Studies of Immunization With Living Rubella Virus

Trials in Children With a Strain Cultured From an Aborted Fetus

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A SEVERE epidemic of rubella appeared throughout a large part of the United States early in 1964, resulting in infection of many women.[1,2] During the last months 1964 and early in 1965 numerous infants were born with congenital abnormalities, including cataracts, congenital heart disease, thrombocytopenia, hepatosplenomegaly, bone lesions, and central nervous system damage.[3,4] A causal relationship between maternal rubella and congenital abnormalities had been established over the past 20 years by clinical and epidemiologic observation.[5-9] More recently, the relationship has been substantiated by serological studies of newborn infants with abnormalities[10-12] as well as by the frequent recovery of rubella virus from these same infants.[3,4,13,14] The incidence of congenital abnormalities due to the 1964 rubella epidemic is as yet incompletely assessed, but early estimates have ranged between 0.3% to 4.0% of infants in utero during the epidemic.[4,15,16]

This current experience, in addition to that in past epidemics of rubella, provides persuasive evidence of the need for an immunizing agent which could be administered to girls before they reach the childbearing age. The adaptation of rubella virus to growth in tissue culture[17,18] in 1962 represented a technologic advance greatly facilitating experimental approaches to the development of a vaccine. Since the isolation of the agent, production of both clinical and subclinical infection by inoculation of living virus has been reported by Sever and Schiff[19] and Green et al.[20] These reports, however, concerned the administration of rubella virus either contained in the acute serum from a patient, or passaged briefly in monkey kidney cell culture. Recently, we have begun studies seeking to develop attenuated variants of living rubella virus in tissue culture for use as an immunizing agent. This paper records the result of administration to children of a strain isolated from a fetus and cultivated in human cells. Children were followed to determine: (1) the nature of clinical reaction to the agent; (2) the extent of virus excretion with infections; (3) the antibody response to infection; (4) the protective effect of inoculation against reinfection; and (5) the presence of secondary spread of rubella to uninoculated children.

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Materials and Methods

Derivation of Rubella Virus for Administration to Children.—Virological Techniques: The nutrient medium used for tissue culture was Eagle's Basal Medium with 10% inactivated calf serum added when cell growth was desired. Double strength of amino acids and vitamins was incorporated into the medium for culture of explants. The concentrations of antibiotics in each milliliter of medium were 100μg of penicillin, 40μg of streptomycin, 50μg of chlortetracycline or 50μg of neomycin, and 20μg of nystatin. Subcultivation of cells was performed with the aid of 0.2% trypsin, and the cells were split 1:2. The organ explant technique used was that of Jensen et al,[21] in which bits of tissue are placed on metal grids at an air-nutrient medium interface.

Source of Virus: Virus was obtained from an aborted rubella-infected human fetus. The 25-year-old mother was exposed to rubella eight weeks after the last menstrual period. A macular rash and lymphadenopathy developed 16 days after exposure, and rubella virus was isolated from her nasopharynx on the second day of rash. Serum neutralizing antibodies to rubella virus increased from <4, at the time of rash, to 16, three weeks later.

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The fetus was surgically ahorted 17 days after the maternal illness and dissected immediately. Explants from several organs were cultured and successful cell growth was achieved from lung, skin, and kidney. All cell strains were found to bc carrying rubella virus. As illustrated in Fig 1, fibroblastic cells from the kidney explant were subcultivated four times by trypsinization and 1:2 split, after which the supernatant fluid was harvested. This harvest was inoculated on stationary WI-38 diploid lung fibroblasts, to initiate infection in these cells. Three further passages were subsequently performed by serially inoculating supernatant tissue culture fluids on fresh WI-38 cultures. Two weeks after inoculation of the fourth set of WI-38 cell cultures, the supernatant fluid was harvested, pooled, and divided into aliquots.

Safety Tests: Before use in children, the following safety tests were performed: The material was tested for the presence of bacteria, fungi, and *Mycoplasma* by inoculation onto appropriate artificial media. Tests for safety in animals included injection of aliquots of the pool into adult mice (intraperitoneally and intracerebrally), suckling mice (intraperitoneally and intracerebrally), guinea pigs (intraperitoneally), and rabbits (subcutaneously). All animals remained well for six weeks.

Further tests for identity and for the absence of contaminating agents were performed in tissue culture. Primary African green monkey, human embryo kidney, primary rabbit kidney, and WI-38 human diploid cells all were inoculated with aliquots of virus, either directly or after neutralization for one hour at 37 C with rabbit antirubella virus serum. There was no evidence of any agent other than rubella in the pool. The material titered $10^{2.5}$ (InD_{50}*) in African monkey kidney by interference.

Procedures for Test of Clinical Specimens.—The methods used for collection of specimens, isolation of virus from clinical materials, and for determination of neutralizing antibody have been described in detail elsewhere." Suffice it to say that the cell system used was African green monkey kidney cells and that virus was detected by interference with echovirus 11.[17] The specimens tested included throat swabs collected in Hanks medium and sera, both of which were stored at —20 C until tested.

Population: The subjects were 31 healthy, normal children, aged 14 to 29 months (average, 21 months), from an orphanage supervised by the Archdiocese of Philadelphia. Permission to include the children in this study was obtained from parents or guardians. The sex distribution was predominantly male (23 boys and eight girls). One third of the group was Negro, and two thirds were Caucasian.

Past medical histories regarding possible clinical rubella were not available. The study group was separated from the remainder of the orphanage population by confinement to one floor of two wings. There was no direct contact between children in the two wings, and their caretakers were all immune to rubella. The children in each wing ate and played together. They slept in one of two common sleeping areas each measuring approximately 256 sq ft and containing eight cribs.

Design of the Trials: Inoculation of rubella virus in children were performed either subcutaneously or intranasally. The subcutaneous technique consisted of the inoculation of 0.1 or

* Interference dose 50.

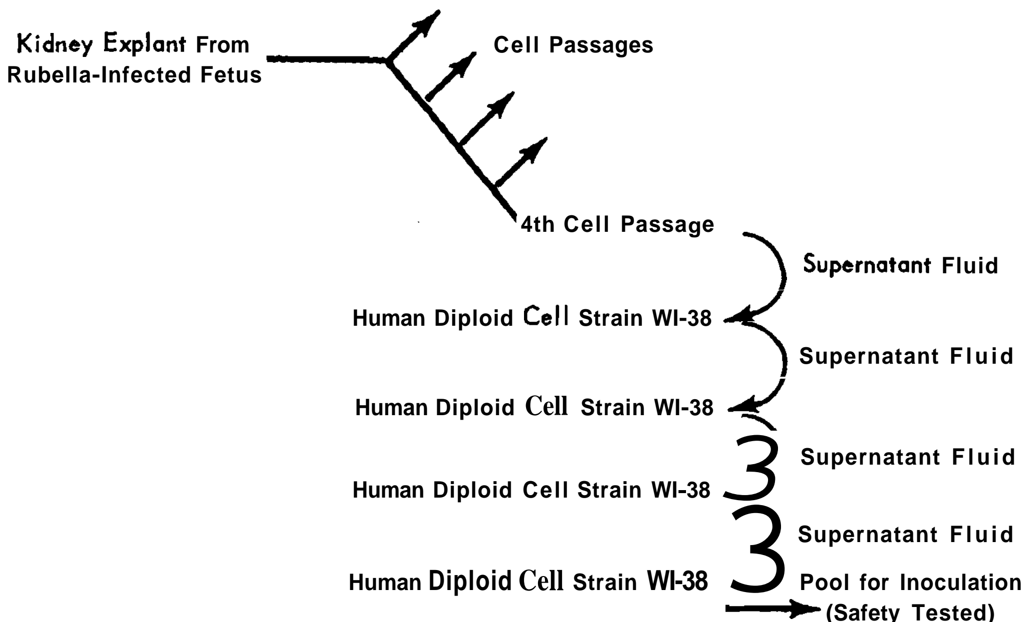

Fig 1.—Derivation of rubella virus pool for inoculation.

Summary

Rubella virus isolated from an aborted human fetus and grown in human diploid lung cells (WI-38) was used to induce infection in children. Subcutaneous inoculation of the virus regularly produced infection in seronegative children, while intranasal instillation gave relatively poor results. Neutralizing antibodies produced by infection prevented reinfection in three children who were reinoculated with a larger dose of virus. The clinical illness induced was mild in all children.

Contact infections were noted, however, in some of the children exposed to primary cases of rubella, precluding the use of this agent for immunization of the general population.

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Generic and Trade Name of Drug

Nystatin—*Mycostatin*.

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