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Attenuation of Rubella Virus by Serial Passage in Primary Rabbit Kidney Cells

III. Clinical Trials in Infants

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Extract

High passage Cendehill strain of rubella virus, possessing *in vivo* and *in vitro* characteristics of an attenuated virus, was tested for efficacy in 28 seronegative infants, 3 to 23 months old. Thirteen seronegative infants received one subcutaneous injection of 0.5 ml of the vaccine preparation containing $10^{3.7}$ plaque-forming units (PFU) per ml. Fifteen seronegative infants served as controls for viral spread and were kept in intimate contact with the vaccinees for a period of 6 weeks.

All thirteen vaccinees developed high levels of hemagglutination-inhibiting antibodies, from $^1/_{128}$ to $^1/_{2048}$, confirming the immunogenicity of the vaccine. No clinical reactions were observed in the vaccinees. The fifteen contacts remained seronegative, indicating that no viral spread occurred from the vaccinees to the susceptible contacts.

The present clinical trial provides evidence that high-passage Cendehill strain presents characteristics of immunogenicity, nonreactogenicity and noncommunicability, making it a prospective candidate for a live attenuated rubella virus vaccine.

Speculation

When the results of preliminary immunization trials with the attenuated Cendehill strain of rubella virus are confirmed in larger groups, this strain may meet all the criteria required for a safe and efficient immunizing agent against rubella.

Introduction

Over the last few years marked progress has been made in the rubella problem. Since the isolation of rubella virus by PARKMAN *et al.* [6] and WELLER and NEVA [15], *in vitro* methods for the cultivation of rubella virus and for titration of specific antibodies have become available. The virologic and serologic investigations recently carried out have confirmed the potential teratogenic effect of this virus as observed by GREGG [2]. The discovery of several tissue culture systems for the propagation of the virus has now made the development of a rubella virus vaccine theoretically possible.

In previous reports [3], we described the characteristics of a rubella virus strain (Cendehill strain), which has been markedly modified in its biological properties after serial passage in primary rabbit kidney cell cultures.

In comparison with the original parent strain, high-passage Cendehill strain was shown to produce an early cytopathic effect in primary rabbit kidney cultures and to have lost its capacity to evoke antibodies

in rabbits inoculated subcutaneously or in monkeys inoculated intranasally [3, 8]. It also forms distinct plaques in RK_{13} cells and induces tenfold the amount of interferon in rabbit cell cultures [10]. At its 21st passage level, Cendehill strain was tested for safety, and preliminary clinical trials were performed in seronegative children with promising results [10]. All vaccinated children showed seroconversion, and no viral spread occurred in seronegative children kept in contact with the vaccinees. Only one of the 25 vaccinees reacted with mild but typical symptoms of rubella. In subsequent trials, higher passage levels of Cendehill strain were used [10]. This report gives the results obtained in seronegative infants inoculated with Cendehill strain at the 51st passage level.

Materials and Methods

Study Group

These studies were performed, with the consent of parents, on normal children in two institutions in Switzerland.

The age of the infants ranged between 3 and 23 months. Serum samples taken from infants in the two institutions (1st trial and 2nd trial), were examined serologically by the hemagglutination-inhibition test. In each institution, a group of seronegative infants was vaccinated and another group of seronegative infants was kept in intimate contact with the vaccinees over a period of 6 weeks. All infants were examined daily by one of us for the presence of clinical symptoms. At the end of the trial, a serum sample was again obtained from the infants. Antibody titers on paired serum samples were determined by the hemagglutination-inhibition test (HI).

Vaccine Preparation

The preparation of the freeze-dried vaccine has been described in detail previously [8]. Safety tests performed on the experimental vaccine were similar to those required for live measles vaccine. Each vial contained $10^{3.7}$ plaque-forming units (PFU) of rubella virus as assayed in RK_{13} cells.

Administration of Vaccine

Each vial containing freeze-dried vaccine was reconstituted to its original volume with 1 ml of distilled water; 0.5 ml of the virus suspension (corresponding to $10^{3.4}$ PFU) was administered subcutaneously to each child.

Serologic Tests

For the determination of the antibody titers, we used a modification of the hemagglutination-inhibition (HI) technique described by STEWART *et al.* [13]. Pigeon erythrocytes were used instead of erythrocytes from one-day-old chicks [9]. To remove nonspecific inhibitors, all serum samples were first treated with pigeon erythrocytes, then with acid-washed kaolin. Four hemagglutinating units were used per tube. HI titers were expressed as the reciprocals of the highest serum dilutions which completely inhibited hemagglutination. Positive and negative reference sera of human origin were used in all tests.

Table I. Hemagglutination-inhibition (HI) antibody titers in prevaccination and postvaccination sera of infants vaccinated against rubella

Child No.	Age in months	Vaccinated or contact	Pre-vaccination HI titer[1]	Post-vaccination HI titer[2]
1st trial				
1	11	vaccinated	<8	512
5	22	vaccinated	<8	1024
6	15	vaccinated	<8	1024
8	18	vaccinated	<8	128
9	5	vaccinated	<8	256
10	10	vaccinated	<8	1024
4	12	contact	<8	<8
7	15	contact	<8	<8
14	11	contact	<8	<8
16	23	contact	<8	<8
92	8	contact	<8	<8
2nd trial				
65	13	vaccinated	<8	128
70	3	vaccinated	<8	512
74	17	vaccinated	<8	128
77	7	vaccinated	<8	256
80	12	vaccinated	<8	512
82	7	vaccinated	<8	2048
83	8	vaccinated	<8	1024
64	15	contact	<8	<8
66	8	contact	<8	<8
67	8	contact	<8	<8
68	7	contact	<8	<8
69	10	contact	<8	<8
71	12	contact	<8	<8
72	14	contact	<8	<8
78	8	contact	<8	<8
79	9	contact	<8	<8
81	9	contact	<8	<8
76[3]	8	vaccinated	8	4

[1] Prevaccination sera were taken on the day of vaccination.

[2] Sera were taken respectively on the 45th (1st trial) and 43rd (2nd trial) day postvaccination.

[3] Infant with maternal antibodies prior to vaccination.

Results

The results of the hemagglutination-inhibition tests are summarized in the accompanying table. In the first institution, six seronegative children were vaccinated and five seronegative children served as controls. As shown in the table, all vaccinees had developed HI antibody titers of $^1/_{128}$ to $^1/_{1024}$ by the 45th day postvaccination. All contact children remained seronegative.

In the second trial, seven seronegative children were vaccinated and kept in contact with ten seronegative controls. By the 43rd day postvaccination, all vaccinees had developed HI antibody titers of $^1/_{128}$ to $^1/_{2048}$, whereas all contacts had remained negative. The table also includes the case of a child (No. 76) who was not a seronegative subject but who still had maternal antibodies ($^1/_8$) prior to vaccination. This was the only infant in the whole group who did not respond to the vaccine; in the serum sample from this child taken 43 days postvaccination, a very low titer of maternal antibodies was still detectable ($^1/_4$).

Close observation of all infants during the whole period of the trial failed to show any clinical symptoms.

Discussion

The early observations by GREGG [2] on the potential teratogenic effect of rubella virus have been confirmed by numerous investigators [12], especially during the 1964 rubella epidemic in the United States. On the other hand, extensive serological surveys have shown that at least 10–20 % of all women in the child-bearing age are seronegative and thus exposed to the risks of rubella virus infection during pregnancy.

Active immunization of the female population before child-bearing age would, therefore, be the most logical approach to prevent this risk. Since all attempts to produce a killed virus vaccine have been unsuccessful because of insufficient potency, research has been directed towards the development of attenuated live vaccines. Preliminary data have already been reported on rubella virus attenuated on green monkey kidney cultures [5, 7], on duck embryo cells [14], on human diploid cells [11], and on primary rabbit kidney cultures [3, 8].

Theoretically, an acceptable live vaccine against rubella should meet with a number of criteria: (1) It should induce strong and long-lasting immunity. (2) It should be nontransmissible, giving no spread from vaccinated persons to nonvaccinated susceptible contacts. (3) It should be devoid of reactogenicity. (4) It should be produced in a tissue culture system free of adventitious viral agents. (5) It should possess virological 'markers' by which it can be readily identified and differentiated from wild rubella virus strains. (6) It should be stable during storage. We intend in this discussion to review to what extent the Cendehill strain (passage 51) meets these requirements.

The results reported by PLOTKIN *et al.* [10] and those described in this paper demonstrate that the Cendehill strain (51st passage level) induces a good antibody response. All vaccinated children showed seroconversion. In the trial conducted by PLOTKIN *et al.* [10], the seroneutralization test was used to measure the antibody titers. In the trial reported here, the hemagglutination-inhibition test was used. This test has been shown to be 2 to 16 times more sensitive than the seroneutralization test [13]. The duration of immunity can only be established at some time in the future by periodically measuring the antibody titers in the vaccinees.

As observed for other virus vaccines, the presence of maternal antibodies seems to inhibit the development of active immunity against rubella since one infant who still had maternal antibodies at the time of vaccination failed to respond to the vaccine.

In the absence of data on the potential teratogenic properties of the vaccine virus, the lack of spreading capacity is, at this stage, the most important criterion in the evaluation of a live rubella virus vaccine. In the trials performed so far with the Cendehill strain, all contact children remained completely free of antibodies against the rubella virus. This may be considered as evidence of noncommunicability, especially when a very sensitive serological test, such as the HI test, is used for checking the absence of antibody formation in the contacts. The Cendehill strain probably lost its spreading capacity at an early passage level, since in earlier trials, none of the children vaccinated with the 21st passage virus transmitted the virus to susceptible contacts [10].

The nontransmissibility of a rubella vaccine virus is probably dependent upon two factors: (1) the amount of vaccine virus shed by the vaccinees, and (2) the tropism of the virus strain for the naso-pharyngeal mucosa.

The first aspect, virus-shedding, was investigated by PLOTKIN *et al.* in a trial involving seven seronegative children vaccinated with Cendehill strain (passage 51) and seven seronegative children kept in contact with the vaccinees [10]. Fourteen swab samples were collected from the nasopharynx of each child between day 7 and day 42 postvaccination. From a total of 196 swabs examined, virus was recovered 5 times between the 9th and 11th day, and only after blind passages in tissue culture. In three vaccinees, virus was isolated only once, in one vaccine twice, the other three vaccinees remaining negative. No virus was isolated from any vaccinee before the 9th or after the 11th day post-

vaccination or from the contact children at any time.

The other factor, ability to infect by the nasal route, was investigated by us in rhesus monkeys. These animals have been shown to react serologically to experimental infection using intranasal instillation of the rubella virus [5]. In our attempts to infect monkeys intranasally with high-titered inocula of the 51st passage Cendehill virus, none of the inoculated monkeys showed any evidence of serologic response, whereas control monkeys inoculated with nonattenuated virus developed high antibody titers [3]. This suggests that the attenuated virus has lost its capacity to multiply in the nasopharyngeal mucosa. This factor, together with the low degree of excretion, may explain the noncommunicability of the attenuated virus in humans.

Although rubella is usually a mild disease in children, preference should be given to a vaccine causing no clinical symptoms in vaccinees. As reported previously, the 21st passage level of the Cendehill strain still induced mild rubella symptoms in one of the 25 vaccinated children [10].

In all the children vaccinated with Cendehill strain at its 51st passage level, no reaction has been observed. More specifically, there have been no cases with rash, lymphadenopathy, or any other clinical symptom suggestive of a rubella infection.

From the viewpoint of safety, a live attenuated vaccine should preferably be grown in a nonsimian tissue, since there is considerable evidence that monkey cells are frequently contaminated with latent agents. Primary rabbit kidney (PRK) cell system has not been examined as thoroughly or as extensively as simian cells but this cell substrate, used for many years in live polio-vaccine tissue culture controls, has been found to be remarkably free of adventitious viral agents [1, 4, 8]. Furthermore, rabbit kidneys can be readily obtained from healthy young animals bred in closed colonies under optimal conditions of isolation and hygiene. From our experience, PRK system appears to be a 'clean' tissue culture substrate for vaccine preparation.

The high passage Cendehill strain can be easily identified and differentiated from wild rubella virus by its characteristics. *In vitro*, it produces an early cytopathic effect in PRK cells and forms in RK_{13} cells distinct plaques specifically inhibited by low bicarbonate concentrations. *In vivo*, it is characterized by its lack of immunogenicity in the rabbit after subcutaneous inoculation or in the rhesus monkey after intranasal administration.

Finally, several experimental batches of high-passage Cendehill strain vaccine were freeze-dried and stored. Such preparations have been shown to be stable for at least several months at 4°.

All data so far available indicate that Cendehill strain (passage 51) is a prospective candidate strain for a safe and efficient live rubella virus vaccine. Further work is needed on a larger scale to confirm the data reported here and to fulfill all other criteria for an acceptable vaccine.

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