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EVALUATION OF THREE RUBELLA VACCINES IN CONFERRING PROTECTION[1]

K. Naficy[2], R. Nategh[3], M. Khakpour[3],
M. Mohagheghpour[3] and N. Sabouri[3]

ABSTRACT

The efficacy of three rubella vaccines, HPV/77/DE-5, Cendehill-51 and Wistar RA/27/3 in conferring immunity against unattenuated rubella virus was compared. Vaccination resulted in 90-100 per cent protection against clinical rubella. Intranasal challenge with the unattenuated strain of rubella virus resulted in serologic evidence of inapparent reinfection in three of the ten Cendehill-51 vaccinees; the virus was isolated from the throat specimens of two, and one developed adenopathy. Two of the eleven RA/27/3 vaccinees became reinfected. Virus was isolated from the throat specimen of one and a booster response occurred in the other; the latter developed rash. All of the HPV/77/DE-5 vaccinees remained symptomless after the challenge. None demonstrated booster response and shed virus.

INTRODUCTION

A considerable number of investigations have indicated that the available attenuated rubella vaccines do not always confer immunity since reinfection with wild rubella virus, as measured by the rise in the antibody titer and

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2- Deceased in November 1972.

3- Department of Epidemiology and Pathobiology, School of Public Health and Institute of Public Health Research, University of Teheran, P.O. Box 1310, Teheran, Iran.

isolation of the virus from the throat, can occur, especially in vaccinees with low antibody titer [1–6].

In the present study we have compared the quality of immunity induced by three rubella vaccines: HPV/77/DE-5, Cendehill-51 and Wistar RA/27/3. We have investigated this by comparing the rate of seroconversion and antibody titer after vaccination and severity and nature of clinical reactions, as well as reinfection rates, after challenge with an unattenuated strain of rubella virus in vaccinated and susceptible children residing in a closed comunity.

MATERIALS AND METHODS

Vaccines. — HPV/77/DE-5, Cendehill-51 and Wistar RA/27/3 rubella vaccines produced by serial passage of rubella virus on green monkey kidney and duck embryo kidney, primary rabbit kidney and WI-38 human diploid cells, respectively, were used in this study. The HPV/77/DE-5 vaccine had a titer of $10^{2.9}$ TCID50 by the interference method, the Cendehill-51 had a titer of 10^{3} TCID50 and the Wistar RA/27/3 had a titer of $10^{3.2}$ TCID50.

Unattenuated Virus. – Brown strain of rubella virus with a titer of 2×10^{3} tissue culture infective dose (TCID50) (monkey kidney echovirus 11 interference method) was employed as the challenging virus. The virus had undergone five passages in primary African green monkey kidney (GMK). For intranasal administration, posterior pharynx roughing was carried out with a sterile cotton swab prior to dripping 0.25 ml of the virus into each nostril of the subjects. All subjects remained supine for at least 30 seconds after virus administration.

Study Population. – Forty-four seronegative (no detectable rubella hemagglutination inhibition antibody titer at 1 :10) and 16 seropositive children, ranging in age from 3.5 to 7 yearss, were drawn from an orphanage near Teheran. A full written consent was obtained from the relevant authorities. All the attendant men and women were seropositive.

Experimental Design:

The first phase of the study. – The seronegative subjects were divided into four study groups: Group 1, nine children were immunized with HPV/77/DE-5; Group 2, ten children received Cendehill-51 rubella vaccine; Group 3, eleven children received RA/27/3 rubella vaccines; Group 4, fourteen seronegative children; and Group 5, sixteen naturally-immune individuals served as an unvaccinated control.

Follow-up. – From day 1 to 28 of vaccination the reactions to vaccination were assessed daily by taking rectal temperature and performing physical examination. Special attention was given to the development of adenopathy, rashes and joint manifestations. Temperatures and results of physical

examination were recorded on individual follow-up cards.

Blood samples for Antibody Assay. – Blood samples for an antibody assay were obtained on filter paper from all subjects on the day of vaccination (day O) and four weeks after viral administration.[7]

The second phase of the study. – Five months after vaccination (day before challenge), blood samples were obtained from the vaccinees and the seronegative control subjects for antibody assay. The entire population, that is, the vaccinated and seronegative uninoculated children, then received unattenuated rubella virus.

Follow-up. – Again from day 5 to 20 of the challenge, physical examination of all children was performed daily.

Blood Samples for Antibody Assay. – Blood samples were collected 6 weeks after challenge.

Collection of Specimens for Viral Isolation. Throat swab specimens were obtained on days 7, 10, 12, 13, 14, 15, 16, 17, 18, 19 and 20, and blood samples on days 7, 10, 13, 14 and 15 of viral inoculation as described previously.[8]

Rubella Antibody Titer. – Rubella hemagglutination inhibition antibody titration was performed as described before.[8]

Viral Isolation. – Two-tenths of a milliliter of each specimen was inoculated into two tubes each of GMK culture and incubated at 34-35°C. Ten days after the inoculation, the cells were scraped and passed into two GMK cell cultures. On the tenth day of the passage each culture was challenged with 100 TCID50 of Echovirus 11.

RESULTS

Clinical Response to the Vaccines. — None of the vaccinees showed any clinical signs or syptoms which differed significantly from the naturally-immune control subjects (Table 1). The occurrence of a considerable amount of fever in control and vaccinated children could be due to other agents, as this study was begun during the second month of winter.

Antibody Response to Vaccines. – Four weeks after vaccination, all of the susceptible vaccinees had developed detectable antibodies. As shown in Table 2, children vaccinated with HPV/77/DE-5 had the highest titers as compared with those receiving Cendehill-51 and RA/27/3.

Clinical Response to the Unattenuated Rubella Virus. – Table 3 summarizes the occurrence of fever, adenopathy and rashes in the susceptible and vaccinated children following administration of the unattenuated rubella virus. Four out of 14 seronegative control group children demonstrated fever. Seven of them demonstrated lymph node enlargement and five developed rashes. In contrast, none of the vaccines demonstrated fever. Only one of the subjects in the Cendehill-51 group who had a low antibody titer (1: 10)

demonstrated adenopathy, and one individual in the RA/27/3 group developed rashes.

Antibody Response to Unattenuated Rubella Vaccines. – The serological response of 14 seaonegative children after viral administration are summarized in Table 4. While six (43%) failed to acquire rubella HI antibodies, in eight (57%) titers were in the range of 40 to 1280 six weeks after administration of the virus. Table 5, 6 and 7 show the HI antibody titer in vaccinated children at intervals of four weeks and five months after vaccination, and also six weeks after the challenge. As can be seen, a booster response of four-fold rise or more occurred in one of the 10 children who were vaccinated with RA/27/3 (Table 5) and three of those who received Cendehill-51 (Table 6).

Viral Isolation. — Rubella virus was isolated from the throat swab specimens of seven seronegative control subjects (50%). The timing of these positive cultures is shown in Table 4. In some of the children, the virus was isolated repeatedly on different days. All but one (Subject No. 9) of the susceptible children who excreted virus during the study period had rubella antibody titers in the range of 40 to — 1280 (Table 4). Three of the subjects who had a positive culture from the throat swab specimens showed viremia. Virus was isolated from the blood of subject No. 9 who, as indicated above, failed to demonstrate seroconversion. After challenge, no virus was isolated from the blood or throat swab specimens of the nine children who were vaccinated with HPV/77/DE-5 (Table 7). However, the virus was isolated from the throat specimens of two subjects who were immunized with Cendehill-51 (Table 6) and one of those who received RA/27/3 vaccine (Table 5). Virus was isolated on days 7, 13 and 16 of the challenge.

COMMENTS

The effectiveness of subcutaneous administration of HPV/77/DE-5, Cendehill-51 and Wistar RA/27/3 strains of rubella vaccines in conferring protection against artificial challenge with the rubella virus was studied. The results reported here indicate that these three vaccines have 90 to 100 per cent protective effect against clinical rubella, as judged by the development of adenopathy, rashes and occurrence of fever in the vaccinees. Although the numbers are small, our findings are similar to those of others who have reported that clinical protection is usually conferred by these vaccines.[1,4,8-11]

None of the HPV/77/DE-5 vaccinees experienced inapparent reinfection, as manifested by a significant rise in antibody titer or detection of virus from the blood or throat swab specimens. The effectiveness of this vaccine in conferring immunity against the unattenuated virus could be due to its capacity to induce high antibody levels. Our results compare favorably with the findings of others which indicate that, in general, resistance to reinfection

correlates with high antibody titer. [1–3] Reinfection, however, occurred in a few of the Cendehill-51 and RA/27/3 vaccines. Upon challenge, three of the 10 (30%) Cendehill-51 vaccinees deonstrated considerable antibody rise and virus was recovered from the throat swab specimens of two of them. In addition to excretion of virus, one of ten vaccinees demonstrated adenopathy. Only one of the ten RA/27/3 vaccinees demonstrated a booster response. This child did not shed virus, but did develop rashes.

In addition virus was isolated from throat specimen of another subject in the group. Development of rashes or adenopathy in seropositive individuals has been reported on a few occasions.[12–14]

When unattenuated rubella virus was administered by intranasal route, the highest reinfection rate observed was only 30%. Similarly, Farquhar[5] reported that the incidence of subclinical reinfection was very low among RA/27/3 vaccinees and was 26 per cent among the recipients of the Cendehill vaccine. However, some reports indicate that the unattenuated virus is capable of reinfecting 50% to 80% of vaccine-induced immune subjects.[2,4,15] This seemingly low rate of reinfection could be due to the low take of unattenuated virus in this study, since upon inoculation of virus only 57% of the seronegative control children showed evidence of seroconversion in contrast to 70% to 100% reported by others. [16–19]

Virus was isolated from six seronegative children who demonstrated seroconversion after viral inoculation. However, virus was excreted from one individual who had not converted (antibody titer $<$ 1:10) at the time of assay. In this case, the possibility of delayed antibody response cannot be ruled out.

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TABLE 1

Clinical Reactions following Vaccination

Clinical Reaction	Unvaccinated, Naturally Immune (16 subjects)		RA/27/3 Vaccinees (10 subjects)		Cendehill-51 Vaccinees (10 subjects)		HPV/77/DE-5 Vaccinees (9 subjects)	
	No.	%	No.	%	No.	%	No.	%
Fever	8	50	5	50	7	70	4	44
Lympha-denopathy	2	13	1	10	1	10	1	11
Rash	0	0	0	0	0	0	0	0
Joint manifestation	0	0	0	0	0	0	0	0

TABLE 2

Hemagglutination Inhibition Antibody Titer in Rubella Vaccinees

Vaccine Strain	No. of Vaccinees	No. with Sero-conversion	Post Vaccination HI Ab. Titer									Geometric Mean Ab. Titer
			<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1680	
HPV/77/DE-5	9	9 (100%)				1	2	2	1	1	2	1:448
Cende-hill-51	10	10 (100%)		2	1	1	3	2	1			1:96
RA/27/3	10	10 (100%)			2	1	1	6				1:112

TABLE 3

Clinical Reactions in Susceptible and Vaccinated Subjects following administration of Unattenuated Rubella Virus

Clinical Reaction	Susceptible Children (14 subjects)		HPV/77/DE-5 Vaccinees (9 subjects)		Cendehill-51 Vaccinees (10 subjects)		RA/27/3 Vaccinees (10 subjects)	
	No.	%	No.	%	No.	%	No.	%
Fever	4	29	0	0	0	0	0	0
Lympha-denopathy	7	50	0	0	1	10	0	0
Rash	5	36	0	0	0	0	1	10

TABLE 4

Antibody Titer and Viral Isolation from Seronegative Children after administration of Unattenuated Rubella Virus

Group	Subjects	HI Antibody Titer	Days after Challenge										
			7	10	12	13	14	15	16	17	18	19	20
1	1	1:40	o	+		o	o				+		
	2	1:160											
	3	> 1:1280			+	●		+					
	4	> 1:1280	+									+	
	5	1:40	+						+			+	+
	6	1:160								+		+	+
	7	1:40											
	8	1:320				+		+	+				
2	9	< 1:10					o		+				
	10	"											
	11	"											
	12	"											
	13	"											
	14	"											

\+ Viral isolation from throat

o Viral isolation from blood

● Viral isolation from throat and blood

TABLE 5

Antibody titer in RA/27/3 vaccinees before and after challenge with unattenuated Rubella virus and viral isolation after administration of challenging virus

Subjects	HI Antibody Titer				Virus Isolation after Challenge	
	Before Vaccination	4 weeks after Vaccination	Before Challenge (5 months after vaccine)	6 weeks after Challenge	Throat	Blood
1	<1:10	1:20	<1:10	<1:10		
2	"	1:40	<1:10	<1:10		
3	"	1:160	1:20	1:20		
4	"	1:160	1:160	1:160	+	
5	"	1:80	1:40	1:640		
6	"	1:160	1:80	1:80		
7	"	1:160	1:320	1:160		
8	"	1:20	<1:10	<1:10		
9	"	1:160	1:160	1:160		
10	"	1:160	1:80	1:80		

TABLE 6

Antibody titer in Cendehill-51 vaccinees before and after challenge with unattenuated Rubella virus and viral isolation after administration of challenging virus

Subjects	HI Antibody Titer				Virus Isolation after Challenge	
	Before Vaccination	4 weeks after Vaccination	Before Challenge (5 months after vaccine)	6 weeks after Challenge	Throat	Blood
1	< 1:10	1:40	1:20	> 1:640		
2	"	1:10	1:10	1:160	+	
3	"	1:20	< 1:10	< 1:10		
4	"	1:80	1:40	1:20		
5	"	1:320	1:320	1:320		
6	"	1:10	< 1:10	> 1:640	+	
7	"	1:160	1:40	1:40		
8	"	1:30	1:10	1:10		
9	"	1:80	1:80	1:40		
10	"	1:160	1:80	1:80		

TABLE 7

Antibody titer in HPV/77/DE-5 vaccinees before and after challenge with unattenuated Rubella virus and viral isolation after administration of challenging virus

Subjects	HI Antibody titer				Virus Isolation after Challenge	
	Before Vaccination	4 weeks after Vaccination	Before Challenge (5 months after vaccine)	3 weeks after Challenge	Throat	Blood
1	< 1:10	1:40	1:40	1:40		
2	"	1:160	1:20	1:20		
3	"	1:80	1:80	1:320		
4	"	1:80	1:80	1:40		
5	"	1:640	1:320	1:320		
6	"	1:1280	1:640	1:640		
7	"	1:1280	1:320	1:320		
8	"	1:160	1:160	1:80		
9	"	1:320	1:640	1:640		