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STUDIES ON LIVE RUBELLA VACCINE. VI. SEROEPIDEMIOLOGICAL SURVEILLANCE AND IMMUNIZATION OF ADOLESCENTS IN HIGH SCHOOL

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SUMMARY The rubella seroimmunity status of a total of 1,204 students aged 12 to 19 in a junior and a senior high school in Osaka district was surveyed. Among these, 487 students (40.5%) were found to be seronegative (<1 : 8) by the hemagglutination inhibition (HI) test.

A total of 287 students were immunized with live rubella vaccine, Biken lot #7233. This caused an increased titer in all except one of the 262 seronegative students, while among 25 students with an initial HI antibody titer of 1 : 8 it caused more than 4-fold increase in 20 and 2-fold increase in 5. The vaccine caused no clinical manifestations, such as fever, rash or arthralgia.

INTRODUCTION

Three rubella vaccines prepared in different tissue-culture-lines have already been licensed in the United States, namely HPV-77DE-5, HPV-77DK-12 and Cendehill, prepared in duck embryo cell cultures, dog kidney cell cultures and rabbit kidney cell cultures, respectively. It has been reported that more than 28 million people have been vaccinated with these vaccines, without any serious accident (Rauh, Schiff and Johnson, 1972). However, some age-related involvement of the joints in women, such as arthralgia was noticed after

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vaccination, and the frequency of this differed with the kind of host-cells in which the vaccine was propagated (Grand et al., 1972; Wallace and Isacson, 1972; Thompson et al., 1973).

Four vaccine strains were approved for general use after official field trials in Japan in 1973. One of these, Biken's vaccine, prepared from quail embryo fibroblast cells infected with the Matsuura strain, was tested extensively on children and found to be safe and effective (Minekawa et al., 1973). But further studies are required on the efficacy of this vaccine in adolescents and on possible clinical reactions, such as arthritis, before general use of this vaccine.

The present investigation was on the seroimmunity status of adolescents in Osaka district, and on possible side reactions of susceptible subjects to the vaccine.

MATERIALS AND METHODS

1. *Vaccines*

Live rubella vaccine, Biken lot #7233 (E65QEF11) (passaged 65 times in the amniotic cavity of chick embryos and 11 times in quail embryo fibroblast cells) was prepared by freezing and thawing from quail embryo fibroblast (QEF) cells infected with the Matsuura strain, as described previously (Okuno et al., 1968; Minekawa et al., 1968, 1973, 1974).

Live rubella vaccine Meruvax HPV-77DE-5 was purchased from Merck, Sharp & Dohme Co., Inc., U.S.A.

2. *Vaccinees*

Healthy junior or senior high school students aged 12 to 19 years were inoculated subcutaneously in the upper part of the arm with a single dose of 0.5 ml of the vaccine.

3. *Serological assay*

Blood specimens were taken from the subjects before and 6 or 8 weeks after immunization, as described in the text. The antibody titer of rubella was measured by the HI test, as described elsewhere (Suzuki et al., 1973).

RESULTS

1. *Seroepidemiological studies of high school students*

A junior and a senior high school in a northern part of Osaka district were chosen for epidemiological surveillance tests of students for rubella. Blood specimens were taken from 1,204 students and antibody titers against rubella were measured by the HI test. Antibody titers of less than 1 : 8 were evaluated as seronegative. The distributions of titers are shown in Figs. 1 and 2. In T Junior High School (Fig. 1), no remarkable difference could be seen between the distribution patterns of male and female students: many students were seronegative ($<2^3$) or had a very high titer ($\geqq 2^8$) and a few had a low titer (2^3-2^5). The number of students with high titers seemed to

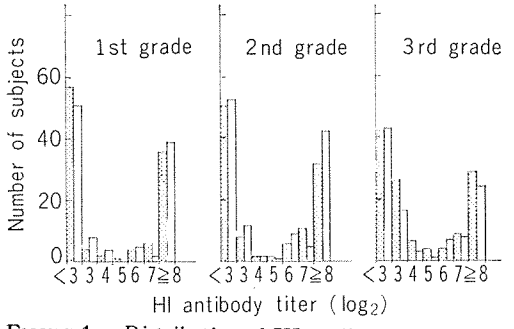

FIGURE 1. *Distribution of HI antibody titers against rubella among T Junior High School strudents.* ▨, *female;* □, *male.*

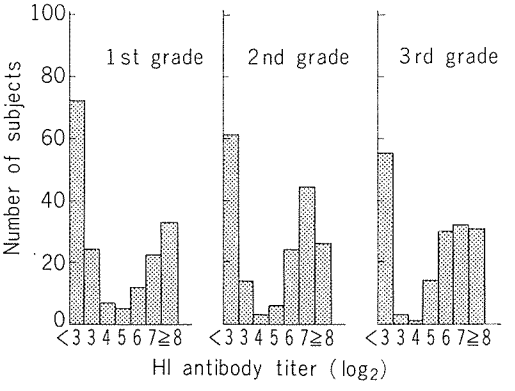

FIGURE 2. *Distribution of HI antibody titers against rubella among female students in I Senior High School.*

decrease with age, suggesting that students with high titers in the 1st grade had recently suffered from rubella infection and that the titer gradually decreased with loss of antibody. The decrease in the number of seronegative students with age suggests the possibility of subclinical infection. In I Senior High School (Fig. 2), the distribution pattern of the titers of each grade was on the whole concave as in T Junior High School. The number of seronegative students also decreased with age, again suggesting the possibility of subclinical infection.

2. *Susceptibilities among high school students*

The susceptibilities and geometric mean HI antibody titers (GMT) of the seroimmune students in the two schools are summarized in Table 1. In T Junior High School, there was not much difference in the seroimmune rates or average HI antibody titers of male and female students, although the proportion of seronegative students was a little higher among female students of the 1st and 2nd grades. The overall average seronegative ratio was 43.6%, which was about 7% higher than that of I Senior High School. In the latter school only female students were surveyed seroepidemiologically. Here the proportion of seronegative students decreased with age as in T Junior High School, and the average proportion was 36.2%. The GMT's of the total seropositive students in the two schools were about the same, being $2^{6.3}$ and $2^{6.4}$ or more.

The GMT of students in T Junior High School decreased with age while in I Senior High School it increased with age. This cannot be explained at present.

3. *Immunization of seronegative students in T Junior High School*

In all 155 seronegative volunteers in T Junior High School were immunized with either Biken lot #7233 or Meruvax HPV-77DE-5 vaccine. The results are shown in Table 2. All but one of the 145 students tested (99.3%) responded to Biken vaccine while all 10 students tested showed seroconversion to Meruvax vaccine. There were no noticeable complications, such as rash, fever or arthralgia. With Biken vaccine, there was not much difference between the seroconversion rates or GMT's of male and female students, but the overall GMT's of the 2nd and 3rd grades were

TABLE 1. *Seroimmunity rates to rubella in junior and senior high school students*

Name of school	Age of students (years)	Sex	HI Ab titer	Number of subjects			
				1st grade	2nd grade	3rd grade	Total
T Junior High School	12–16	M	<1:8	51/109 (46.8%)	53/125 (42.4%)	44/105 (41.9%)	148/339 (43.7%)
			≧1:8	58/109	72/125	61/105	191/339
			(GMT)[a]	(≧6.8)	(≧6.7)	(≧6.0)	(≧6.5)
		F	<1:8	57/110 (51.8%)	51/112 (45.5%)	43/124 (34.7%)	151/346 (43.6%)
			≧1:8	53/110	61/112	81/124	195/346
			(GMT)	(≧7.2)	(≧6.7)	(≧5.6)	(≧6.4)
		Total	<1:8	108/219 (49.3%)	104/237 (43.9%)	87/229 (38.0%)	299/685 (43.6%)
			≧1:8	111/219	133/237	142/229	386/685
			(GMT)	(≧6.9)	(≧6.7)	(≧5.8)	(≧6.4)
I Senior High School	15–19	F	<1:8	72/175 (41.4%)	61/178 (34.2%)	55/166 (33.2%)	188/519 (36.2%)
			≧1:8	103/175	117/178	111/166	331/519
			(GMT)	(≧6.0)	(≧6.4)	(≧6.6)	(≧6.3)

[a] Geometric mean HI antibody titer, expressed as $\log_2$.

TABLE 2. *Antibody response of seronegative students in T Junior High School to live rubella Biken lot # 7233 and Meruvax HPV-77DE-5 vaccines*

Name of vaccine	Grade	Sex	Number of vaccinees	Number showing seroconversion	GMT[a] at 8 weeks postvaccination
Biken lot # 7233	2nd	M	29	28 (96.7%)	4.4
		F	49	49 (100%)	4.3
		total	78	77 (98.7%)	4.4
	3rd	M	31	31 (100%)	5.7
		F	36	36 (100%)	5.7
		total	67	67 (100%)	5.7
	Total		145	144 (99.3%)	5.0
Meruvax HPV-77DE-5	3rd	F	10	10 (100%)	5.9

[a] Geometric mean HI antibody titer ($\log_2$).

rather different for some unknown reason. However, GMT of the female students of the 3rd grade vaccinated with Biken lot #7233 vaccine was not much different from that of students vaccinated with Meruvax HPV-77DE-5 vaccine, the latter being $2^{0.2}$ higher than the former.

4. *Immunization of female students in I Senior High School*

In all 142 female students (117 seronegative students and 25 students with an HI antibody titer of 1 : 8) were vaccinated with live rubella vaccine, Biken lot #7233. Six weeks after vaccination the 117 students who were initially seronegative showed 100% seroconversion and 20 of the 25 students who had an initial HI antibody titer of 1 : 8 showed more than 4-fold increase, the other 5 showing 2-fold increase (Table 3). The GMT's of the two groups were about the same, irrespective of their initial antibody titers. No clinical manifestations were observed, either subjectively or objectively.

TABLE 3. *Seroconversion of I Senior High School students to live rubella Biken lot# 7233 vaccine*

Grade	Ab titer prevaccination	Number of vaccinees	Increase of Ab titer		GMT[a] at 6 weeks postvaccination
			≧×4	×2	
1st	<1 : 8	35	35 (100%)	0	5.2
	1 : 8	13	9 (69%)	4 (31%)	5.0
2nd	<1 : 8	44	44 (100%)	0	5.1
	1 : 8	9	8 (88%)	1 (22%)	5.0
3rd	<1 : 8	38	38[b] (100%)	0	4.8
	1 : 8	3	3 (100%)	0	5.0
Subtotal	<1 : 8	117	117 (100%)	0	5.0
	1 : 8	25	20 (80%)	5 (20%)	5.0
Total		142	137 (97%)	5 (3%)	5.0

[a] Geometric mean HI antibody titer, expressed as $\log_2$.

[b] Including 2 persons whose HI antibody titers were initially less than 1 : 8 and increased to 1 : 8 after vaccination.

DISCUSSION

The seronegative ratios and GMT's of different grades of high school students observed in the present study suggested the possible occurrence of subclinical infection among these students. The incidence of viremia or virus shedding by subclinical infection is still obscure (Peckham, 1974) and the contagiousness of subclinical infection is unknown. However, when the seronegative ratio becomes high some epidemic causing clinical infection may occur. For example, a rubella epidemic was reported in Bermuda where 65.5% of 296 children aged 4 through 18 years were susceptible (Judelsohn and Wyll, 1973). It was also reported that an island-wide vaccination program was successful in halting further spread of rubella. In Japan adolescent girls in high school seem to be pertinent subjects for mass vaccination to prevent congenital malformation due to rubella infection, but more seroepidemiological data on them must be accumulated before drawing up nation-wide vaccination schedules.

Meruvax HPV-77DE-5 vaccine was reported to cause some joint involvements in4.3% and 10.3% of groups of vaccinees aged 12–15 and 16–19, respectively (Weibel et al., 1972), and joint and muscular symptoms in 5.1% of the vaccinees (Grand et al., 1972). However, it had no detectable side effects in 10 vaccinees in T Junior High School. Biken vaccine also caused not even slight febrile reactions but the number of Meruvax vaccinees was too small to allow a definite comparison of the two vaccines. Thus no differences between these two vaccines prepared in quail embryo and duck embryo cells could be demonstrated by the present field trial. Administration of these vaccines to larger numbers of older women might be helpful in differentiating between them because Meruvax HPV-77DE-5 vaccine was reported to cause a higher incidence of joint involvement in older women (Weibel et al., 1972) while Biken lot #7233 vaccine did not cause any clinical manifestations in older vaccinees, as reported previously (Minekawa et al., 1973). From the results of the present field trial, it is concluded that QEF-passaged Biken vaccine is safe and immunogenically potent at least when used for adolescent girls.

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REFERENCES

Grand, M. G., S. A. Wyll, S. H. Gehlbach, P. J. Landrigan, R. G. Judelsohn, S. A. Zendel, and J. J. Witte. 1972. Clinical reactions following rubella vaccination. A prospective analysis of joint, muscular, and neuritic symptoms. JAMA 220: 1569–1572.

Judelsohn, R. G., and S. A. Wyll. 1973. Rubella in Bermuda. Termination of an epidemic by mass vaccination. JAMA 223 : 401–406.

Minekawa, Y., N. Suzuki, M. Takahashi, and Y. Okuno. 1968. Studies on live rubella vaccine. II. Field trials with two experimental vaccine strains. Biken J. 11 : 243–250.

Minekawa, Y., N. Suzuki, J. Osame, S. Ueda, K. Yamanishi, M. Takahashi, Y. Okuno, T. Kan, N. Onishi, and H. Toyama. 1973. Studies on live rubella vaccine. IV. Comparative field trials with attenuated vaccines at different passage levels in Japanese quail embryo fibroblast cells. Biken J. 16: 155–160.

Minekawa, Y., S. Ueda, K. Yamanishi, T. Ogino, M. Takahashi, Y. Okuno, T. Kan, and T. Sasada. 1974. Studies on live rubella vaccine. V. Quantitative aspects of interference between rubella, measles and mumps viruses in their trivalent vaccine. Biken J. 17 : 161–167.

Okuno, Y., Y. Minekawa, N. Suzuki, and M. Takahashi. 1968. Studies on live rubella vaccine. I. Propagation of rubella virus in developing chick embryos and its laboratory characterization. Biken J. 11 : 235–241.

Peckham, C. S. 1974. Clinical and serological assessment of children exposed in utero to confirmed maternal rubella. Br. Med. J. 1 : 259–261.

Rauh, J. L., G. M. Schiff, and L. B. Johnson. 1972. Rubella surveillance and immunization among adolescent girls in Cincinnati. Am. J. Dis. Child. 124 : 71–75.

Suzuki, N., Y. Minekawa, J. Osame, S. Ueda, T. Ogino, K. Yamanishi, M. Takahashi, Y. Okuno, T. Kan, N. Onishi, and K. Koyama. 1973. Studies on live rubella vaccine. III. Field trials with vaccines attenuated by serial passages in primary pig kidney cells. Biken J. 16 : 149–154.

Thompson, G. R., J. J. Weise, J. L. Shillis, and R. G. Brackett. 1973. Intermittent arthritis following rubella vaccination. A three-year follow-up. Am. J. Dis. Child. 125 : 526–530.

Wallace, R. B., and P. Isacson. 1972. Comparative trial of HPV-77, DE-5 and RA27/3 live-attenuated rubella vaccines. Am. J. Dis. Child. 124 : 536–538.

Weibel, R. E., J. Stokes, Jr., E. B. Buynak, and M. R. Hilleman. 1972. Influence of age on clinical response to HPV-77 duck rubella vaccine. JAMA 222 : 805–807.