virus isolates.

1. Introduction

Several marker tests have been described for the identification of rubella virus vaccine strains (1, 5, 10). The in vitro markers were based on increased interferon stimulation and large plaque morphology in RK13 cells by the Cendehill and HPV-77 vaccine strains (1, 10). Some studies demonstrated, however, that these marker tests were not always capable of distinguishing the attenuated rubella strains from 'wild' virus (6, 11).

The *in vivo* markers in animals appeared to be a more reliable index of attenuation (7, 9), particularly with respect to the Cendehill rubella virus vaccine (4, 12) but the test results depended to a large extent on the techniques employed. Serial passage of the Cendehill rubella strain in primary rabbit kidney cells led to a loss of its immunogenicity for several animal species (12). The absence of seroconversion in rabbits inoculated subcutaneously with the Cendehill vaccine is well documented (12) and constitutes the basis of the rabbit marker test for Cendehill rubella vaccines. While this marker test has proven reliable for the characterization of the vaccine prepared in the laboratory, there are few data concerning the stability of this marker following passage of the vaccine virus in the human host, information essential for identification of a rubella virus isolate of unknown origin (9).

In this study the antibody response of rabbits to rubella 'wild' or vaccine (Cendehill, HPV-77) strains was monitored biweekly for a period of 8 to 14 weeks to establish the reproducibility of the immunogenic marker test. Attempts were made to determine in rabbits the immunogenic characteristics of rubella virus strains that were isolated from persons who had received Cendehill vaccine.

### 2. Materials and Methods

#### 2.1. Virus Strains

The Cendehill rubella vaccine (passage 53 in primary rabbit kidney cells) was obtained in lyophilized form from Smith Kline and French Canada Ltd., Montreal. In one experiment three preparations of this vaccine strain were obtained by two passages in RK13, Vero, or African green monkey kidney (AGMK) tissue cultures. The M33 rubella virus (pass. 4 in BSC-1 cells) and its vaccine derivative HPV-77 (pass. 77 in AGMK) were kindly supplied by Dr. Paul Parkman, Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland, and were passaged once in our laboratory in AGMK cells. The duck embryo (DE) tissue culture strain of HPV-77 (pass. 5 in DE), Meruvax, was obtained from Merck Sharp and Dohme, West Point, Pennsylvania. “Catham” was chosen as a ‘wild’ virus strain isolated in our laboratory from a clinical case of rubella (pass. 3 in RK13). Eight rubella virus strains (Isolates 1—8) isolated from 5 Cendehill vaccinees 8 to 12 days post-vaccination, were kindly provided by Dr. C. H. Taylor-Robinson, Liverpool, England. These strains were isolated in Vero cell cultures from throat, nasal, and cervical swabs, and passaged a second time in our laboratories in the same type of cell cultures. Another rubella virus strain (Isolate-9) was forwarded to us by Dr. L. A. Hatch, London, Ontario. This virus was isolated in RK13 cells from the fetus of a woman who was in contact within her household with a Cendehill vaccinee during early pregnancy and about 18 days later presented a clinical infection of rubella. Isolate-9 was passaged once more in our laboratory in RK13 cells.

#### 2.2. Virus Infectivity Titrations

Virus preparations were titrated in RK13 cells by a previously described micro tissue culture assay (2).

#### 2.3. Immunization Procedure

Male New Zealand white rabbits weighing 5—7 lbs. were obtained from the Animal Care and Research Division of our Department. Unless otherwise specified, rabbits were inoculated in accordance with procedures used in the safety testing of Cendehill rubella vaccine (12): 2.0 ml of virus suspension subcutaneously, with an additional 1.0 ml intradermally at various sites. Since the titer of the reconstituted Cendehill vaccine in RK13 tissue was $10^{4.3}$ TCID$_{50}$/ml, other virus preparations were diluted to a comparable titer. Animals were kept under observation for 29—30 days at which time a second series of inoculations was made. Rabbits were bled from the marginal ear