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Experimental Active and Passive Immunization against Rubella

By

J. Budai, Margit Tóth and L. Melis

Central Hospital for Infectious Diseases, Budapest

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Active and passive immunization against rubella has been studied. The Cendehill 51 live attenuated vaccine caused no clinical symptoms in seronegative inmates of a children's home and induced an antibody production which resulted in appropriately high HI titres and seemed to be of long duration. In accordance with literary data the vaccine virus could be re-isolated from the throat of some of the vaccinees without any dissemination of the virus. The rubella HI titre of the originally seropositive vaccines showed a slight increase. The protective effect of the Cendehill 51 vaccine has been proved experimentally; the vaccine meets the requirements for a rubella vaccine.

For experimental passive immunization, a gamma-globulin preparation of high HI antibody titre was used. In children given the dose/kg body weight usually administered to exposed women, even if it was injected before active immunization with the Cendehill 51 vaccine, the infection was not influenced notably. Three times larger doses prevented seroconversion in half of the children if administered before vaccination, but the protective effect was minimal if given five days after vaccination.

The public health importance of rubella is primarily due to its teratogenic effect. Although the participation of maternal rubella in human teratogeny is as low as 3—5%, control of the disease is of importance because

(a) 15—20% of women in the generative age are susceptible to rubella [3, 30, 36];

(b) importation of rubella virus strains more teratogenic than the presently prevalent ones cannot be excluded [18];

(c) an increase in number of defective children as a consequence of rubella epidemics may result in great social, public health and other problems as well as economic loss [4];

(d) rubella is the only teratogenic agent which can be controlled without risk, *viz.*, by active immunization.

The pathological character of intrauterine rubella explains why the fetus is protected if the specific antibody level in the blood of the mother is sufficiently high to prevent maternal viraemia.

It is highly probable that rubella infection, either subclinical or accompanied by illness, is followed by an immunity which completely protects the fetuses of subsequent pregnancies. In immune subjects viraemia does not develop even if the serum antibody level is too low to prevent re-infection, *i.e.*, viral multiplication

in the pharynx [24, 33, 39]. Such reinfection may exert a booster effect.

In the present work children were immunized with the Cendehill 51 vaccine. This was the first use of rubella vaccine in Hungary. Besides, the usual method of passive immunization of pregnant women was checked.

I. Active Immunization of Children with the Cendehill 51 Vaccine

Materials and methods

The vaccine, prepared and kindly supplied by the Belgian RIT Vaccine Institute, contained live attenuated rubella virus in the lyophilized state. Each dose, containing 3×10^3 TC_{50} of virus, was suspended in 0.5 ml distilled water. The vaccine was administered subcutaneously.

Children from one to three years of age were elected from the inmates of a Budapest children home. The children lived in several cottages built 50 to 100 m apart. The children in each cottage were staying in the same room.

Bodily and mentally healthy children with no history of rubella were chosen. Of these 107 were tested for haemagglutination-inhibiting (HI) antibodies. The vaccinees and their contacts were subjected to regular medical observation during the period when vaccination illness was expected.

Serological tests

HI antibodies to rubella virus were determined according to Stewart's method [35] as modified by Peetermans and Huygelen [27]. A haemagglutinating extract from rubella virus (lyophilized preparation from the RIT Institute) [28] and pigeon erythrocytes were used. From the serum samples to be tested the non-specific inhibitors and the disturbing normal agglutinins were removed by absorption with kaolin and pigeon red cells, respectively.

Blood samples were taken before and 6 to 8 weeks after vaccination from each vaccinee, and at the same time from all the children included in the experiment.

Virus isolation

Nasal and pharyngeal swabs were taken on each of the 7th to 12th postvaccination days. The swabs were then washed into a tube containing gelatin in phosphate buffer. The suspension thus obtained was centrifuged and, with antibiotics added, inoculated into RK_{13} cell cultures. Nine days later the cultures were frozen and thawed and a subpassage was made in each of RK_{13}, RC, HEp-2 and primary monkey-kidney cell cultures. The isolates which were thought to be rubella virus on the basis of their cytopathic effect in RC cell cultures were identified in neutralization tests carried out in RK_{13} cell cultures, using anti-rubella monkey and rabbit sera.

Gamma globulin

The 10% gamma globulin preparation batch No. 577 of the Institute for Serobacteriological Production and Research, Human, Budapest, was used throughout. The HI titre of the preparation was 1:1024.

Results

Of the 107 children with no history of rubella 68 proved to be seronegative. The remaining 39 presumably had had a symptomless rubella infection.

First trial

In November, 1968, four groups each consisting of 10 children were set up. Group 1 included seronegative

children to be vaccinated, group 2 seronegative children to serve as contact controls, group 3 included seropositive children to be vaccinated, and group 4 seropositive children to serve as clinical controls.

Among the 10 seronegative vaccinees one had left the children home before the end of the experiment. None of the others showed any sign or symptom of rubella or any other disease. Each of the 9 vaccinees developed HI antibodies. The postvaccination titres ranged between 1 : 64 and 1 : 512, with a geometric mean of 1 : 161.

The virus was re-isolated from three children; one nasal and one pharyngeal swab taken on the 9th day and two nasal and two pharyngeal swabs taken on the 12th day proved to contain the virus.

In the group of seronegative unvaccinated children all children remained symptomless. None of them had developed antibodies.

The seropositive vaccinees remained symptomless and had no complaints during the observation period. The geometric mean of their HI titres rose from 1 : 116 to 1 : 256.

In the seropositive clinical control group the children remained symptomless.

TABLE I

Immunization with Cendehill 51 vaccine. First trial.
Vaccination: November 25, 1968.
Blood sampling: January 17, 1969.

	Vaccinated children		Unvaccinated controls	
	seronegative	seropositive	seronegative	seropositive
No. of children	9	10	10	10
Clinical symptoms	0	0	0	0
Seroconverison	9/9	—	0/10	—
Geometric mean of prevaccination/ postvaccination titres	0/161	116/256	0/0	—
Re-isolation of virus	3/9	—	0	—

Second trial

Thirty-two seronegative children were vaccinated on February 25, 1969; 15 of them lived in cottage II and 17 in cottage III. At the same time 9 and 6 seronegative children, respectively, were put under observation.

The children in cottage II remained symptomless and all had developed antibodies. The HI titre ranged between 1 : 128 and 1 : 2048, with a geometric mean of 1 : 851.

Among the control children as well as among the children living in the same cottage but not included in the trial, a rubella outbreak occurred one

month after the vaccinations. In the control group two children developed typical rubella, two further children had fever without exanthem. All these children and two symptomless control children (a total 6 of the 9 controls) had developed antibodies. The HI titre ranged between 1 : 256 and 1 : 2048, with a geometric mean of 1 : 1320.

All the vaccinees in cottage III developed HI antibodies, without any clinical symptom. The extreme titres were 1 : 64 and 1 : 2048. The geometric mean was 1 : 472.

One member of the control group was transferred to cottage III from cottage II. At that time this child had fever. His second blood sample had a HI titre of 1 : 2048. The other members of the control group in cottage III remained symptomless and showed no seroconversion.

Discussion

The requirements for a live attenuated rubella vaccine are covered by four different vaccines. Each of these were evolved in different laboratories and prepared from different strains in different cell cultures. The vaccine strains are as follow. (a) Derivatives of the strain HPV-77; (b) members of the Merck—Benoit group; (c) the Cedenhill 51 strain; and (d) the RA 27/3 strain [24].

The Cedenhill 51 strain was isolated in primary monkey-kidney cell culture from the urine of a patient with rubella. After four further passages

TABLE II

Immunization with Cendehill vaccine. Second trial.
Vaccination: February 25, 1969.
Blood sampling: April 19, 1969.

	Cottage II	Cottage III
Seronegative vaccinees		
No. of children	15	17
Seroconversion	15/15	17/17
Geometric mean of posvaccination HI titres	851 (128—2048)	472 (64—2048)
Clinical symptoms	0	0
Seronegative control children		
No. of children	9	6
Seroconversion	6/9	1/6
Geometric mean of HI titres of second blood samples	1320 (256—2048)	2048
Clinical observations		
typical rubella*	2	0
atypical rubella*	2	1
Symptomless seroconversion	2	0

* Late in March, 1969.

in monkey-kidney cell cultures the strain was adapted to primary rabbit-kidney cell culture. Among the different passage levels of the strain those having undergone 51 or 53 passages proved to be most suitable for vaccination. These caused no clinical symptoms and evoked a high-level immunity which seems to be of long duration. Although the vaccine virus can be recovered from the throat of some of the vaccinees, contact infection does not occur [13, 19, 21, 26, 29]. It should be emphasized that the primary rabbit-kidney culture is considered to be free from viruses attacking man [15, 19].

The Cendehill 51 virus has a further advantage; unlike other rubella vaccine strains it rarely causes joint complaints in adults [2, 5, 8, 10, 11, 17].

The Cendehill 51 vaccine has been licensed in the USA and introduced in several West-European countries as well as in Australia [9]. More than 30,000 subjects have been immunized with this vaccine.

The results of our first trial have confirmed earlier observations with the administration of this vaccine. It caused no symptoms in the children. The numerical data of the antibody response were consistent with literary data, proving the good antigenicity of the vaccine. The high post-vaccination titres are suggestive of a long-lasting immunity. According to several literary data, the post-vaccination titres remained practically unchanged for years, running near the titre curves characterizing the immunity following natural rubella [20, 29].

The re-isolations in the present study were consistent with earlier observations. Although the virus was present in the throat of some vaccinees 9 or 12 days after vaccination, no secondary cases occurred in the seronegative contacts as shown by the absence of HI antibody response. In the lack of implantation of the vaccine virus in the throat of the contact children besides the changed qualities of the vaccine strain, quantitative factors may also play a role. The virus titre in the throat is much lower than during natural rubella [8, 24, 29], when 80% contagiosity may occur [12, 31]. The lack of virus dissemination excludes the accidental infection of pregnant women with the vaccine virus as well as the possibility that after uncontrollable passages the vaccine virus might regain its virulence. The exclusion of the former risk is especially important because there is no evidence for the lack of teratogenicity of the vaccine strain [29, 38].

The titre increase after the vaccination of seropositive children confirms that re-infection may occur in subjects having acquired immunity to rubella in the natural way. It may be noted that there is no evidence of such re-infection in the case of measles. Natural re-infection followed by booster effect may occur even with wild strains of the rubella virus, but it is not accompanied by viraemia, the phenomenon most important in the mechanism of embryopathy [39].

Evaluation of our second trial was disturbed by the rubella outbreak that occurred one month after the vaccinations. On the other hand, the outbreak enabled us to confirm earlier literary data suggesting that natural rubella infection is followed by a higher antibody response than vaccination. In this respect it was remarkable that in cottage II even the vaccinees who showed no symptom of rubella had developed higher titres than the vaccinees in cottage III, suggesting that in the cottage in which six cases occurred among the controls some of the vaccinees may had been re-infected with the wild virus. The fact that none of the re-infected children became ill suggests that the children were already immune in the early postvaccination period. In this respect it should be emphasized that among the 15 seronegative controls of the second trial 5 became ill with rubella while none of the 32 children vaccinated one month earlier showed any symptom of the disease. A similar early protective effect of other rubella vaccines has already been observed [6].

II. The Protective Effect of Gamma Globulin

The protective effect of commercial human gamma globulin preparations is equivocal. Besides favourable data [25] equivocal [1, 16, 22, 23, 30] and, also, negative results [7, 12, 31, 32] have been published. In some of the studies that yielded negative results, virological methods were also applied. The introduced antibody could not be detected in the blood serum [31, 37].

The present studies were undertaken to investigate whether various amounts of gamma globulin administered before or soon after vaccination with the Cendehill 51 strain were able to influence the course of the infection.

Results

(1) Three seronegative children weighing 8—10 kg each were given 2.5 ml gamma globulin two days before vaccination. Seroconversion ensued after vaccination in all the three cases. The titres in the serum samples taken 40 days after vaccination gave a geometric mean of 1:201.

(2) Each of six seronegative children of about the same body weight were given 7.5 ml gamma globulin. On the second postvaccination day we were able to detect the introduced HI antibody in the blood of two of the children (titre, 1 : 8 and 1 : 8). By the 12th day the antibody had disappeared.

Blood samples taken on the 40th postvaccination day showed that seroconversion had ensued in three of the six children. The geometric mean of the positive titres was 1 : 12.

We succeeded in re-isolating the vaccine virus from the throat of one of the children on the 12th postvaccination day.

(3) Each of nine initially seronegative children were given 7.5 ml

gamma globulin five days after vaccination. Eight children had developed antibodies; their postvaccination HI titre ranged between 1 : 8 and 1 : 64, with a geometric mean of 1 : 24.

The virus was re-isolated from the throat of two children.

TABLE III

Effect of gamma globulin* on artificial Cendehill 51 infection

No. of children observed	Gamma globulin		Passive titre on postvaccination day		Sero-conversion by day 40	Geometric mean of postvaccination HI titres 1:	Re-isolation of virus
	dose ml	time of injection	2	12			
3	2.5	Two days before vaccination	—	—	3/3	201 (32—256)	—
6	7.5	Two days before vaccination	2/6 (1:8, 1:8)	0	3/6	12 (8—32)	1/6
9	7.5	Five days after vaccination	—	—	8/9	24 (8—64)	2/9

* Anti-rubella HI titre, 1:1024.

It should be noted that the neutralization titre of a serum is 4—16 times higher than its HI titre.

Discussion

The degree of passive protection against rubella is a function of at least two variables, *viz.*, the quantity of the introduced antibody and the time of its administration. SCHIFF [34] found that 20 ml of a gamma globulin preparation with a neutralizing antibody titre as high as 1 : 4096 prevented the infection if the gamma globulin was administered before, or within 24 hours after infection. No protective effect was observed when the same volume of low-titre (1 : 256) gamma globulin was administered.

Choosing of the most suitable time is in general difficult. Patients are shedding the virus as early as 7 days before the eruptive phase, *i.e.*, at the time of recognizing a case of rubella, contact cases may have reached the 7th day of the incubation period, thus one or two days before the onset of viraemia, the most important factor in inducing embryopathy. Such a short period may not suffice for completing passive protection.

The anti-rubella antibody titre of different gamma globulin preparations is rather variable [35]. The different batches of the gamma globulin recommended for rubella prophylaxis in Hungary vary in HI titre from 1 : 320 to 1 : 1280. Consequently the introduced antibody often escapes detection in the blood serum [32]. The usual 20 ml gamma globulin if injected into a pregnant woman of 60 kg

body weight will be diluted in her extracellular fluid of approximately 12 litre 1 to 600. Taken into consideration that the lowest detectable titre is 1 : 8, a preparation of 1 : 4800 titre must be used for being detectable in blood.

In accordance with this calculation, we could not detect the introduced antibodies in the sera of the children, except for two children who had received at least three times the dose administered to pregnant women as calculated for body weight (about 0.9 ml/kg as compared to 0.3 ml/kg). In the serum of even these two children the antibody level soon fell below the threshold of detectability.

The 0.3 ml/kg dose of a gamma globulin preparation of relatively high titre (1 : 1024) failed to influence subsequent infection with the attenuated rubella virus. The three times larger dose prevented the subsequent infection in half of the cases, and partially suppressed it in the rest. It may therefore be supposed that administration of an elevated dose of high-titre gamma globulin to a pregnant woman before or at the time of exposure might prevent viraemia and its teratogenic consequences.

Five days after the vaccination even the elevated dose of gamma globulin was without considerable effect.

It may be concluded that the possibilities of passive immunization against rubella are limited. The only practicable way of specific control is the active immunization with live attenuated virus vaccine.

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Dr. J. Budai
Gyáli út 5
Budapest IX., Hungary