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Clinical and Laboratory Studies of Live Attenuated RA 27/3 and HPV 77-DE Rubella Virus Vaccines (40931)

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Abstract. Comparative studies of RA 27/3 and HPV 77-DE rubella virus vaccines were carried out in children and adults. RA 27/3 vaccine induced antibody in a larger proportion of individuals and at a substantially higher titer level than did HPV 77-DE vaccine. Additionally, the RA 27/3 vaccine induced theta and iota antibodies that are commonly found after infection with rubella virus in nature. The greater serologic responses to RA 27/3 virus were achieved without any important increase in clinical reactions and there was no evidence for contagious spread of the infection. There was slightly greater occurrence of rash and lymphadenopathy after RA 27/3 vaccine compared with HPV 77-DE but the rate for occurrence of arthritis was substantially lower. Nearly 8000 initially seronegative persons received the RA 27/3 vaccine in the present studies. The seroconversion rate was 98% and there were no clinical reactions of importance. Follow-up studies showed undiminished retention of antibody titer for at least 2 years following vaccination with RA 27/3 vaccine.

Live attenuated rubella virus vaccine, prepared using the HPV 77 strain grown in duck embryo cell culture (HPV 77-DE) (1–3) was licensed for general use in the United States in June 1969. Its purpose was to prevent rubella and fetal infections in primary recipients of the vaccine and to reduce the reservoir of infected individuals who were the source of infection to susceptible pregnant women. This goal was accomplished (4) by routine immunization of all children from 1 to 12 years of age and by selective immunization of women of childbearing age. To date, 104.6 million doses of rubella vaccine alone or combined with measles and mumps virus vaccines have been distributed in the United States. The number of cases of rubella has been markedly reduced in the United States since the time of introducing the vaccine and there has been no major epidemic of rubella since 1965 such as had been shown to occur at 6- to 9-year intervals between 1935 and 1964 (5). The number of cases of congenital rubella has been maintained at low level since the time of the 1964 epidemic, when several thousand cases occurred (6).

Another live attenuated rubella virus vaccine prepared using Plotkin's RA 27/3 rubella virus strain (7) grown in human diploid fibroblast cells was licensed in the United States during 1978. In clinical studies (8, 9) this virus was shown to induce antibody in quantity and quality more closely approximating that following natural rubella virus infections than obtained following use of previously available vaccines. Additionally, there was a lesser subclinical reinfection rate with natural rubella virus following the RA 27/3 vaccine compared with other rubella vaccines (10).

This report presents the findings in extensive clinical and laboratory studies of RA 27/3 and HPV 77-DE rubella vaccines. Though both vaccines are highly effective in routine use, the RA 27/3 vaccine induced measurably higher titer antibody and antibodies of greater variety in a greater percentage of persons than did HPV 77-DE vaccine.

Materials and methods. Vaccines. Live RA 27/3 and HPV 77-DE vaccines were prepared at the Merck Institute for Therapeutic Research or in commercial

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production employing RA 27/3 seed virus supplied by Dr. Stanley A. Plotkin or HPV 77-DE virus described earlier (1, 7). All vaccines met the quality standards specified by the Bureau of Biologics, U.S. Food and Drug Administration.

Serology. Hemagglutination-inhibiting (HI) antibody titers were measured according to Stewart *et al.* (11) employing an initial serum dilution of 1:8. Tests for theta and iota antibodies were performed by the procedure described by Le Bouvier (12).

Clinical studies. The large-scale trials of the RA 27/3 vaccine (Table III) were carried out in greater Philadelphia (R.E.W.), Costa Rica (V.M.V.), and New York City (E.B.K.). The more detailed studies, for which the findings are given in Tables I, II, IV, and V and in Fig. 1, were carried out by one of us (R.E.W.) among healthy persons in the greater Philadelphia area. Each vaccine was given subcutaneously in a single 0.5-ml dose. Postpubertal females who were found to be nonpregnant were advised not to become pregnant for 3 months after vaccination and none did become pregnant. Temperatures and complaints were recorded daily by the subjects or by their parents or guardians and all important clinical findings were confirmed by qualified medical personnel. Routine samples of blood for serologic testing were taken immediately prior to vaccination and 6 to 8 weeks later. The sera were stored frozen until assayed for antibody. All clinical studies were carried out with informed written consent and in compliance with the Investigative New Drug laws.

Results. Comparison of RA 27/3 and HPV 77-DE vaccines given to initially seronegative children and adults (Studies 444 and 430). A total of 153 children aged 11 months to 16 years (mean, 3.5 years) were given 0.5 ml of RA 27/3 vaccine (Lot 579) and 156 children aged 11 months to 16 years (mean, 2.7 years) were given a like volume of HPV 77-DE vaccine (Lot 1414T, 0649V, or 0828V). These healthy children were cared for in public and private facilities and resided in the open community. Ninety-nine adult females aged 17 to 38 years (mean, 23.5 years) received the RA 27/3 vaccine and 94 adult females aged 17 to 43 (mean, 24.3 years) were given HPV 77-DE vaccine. All resided in open communities. The serologic findings are given in Table I and the clinical observations are summarized in Table II. The seroconversion rates were greater following RA 27/3 vaccine (100 and 99%, respectively, in children and adults) than following HPV 77-DE vaccine (97 and 90%, respectively). The geometric mean antibody titers were roughly two times greater following RA 27/3 than after HPV 77-DE vaccine.

The clinical findings among the children and adults are summarized in Table II. The temperature patterns were essentially the

TABLE I. COMPARISON OF HEMAGGLUTINATION-INHIBITING (HI) ANTIBODY RESPONSES AMONG INITIALLY SERONEGATIVE CHILDREN AND ADULTS WHO RECEIVED RA 27/3 OR HPV 77 DUCK CELL-MODIFIED RUBELLA VIRUS VACCINE

Vaccine	Hemagglutination-inhibiting antibody responses in recipients					
	Children (Study 444)			Adults (Study 430)		
	No. seroconverting/ total	HI titer		No. seroconverting/ total	HI titer	
		Range	Mean		Range	Mean
RA 27/3 (Lot 579)	153/153 (100%)	8–1024	153*	98/99 (99%)	<8–512	84*
HPV 77-DE duck**	152/156 (97%)	<8–512	81	85/94 (90%)	<8–512	35

* Significantly greater than geometric mean titer for HPV 77-DE group ($P < 0.001$).
** Lots 1414T, 0649V, and 0828V were used.

TABLE II. CLINICAL REACTIONS RECORDED AMONG INITIALLY SERONEGATIVE CHILDREN AND ADULTS WHO RECEIVED RA 27/3 OR HPV 77 DUCK CELL-MODIFIED RUBELLA VIRUS VACCINE

Group	Complaint	No. persons with complaint according to time (days) after receiving vaccine							
		RA 27/3 (153 persons)				HPV 77-DE (156 persons)			
		5–12	13–18	19–28	29–42	5–12	13–18	19–28	29–42
Child (Study 444)	Fever (oral)								
	<99°	89	103	95	101	89	102	90	91
	99–100.9	43	28	31	27	48	33	36	37
	101–102.9	4	5	7	5	—	2	8	4
	103–104.8	1	—	—	1	3	—	3	3
	Rubella-like rash	7	8	3	—	1	1	—	—
	Lymphadenopathy	2	3	2	1	1	1	2	1
	Arthralgia	1	—	1	—	2	—	—	1
	Myalgia	1	1	3	3	2	—	—	1
	Malaise	—	—	—	—	—	1	—	—
	Anorexia	6	8	10	8	12	9	12	11
		RA 27/3 (105 persons)				HPV 77-DE (96 persons)			
Adult (Study 430)	Fever (oral)								
	<99°	48	66	65	65	64	65	58	65
	99–100.9	44	29	30	29	28	27	33	24
	101–102	3	2	1	—	—	—	1	2
	103	2	—	—	1	—	—	—	—
	Rubella-like rash	11	17	—	—	5	6	1	—
	Lymphadenopathy	28	20	7	6	9	9	5	5
	Arthritis	—	3	3	—	—	10	7	2
	Arthralgia only	7	5	6	4	3	12	6	4
	Myalgia	12	6	5	4	8	9	6	3
	Malaise	—	—	1	—	—	—	—	—
	Anorexia	5	1	2	3	2	3	2	—

same among the children following the two vaccines. No arthritis followed either vaccine. Transient mild arthralgia was noted in two children following RA 27/3 and in three children following HPV 77-DE vaccine. Anorexia was present in a minority of subjects following both vaccines and this extended through the full 42 days of observation. The principal difference between the two vaccines was the infrequent but greater occurrence of the inconsequential rubella-like rash and lymphadenopathy in the children who received the RA 27/3 vaccine. The rash and lymphadenopathy were not associated with temperature elevation or other complaints.

Among the adults, there was no marked difference in temperature pattern or occurrence of myalgia, malaise, or anorexia following the two vaccines. Rubella-like rash and lymphadenopathy occurred more frequently after RA 27/3 vaccine. These were not important clinically. By contrast, arthritis was more commonly present among adult recipients of HPV 77-DE vaccine (13 persons) than RA 27/3 vaccine (4 persons). Arthritis, judged to be vaccine-related, began on days 13 to 19 following RA 27/3 vaccine and lasted 2 to 11 days. It began 13 to 24 days after HPV 77-DE vaccine and lasted 3 to 12 days. There was no difference in severity of the arthritis following the two vaccines. The fingers were most frequently involved with less common occurrence in the wrists and least in the knees. Arthralgia was present with roughly the same frequency, severity, and site following the two vaccines. It began 10 to 25 days after vaccination and persisted for 1 to 9 days.

Large-scale tests for six lots of RA 27/3 vaccine. A total of 10,823 children and adults in greater Philadelphia (1740 per-

TABLE III. SEROCONVERSION FINDINGS AMONG 10,823 PERSONS WHO RECEIVED ONE OF SIX DIFFERENT LOTS OF RA 27/3 STRAIN RUBELLA VACCINE

Lot No.	No. vaccinated			HI test results		Calculated No. of initial seronegatives vaccinated
	Children	Adult	Total	No. seroconverted/ No. tested	Percentage sero-converting	
579	4799	547	5346	1686/1696	99	3630
60149	1167	0	1167	214/219	98	1006
60150	1028	0	1028	176/180	98	752
60151	1083	0	1083	210/220	95	790
60152	1089	0	1089	203/205	99	860
60640	1069	41	1110	226/238	95	838
Total	10,235	588	10,823	2715/2758	98	7876

sons), Costa Rica (8798 persons), New York City (261 persons), and Nebraska[1] (24 persons) received a dose of one of six different lots of RA 27/3 vaccine in a total of 28 separate studies. The tests in the adults were carried out in Philadelphia, New York, and Costa Rica. Paired serum samples were taken from 2758 (25%) of the total group and the seroconversion rates were determined. The total numbers of initially seronegative persons that were vaccinated were calculated based on the percentage of initially seronegative persons found in each group that were tested. The findings presented in Table III show that roughly 7876 initially seronegative persons were given the vaccine. The seroconversion rates were essentially the same for all vaccine lots ranging from 95 to 99% and averaging 98%. Clinical reactions were inconsequentiai and the findings in the sample shown in Table II were representative of those seen in the total group.

Tests for contagious spread. In a study carried out in the Philadelphia community (Study 447), a total of 22 seronegative children (age 1 to 11) and three seronegative mothers were exposed by close familial contact to 22 initially seronegative children who were vaccinated with RA 27/3 rubella vaccine and who developed rubella antibody. None of the exposed seronegative persons developed rubella antibody during the 6-week period after the vaccine was given, providing evidence of lack of contagious transmission of the vaccine virus infection even though virus may be present in the throat (Table IV).

In another study (536) not reported in detail here, initially seronegative children who had been given RA 27/3 vaccine were tested periodically for presence of rubella virus in their throats. Three of six children given RA 27/3 vaccine had a positive rubella virus culture on one occasion 11 to 18 days after vaccination. Four of five children given HPV 77-DE vaccine were shown to have virus present 11 to 18 days after

TABLE IV. LACK OF CONTAGIOUS TRANSMISSION OF RA 27/3 RUBELLA VACCINE VIRUS INFECTION FROM VACCINEES TO SUSCEPTIBLE FAMILY CONTACTS (STUDY 447)

Age of subject (years)	Vaccinees (No. seroconvert/ total vaccinated)	Seronegative contacts (No. seroconvert/ total exposed)
1	2/2	
2	3/3	
3	2/2	
4	4/4	0/3
5	1/1	0/1
6	2/2	0/2
7	4/4	0/4
8	2/2	0/5
9		0/1
10	2/2	0/2
11		0/4
12		
28		0/1
34		0/1
37		0/1
Totals	22/22	0/25

[1] These persons were vaccinated and observed by Dr. S. Lerman.

vaccination. Very small amounts of virus were present and concentration of virus from most of the throat specimens was required in order to detect it.

Quality of antibody response. Persons who are infected with rubella virus in nature develop neutralizing and HI antibodies and commonly develop antibodies against theta and iota rubella antigens that are measured in precipitin tests (12). Paired sera from 82 initially seronegative children (Study 416) who developed HI antibodies following vaccination with RA 27/3 vaccine were tested for development of anti-theta and anti-iota antibodies. The findings presented in Table V show that 98% of the children developed anti-theta and 80% developed anti-iota antibodies. In previous clinical tests conducted here (unpublished) using HPV 77-DE vaccine, only 17/25 (68%) persons developed anti-theta and 5/25 (20%) persons developed anti-iota antibodies.

Antibody persistence. Sera from 41 initially seronegative children who received RA 27/3 vaccine were tested for rubella antibodies 6 weeks and 2 years after vaccination and the titers were compared. Figure 1 shows the titer value for each person's serum at 6 weeks and 2 years after vaccination. The individual changes were small and the mean antibody titer found 2 years after vaccination was about the same as was measured 6 weeks after vaccination.

Discussion. The induction of immunity against rubella virus by vaccination depends upon the development of antibodies. The HI test measures protective antibody (13–15). Both the RA 27/3 and HPV 77-DE rubella vaccines induce HI antibodies in more than 90% of susceptible recipients but, as shown in these investigations, the

TABLE V. ANTI-THETA AND -IOTA ANTIBODY RESPONSES AMONG 82 INITIALLY SERONEGATIVE CHILDREN WHO RECEIVED RA 27/3 STRAIN RUBELLA VIRUS VACCINE (STUDY 416)

Numbers of children who developed antibody/total	
Anti-theta	Anti-iota
80/82 (98%)	66/82 (80%)

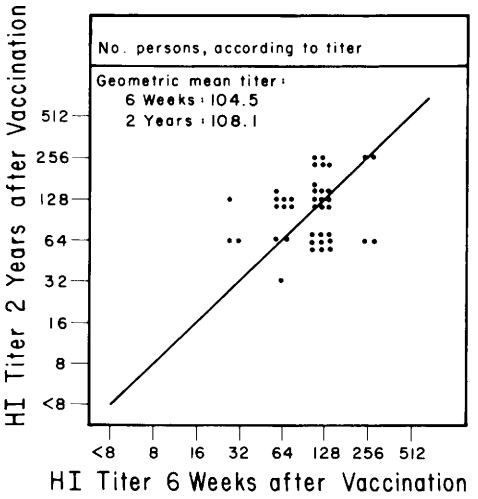

FIG. 1. Persistence of rubella HI antibody in children for 2 years following RA 27/3 rubella virus vaccine (Study 416).

percentage of such responders is slightly greater when the RA 27/3 strain is used and the antibody titers are higher. Published studies (8, 10) have shown that there is greater protection against reinfection with rubella virus in nature following RA 27/3 vaccine than following other rubella vaccines; it should be noted, however, that this may not be of major importance since reinfection is subclinical and since the virus in immune persons is not transmitted to the fetus (16).

The induction of anti-theta and anti-iota antibodies by RA 27/3 vaccine shown in these studies is consistent with findings reported previously (17). The induction of antibodies against these antigens, together with the greater seroconversion rate and the higher levels of HI antibody, provides evidence that the RA 27/3 virus is more like wild virus than are the other attenuated vaccine strains (18–23). This appears to correlate with better persistence of antibody (24) and with greater resistance to reinfection in nature with the wild virus (9, 25, 26). The present studies showed persistence in vaccinated subjects of HI antibody without significant decline for 2 years after vaccination. These data provide a basis for expectation that the immunity following vaccination will be lasting.

Importantly, the better serologic results

with the RA 27/3 strain were not obtained at the expense of an increase in reactions that are of clinical consequence. The greater occurrence of rash and lymphadenopathy in recipients of RA 27/3 vaccine compared with HPV 77-DE vaccine is of no great importance since these clinical reactions are extremely mild and there is no apparent illness in the vaccinated person. The less frequent occurrence of arthritis among RA 27/3 vaccinees compared with recipients of HPV 77-DE vaccine is an important attribute of the RA 27/3 vaccine since vaccine-induced arthritis can be severe in some adults (27).

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