

# STUDY OF YOUNG WOMEN VACCINATED AGAINST RUBELLA VIRUS FOR 10 YEARS IN TAIWAN

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**Abstract.** Since the licensing of the rubella virus vaccine (RA 27/3 strain) in 1979, clinical studies on the RA 27/3 strain vaccine, which gives rise to high titer antibody, have been reported. In the present study, this vaccine was used to examine the immune response in young women. Volunteers without the previous immunity to rubella virus screened by hemagglutination inhibition (HAI), latex agglutination (LA), fluorescence immunoassay (FIA) and solid-phase immunoassay (SPIA) tests were injected with Rudivax vaccine or Meruvax II vaccine. Adverse reactions occurred between 10 and 20 days after vaccination in 30% of the volunteers. After 28-35 days, vaccinees developed antibodies against rubella virus. The titer of rubella antibody reached its peak from the 40th day through the 100th day. One year after vaccination, the geometric mean titer (GMT) of rubella virus antibody still remained over 1 : 64 (HAI) and 1 : 38.2 (FIA), and SPIA IgG RI mean was 2.80. Two years later, the antibody titers were 1 : 52 by HAI and 1 : 32.1 by FIA, and SPIA IgG RI mean was 2.75. After 5 years, the antibody titers were 1 : 48.6 (HAI) and 1 : 28.2 (FIA), and SPIA IgG RI mean was 2.74. After 10 years, the anti-rubella virus antibody titers were 1 : 38.9 (HAI) and 1 : 25.1 (FIA), and SPIA IgG RI mean was 2.42. LA antibody still remained seropositive. In conclusion, the rubella vaccine RA 27/3 is safe and efficient, and it is applicable for the control of the rubella in Taiwan.

## INTRODUCTION

In 1941, the previous belief that few diseases were so benign as rubella was shattered by the observation in Sydney by Gregg of congenital defects in infants of mothers who had suffered rubella early in pregnancy (Gregg, 1941). In 1962, the isolation of rubella virus was reported simultaneously by Parkman *et al* (1962), and Weller and Neva (1962). That discovery marked the beginning of modern studies of the natural history of both intrauterine and extrauterine rubella infections and the immune responses to these infections. This breakthrough was a landmark: it made possible an accurate delineation of the clinical epidemiology of the disease, a tool available to determine the behavior of the virus in population groups, and, most significantly, provided the basis for the development of

vaccines for the control of congenital defects due to rubella. Four years later many investigators succeeded in attenuating the rubella virus making possible the development of live virus vaccines (Meyer *et al*, 1966; Parkman *et al*, 1966). The RA 27/3 rubella strain vaccine of Plotkin *et al* (1969), recovered and passaged 25 times in human diploid WI-38 cells was licensed in the United States in 1979 (Plotkin *et al* 1985). At present, only the RA 27/3 rubella strain vaccine is used.

The consequences of rubella infection in pregnant women are varied and unpredictable. Infection of fetus during the first trimester of pregnancy, and to a much lesser degree during the second trimester, may result in congenital defects. The trial of anatomic abnormalities including cataracts, neurosensory deafness, and congenital heart disease have classically been referred as the congenital rubella syndrome (CRS). The rubella vaccination program has been executed in many areas of the world and considerably decrease the incidence of congenital rubella (Horstman, 1989). In Taiwan, a subtropical island with a large population and

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extensive communication with other areas of the world, four large-scale epidemics of rubella occurred in 1944, 1957-1958 (Grayston *et al* 1972), 1968-1969 (Gale *et al*, 1972), and 1977 (Lin *et al*, 1986), respectively. These epidemics of rubella occurred in cycles at intervals of about 10 years. After 1977, a change in epidemic pattern of rubella was identified.

The regular cycle of rubella epidemics with a 10-year interval no longer existed. Instead, small-scale local outbreaks occurred sporadically in various areas in different years (Lin and Chen 1993, 1994). The rubella vaccination program in Taiwan was implemented in 1986. At the beginning, only 9th grade junior high schoolgirls were vaccinated. Since 1991, infants, elementary schoolchildren, 9th grade junior high schoolboys, and women at reproductive ages between 20 and 35 have also been included (Department of Health, 1992). Schoolchildren in elementary and junior high schools are vaccinated in schools which giving a very complete coverage. Infants are vaccinated at healthy baby clinics of hospitals or health centers with a coverage greater than 90%. Women at reproductive ages are vaccinated on a voluntary basis, therefore, giving a lower coverage.

This paper reports the results of a 10-year follow-up study of immunological responses in young women who took part in the first comprehensive study of status of Wistar RA 27/3 strain live attenuated rubella vaccine in Taiwan.

## MATERIALS AND METHODS

### Vaccines

The Wistar RA 27/3 strain live attenuated rubella vaccine was used in this study. The vaccine was obtained by culture on WI-38 tissue cells and titered  $10^3$  50% tissue culture infectious dose (TCID<sub>50</sub>). Two kinds of commercially available vaccines were Rudivax (Merieux Institute, Lyon, France), and Meruvax II (Merck Sharp and Dohme, PA, USA). Lyophilized vaccine was reconstituted with sterile deionized water just before injection, and subcutaneous administration was accomplished by the injection of 0.5 ml RA 27/3 into the deltoid area.

### Selection of study groups and collection of samples

Members of the study group were randomly

selected from 662 young women routinely screened for rubella serology from the female medical college students, and hospital nurses in Taipei (northern Taiwan), Taichung (central Taiwan), and Kaohsiung (southern Taiwan) areas. A total of eighty adult and adolescent females who were rubella seronegative and had no previous history of rubella immunization were selected. All volunteers were told that the safety information and contraindication of vaccine and accepted by themselves. They were vaccinated during February and March, 1984. Among them, 76 young women aged 19-28 years (mean age, 22.6 years) and four girls aged 9-15 years. All individuals underwent immunization with the RA 27/3 rubella vaccine after taking a medical history and preimmunization blood samples. Clinical follow-up was carried out by blood samples at weekly intervals during the first 10 weeks, and then at the 1st, 2nd, 5th, 10th years. Blood samples were collected from each subject and serum specimens were kept at -70°C for the laboratory examination.

### Rubella serology

All sera were collected before and after vaccination, and tested for rubella antibody by the four methods described as follows:

**1. Hemagglutination inhibition (HAI) test:** Sera were tested by the standard HAI test procedure recommended by the CDC (Centers for Disease Control), with duplicate samples and standard batch testing techniques (Palmer *et al*, 1977). Sera were pretreated with heparin-manganous chloride to remove nonspecific inhibitors, using serial twofold serum dilutions at 1 : 4 expression results as the reciprocal of the highest serum dilution that caused complete inhibition of hemagglutination. The absence of detectable HAI antibody at the 1 : 8 level has been interpreted as indicative of susceptibility to rubella.

**2. Fluorescence immunoassay (FIA) test:** The FIA procedure for rubella IgG was performed with IDT (International Diagnostic Technology, Inc, Santa Clara, CA, USA) commercial kit according to manufacture's instructions (Fayram *et al*, 1983). Fluorescence signals were read in a FIAX 100 fluorometer (IDT). A titer of less than 8 indicates no immunity. An equivocal (titers of 8 to 12 inclusive) zone has been established by the manufacturer to avoid false-positive readings, and it is

recommended that all equivocal specimens be retested. Samples which test  $\geq 8$  on repeat testing may be considered positive for rubella antibody.

**3. Latex agglutination (LA) test:** The LA testing was performed, using Rubascan kits (BBL HW and D Microbiology Systems, Cockeysville, MD, USA) according to the manufacture's instructions (Fayram *et al*, 1983). First, 25  $\mu$ l 1:10 dilution of serum was added to a 25  $\mu$ l of suspension containing antigen-coated latex particles. The mixture was rotated in a humidified atmosphere for 8 minutes, and agglutination was read visually.

**4. Solid-phase immunoassay (SPIA) test:** The SPIA procedure for Rubazyme (Abbott Laboratories, North Chicago, IL, USA) was performed according to the manufacture's instructions (Chernesky *et al*, 1986; Mahony and Chernesky, 1992). Sera were tested without pretreatment, and the controls which were supplied in the kit included one negative, and three positive, specimens. Optical densities were read in a Quantum Analyzer (Abbott Laboratories). The immunity or susceptibility was determined by comparing the optical density of the test serum with that of the mean optical density of the immune status controls (Assay index). A rubella index (RI) greater than or equal to 1 was considered positive, as evidence of immunity, while an index less than 1 was considered negative and susceptible.

#### Virus culture

The throat swabs collected from vaccinees within one week post-vaccination were used for virus isolation. Human embryonic cells, African green monkey kidney cells and Vero cells were used as the host cell cultures. The interference test challenged with ECHO type 11 was used for screening the present of rubella virus. The final identification of rubella virus was confirmed by immunofluorescence staining technique.

#### Adverse reactions

Adverse reactions such as malaise, skin rashes, lymphadenopathy, fever (over 38°C), and joint symptoms (pain or swelling) were recorded. All adverse reactions occurred within eight weeks post-vaccination were collected and analyzed. Adverse reactions due to other etiology were ruled out by physical examination and diagnosis by physicians.

#### Statistical methods

Statistical assessment of clinical and laboratory findings was performed for individuals who developed immunological responses (rubella specific and nonspecific). Logarithmic transformation of the data of geometric mean titer (GMT) was carried out when necessary, and statistical comparisons were made with chi-square or Fisher's exact tests.

## RESULTS

#### Adverse reactions of rubella vaccinee

After having been inoculated with Rudivax and Meruvax II of Wistar RA 27/3 strain live attenuated rubella vaccine, 30% (24/80) of the vaccinees had at least one of the rubella symptoms, with malaise excluded. As shown in Table 1, there was no statistical difference ( $p > 0.05$ ) between the adverse reactions using the Rudivax and Meruvax II vaccines. The adverse reaction percentage and occurrence periods are as follows respectively: 22.5% of those vaccinated had malaise during the 10th to 15th day period; 17.5% produced rash during the 11th to 16th day period; 17.5% got lymphadenopathy during the 14th to 20th day period;

Table 1

The adverse reactions of Rudivax and Meruvax II vaccination.

Vaccines Adverse reactions	Vaccines		
	Rudivax (%) <sup>1</sup>	Meruvax II (%) <sup>2</sup>	Total(%) <sup>3</sup>
Malaise	14 (22.6)	4 (22.2)	18 (22.5)
Rash	11 (17.7)	3 (16.7)	14 (17.5)
Fever	8 (12.9)	3 (16.7)	11 (13.8)
Joint symptom	7 (11.3)	2 (11.1)	9 (11.3)
Lymphadenopathy	10 (16.1)	4 (22.2)	14 (17.5)
Total <sup>4</sup>	18 (29.0) <sup>5</sup>	6 (33.3) <sup>5</sup>	24 (30.0)

<sup>1</sup>No. of reactions/injection cases (62)

<sup>2</sup>No. of reactions/injection cases (18)

<sup>3</sup>No. of reactions/injection cases (80)

<sup>4</sup>No. of cases with adverse reaction

<sup>5</sup> $p > 0.05$  by the chi-square test

13.75% had fever during the 10th to 15th day period; and 11.25% obtained joint symptoms during the 14th to 20th day period. As shown in Table 1, the adverse reaction percentages of various symptoms, whether from Rudivax or Meruvax II vaccine, are almost the same. If we analyze the adverse reaction percentage of two age groups inoculated with RA 27/3 vaccine (Table 2), none of the younger volunteers (less than 20 years old) had adverse reactions, except 4 of them who had malaise and were not included in the analysis. Yet 35% (24/68) produced adverse reactions among the 20-28 year old volunteers. The difference in two age groups were statistically significant ( $p < 0.01$ , Fisher's

exact test). The 24 volunteers who were older than 20 years produced adverse reactions, and higher rubella antibody titers (1 : 53) in the early period, whereas, 12 younger volunteers (less than 20 years old) produced no adverse reactions and lower titers (1 : 26.2). For ten years, the women who were inoculated with rubella vaccine never produced rubella symptoms and the children they gave birth to are all normal and healthy.

#### Antibodies responses of rubella vaccinees

IgM antibody seropositive could be detected by SPIA test after rubella vaccination for two weeks, and all sera collected at this time did not show any seropositive by all IgG detection. 19 days post-vaccination, SPIA IgG and HAI antibodies were also found. FIA and LA antibodies were now detected until the 20th day and 21st day, respectively. The days for the appearance of the seropositive for all the volunteers were: 28 days post-vaccination for SPIA IgM with RI mean as 1.32; 28 days for SPIA IgG with RI mean as 1.86; 28 days for HAI with GM titers as 46.8; 31 days for FIA with GM titers as 23.3; 35 days for LA. In a word, all the seropositive could be measured by various

Table 2

Numbers of adverse reaction in two age groups vaccinated with RA 27/3 vaccine.

Age groups (years)	Cases No.	No. with adverse reaction (%)
< 20	12	0 (0) <sup>1</sup>
≥ 20	68	24 (35) <sup>1</sup>

<sup>1</sup> $p < 0.01$  by the Fisher's exact test.

Table 3

The immune response of sera collected at various stages after vaccination.

Vaccinated date	No. of test	HAI		FIA		SPIA IgM		SPIA IgG		LA	
		+	-	+	-	+	-	+	-	+	-
7-10 D	34	0	34	0	34	0	34			0	34
12-17 D	23	0	23	0	23	3	20	0	23	0	23
19-25 D	72	67	5	44	28	70	2	68	4	52	20
28-34 D	72	72	0	72		72	0	72	0	70	2
(28-35 D)	(80)	(80)	(0)	(80)	(0)	(80)	(0)	(80)	(0)	(80)	(0)
35-40 D	11	11	0	11	0	6	5	11	0	11	0
42-45 D	25	25	0	25	0	5	20	25	0	25	0
48-55 D	8	8	0	8	0	1	7	8	0	8	0
56-60 D	17	17	0	17	0	0	17	17	0	17	0
63-100 D	10	10	0	10	0			10	0	10	0
101-360 D	20	20	0	20	0			20	0	20	0
2 Yrs	43	43	0	43	0			42	1	43	0
5 Yrs	28	28	0	28	0			28	0	28	0
10 Yrs	40	40	0	40	0			40	0	40	0

D = days, Yrs = years, GM = geometric mean, RI = rubella index, HAI = hemagglutination inhibition, FIA = fluorescence immunoassay, SPIA = solid-phase immunoassay, LA = latex agglutination

methods after the 35th day when the volunteers were vaccinated. As shown in Table 3 and Fig 1, the peak of the anti-rubella virus antibodies were as follows: SPIA IgM antibody showed on the 28-34th day, and RI mean was 1.39; HAI test on the 42-45th day, and GM titers 71.5; FIA test on the 56-60th day, and GM titers 42.4; However, SPIA IgG antibody formed in slow ascent after three months, and RI mean was 2.84. All the rubella IgG antibodies seropositive could be detected by various methods from the samples taken from those who had been inoculated for 5 or 10 years (Fig 1).

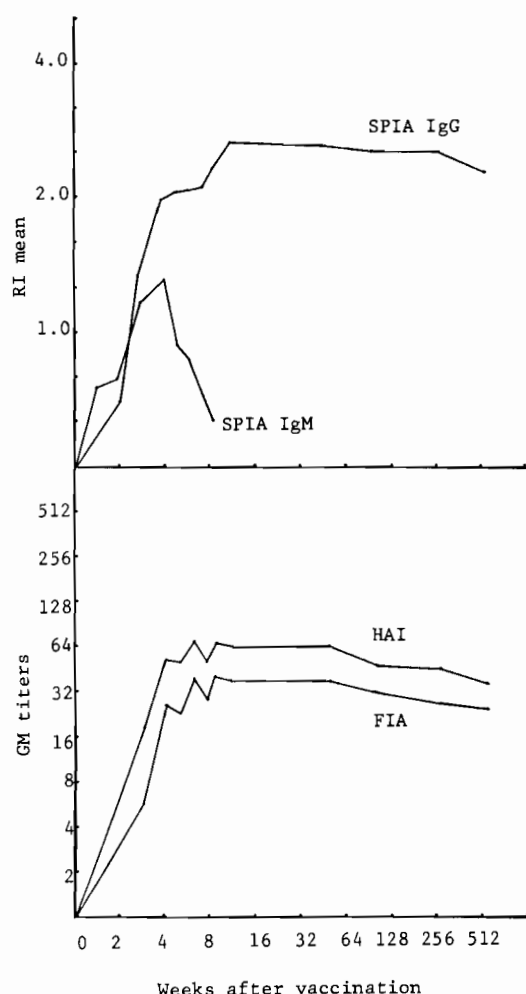


Fig 1—Time course of appearance of antibodies to rubella virus after vaccination with 27/3 vaccine (GM = geometric mean; RI = rubella index; SPIA = solid-phase immunoassay; HAI = hemagglutination inhibition; FIA = fluorescence immunoassay).

## DISCUSSION

In this study, the frequency of lymphadenopathy and rash were higher than other practical adverse reactions in rubella virus vaccinees. Other studies indicated that joint symptoms had the highest frequency (Freestone *et al*, 1971; Tingle *et al*, 1983), and some reports suggested lymphadenopathy was highest (Weidel *et al*, 1980). In general, when one is vaccinated during the childhood, the frequency of rash occurrence is higher (Lerman *et al*, 1981). Weidel *et al* (1980) reported that the RA 27/3 strain produced higher rubella antibody titers than the HPV 77-DE strain. Meanwhile, higher percentages of the occurrence of lymphadenopathy and rash were obtained in the RA 27/3 strain vaccinated group. Although 30% of the volunteers had adverse reactions after 10-20 days of vaccination, no virus was isolated from throat swab specimens collected from this group. The result was similar to that of the report of Liebhaver *et al* (1972), which suggested that infection caused by vaccination of RA 27/3 strain may not have occurred.

All volunteers were medical employees, so their description about reactions were relatively valid. It showed from the questionnaires that the way these volunteers recognized CRS caused by rubella virus was through medical books, unlike other non-medical groups who learned through newspapers or magazines (Lin, 1985; Wang *et al*, 1991). One-third of volunteers remembered that their siblings or close classmates had rubella symptoms before, but in fact they were rubella seronegative. In such cases, their memory can't be used as an evidence. In his survey of adolescent rubella inoculation conducted in the city, Lamprecht *et al* (1982) also found that if there was no detailed record, the parents' or volunteers' own memory of whether they had contracted the rubella virus or had been vaccinated would be unreliable. To reduce the adverse reaction error which resulted from emotional factors, we deliberately told the volunteers that the vaccination would have no side effects, and hence the effect of vaccination could be exactly evaluated in the current stage. The frequency of adverse reactions was 30% (24/80). This percentage was similar to that (23-34%) in other reports (Balfour *et al*, 1976; Fogel *et al*, 1978; Schiff *et al*, 1974). As shown in Table 2, the young age group (less than 20 years old age) had fewer reactions than the older one. Others have also reported the same result

(Balfour *et al*, 1981; Plotkin *et al*, 1973). By contrast, the antibody titers of the age-specific groups (more than 20 years age) were higher than those younger than 20 years old. This result may be relevant to the more conspicuous adverse reactions that those higher age groups had.

In one female case, the rubella IgG levels recorded at the different periods of 2-months, 6-months, 1-year, and 1.5-years post-inoculation were 1.79, 1.97, 2.12 and 1.54 (rubella IgG index), respectively. After 2 years of vaccination, the rubella SPIA IgG antibody was negative (rubella IgG index, 0.67), just after she gave birth to a very healthy baby. Four years post-inoculation, she had a second baby but the rubella IgG index came up only to the borderline value (rubella IgG index, 1.002). When the blood was checked five years later, the rubella IgG index was 1.10; nine years later, the index was 1.09; ten years later, the index was 1.08. It meant that before and after childbirth the rubella IgG indices of this female case were seropositive. But immediately after the baby was born, the index fell rapidly to the seronegative level. Even when HAI and FIA test were used, the index merely maintained at its borderline value.

In 8 cases, it was found that the rubella IgG indices of the samples collected 10 years after vaccination were higher than those collected within 5 years after vaccination. During the period, some obtained revaccination (3 cases) and others maybe were exposed again to rubella by contagious groups, producing only the symptom of malaise. This could result in a booster effect. The rubella antibody titers of the three samples taken on the 22nd day after vaccination were extremely high (5-6 times the GMT or RI mean). The antibody titers of these samples taken before vaccination showed between borderline and negative cut-off points, and were judged to be seronegative. Therefore, it was possible to detect a booster effect after inoculation, with the rapidly rising titers. Others have also drawn the same conclusion in their reports (Fogel *et al*, 1978; Harcourt *et al*, 1980; Plotkin *et al*, 1973; Wyll *et al*, 1971).

The rubella prevalence in Taiwan has increased since 1992. The numbers of cases in 1992 was over ten thousand, (Department of Health, 1993a), as against 309 cases in 1990, 1,796 cases in 1991 (Department of Health, 1992), and 1,444 cases in 1993 (Department of Health, 1994). Meanwhile the number of CRS cases in 1992 was 16 (Department

of Health, 1994), which as against one case in 1991 (Department of Health, 1992). More CRS cases were reported later in 1992 and early 1993 (Department of Health, 1993b). This means that the endemic prevalence of rubella cases in Taiwan caused infection in some pregnant women and the CRS cases rose after term pregnancy. In the meantime, according to recent reports on seroepidemiology of rubella, the susceptibility prevalence of rubella among young women is as high as 38.5-45.2% in Taiwan (Lin and Chen 1993, 1994; Wang *et al*, 1991; Wu *et al*, 1987; Yuan *et al*, 1989). The expansion of the vaccination program to this group is highly recommended. Serosurveillance of rubella virus infection is also important for the monitoring and evaluation of the current vaccination status in Taiwan.

At the early period after vaccination, the antibody titers due to Rudivax vaccine were higher than those of Meruvax II, but two months later after vaccination there were no conspicuous differences between the two groups of titers (Lin *et al*, 1986). The young Chinese women could produce 100% immune response after being vaccinated with RA 27/3 strain rubella vaccine, and similar results (99-100%) were obtained in other reports (Fogel *et al*, 1978; Lerman *et al*, 1981; MacDonald *et al*, 1978; Weidel *et al*, 1980). Though 30% of volunteers produced adverse reactions, the symptoms were very inconspicuous. Most of the reactions appeared about on the 10th day after inoculation, and reactions lasted for 1-5 days. All volunteers had no rubella symptoms in the 10 years since vaccination, and their children had no CRS. It can be concluded that RA 27/3 strain rubella vaccine is safe and reliable.

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