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Antibody titres in women six to eight years after the administration of RA27/3 and Cendehill rubella vaccines

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SUMMARY

Titres of haemagglutination-inhibiting antibody have been measured repeatedly in young women during a period of 6–8 years after the administration of RA27/3 and Cendehill attenuated rubella vaccines. Mean antibody titres were initially 217 after RA27/3 and 159 after Cendehill, but the difference diminished after the first year. Antibody titres were subsequently well maintained in both groups and did not reveal any need for regular revaccination. Mean titres in the Cendehill group were partly maintained by symptomless reinfection which was commoner after Cendehill than after RA27/3. Significant falls in titre were equally common after both vaccines, but low titres of 30 or less were more frequent in subjects who had received Cendehill.

Mean neutralizing antibody titres were initially 15·4 after RA27/3 vaccine and 9 after Cendehill. Titres remained higher after RA27/3 for 3 years, but the difference then diminished and became insignificant during the fifth year.

Revaccination of women with low antibody titres produced significant increases in 69 % of subjects when standard RA27/3 vaccine was used; a special preparation of RA27/3 of higher potency produced a similar number of rises (70 %) but elicited higher titres and might occasionally be useful for revaccinating women who are likely to come into contact with rubella. Challenge with RA27/3 vaccine produced weaker responses in women who had experienced natural infection than in those whose antibody was vaccine-induced.

Rises in antibody titre after revaccination consisted mainly of IgG, but traces of IgM antibody were detected in one vaccinee who had recently experienced natural reinfection and in 1 woman with naturally acquired antibody who had been challenged with high titre RA27/3 vaccine.

INTRODUCTION

In 1970 attenuated rubella vaccine was first licensed for use in the United Kingdom. During the next 2 years the Department of Health and Social Security introduced programmes for mass vaccination of schoolgirls aged 11–14 years and

selective vaccination of adult women who were likely to be exposed to rubella but were found to lack antibody (Department of Health and Social Security, 1970, 1972). Maternal immunity is needed for at least 20 years in order to protect the fetus from infection. It is necessary, therefore, to study the persistence of antibody after vaccination in order to decide if any reinforcement of immunity is desirable. It is also necessary to observe the serological results of revaccination in order to see if this procedure is effective.

We have measured antibody titres in women repeatedly during a period of 6–8 years after the administration of RA27/3 (Almevax) and Cendehill (Cendevax) rubella vaccines and we have studied the ability of these preparations to increase titres in women with low levels of antibody resulting either from previous vaccination or from natural infection.

MATERIALS AND METHODS

Rubella vaccines

Cendehill vaccine was administered subcutaneously in a dose of either $10^{3\cdot48}$ or $10^{4\cdot1}$ TCD 50. RA27/3 vaccine was administered either subcutaneously or intranasally in a routine dose of $10^{3\cdot5}$ TCD 50, but some women with pre-existing antibody were given a special high titre preparation containing $10^{4\cdot7}$ TCD 50.

Subjects studied

The principal study group consisted of 327 seronegative women aged 18–35 years in whom antibody was not detected by either the haemagglutination-inhibition (HI) or neutralization tests at serum dilutions of 1/20 or 1/4 respectively. One hundred and ninety of these women were hospital staff or students; 137 were patients who were vaccinated *post partum* within 4 months of delivery. Cendehill vaccine was administered to 200 women; RA27/3 vaccine of normal potency was given subcutaneously to 96 women and intranasally to 31. The initial antibody responses were estimated in blood samples taken 2–4 months after vaccination. Attempts were made to obtain subsequent specimens at yearly intervals, but it was impossible to bleed each subject annually and the number who were tested diminished progressively each year (Table 1). Testing continued for 6 years in the RA27/3 group and for 8 years after the administration of Cendehill. Women who reported recent exposure to rubella were bled 2–6 weeks after contact.

The ability of rubella vaccine to increase low titres of pre-existing antibody was tested in women who had HI titres of 40 or less from previous vaccination (26 subjects) or natural infection (115 subjects). Ten women in each of these groups were given high titre RA27/3 vaccine. The remaining subjects were challenged with either Cendehill or RA27/3 vaccine of normal potency.

Ninety babies of mothers who had been immunized *post partum* were tested for rubella antibody 10–18 weeks after maternal vaccination in order to detect evidence of transmission of virus from the mothers to their infants. Thirty-nine of these mothers had been given RA27/3 vaccine; 51 had received Cendehill.

Antibody titrations

All sera were tested for HI antibody by the method in routine use in the Manchester Public Health Laboratory (Thompson & Tobin, 1970). During the first 5 years of the study all sera were also tested for neutralizing antibody (Hutchinson & Thompson, 1965). When using either of these methods the latest serum was tested in parallel with the previous specimen, and sometimes with earlier sera, all of which had been stored at −20 °C. We found no evidence of loss of antibody during storage. Some sera were additionally tested for rubella-specific IgG and IgA antibodies by the indirect immunofluorescent technique (Cradock-Watson, Bourne & Vandervelde, 1972). Tests for specific IgM antibody were carried out by applying the HI and immunofluorescent tests to fractions obtained after centrifugation on sucrose density gradients.

RESULTS

Antibody responses in seronegative women

HI antibody was detected after vaccination in 197 out of 200 subjects (98·5%) who received Cendehill vaccine, in all 96 subjects who received RA27/3 vaccine subcutaneously and in 30 out of 31 subjects (96·8%) who received RA27/3 intranasally. The range of HI titres and the geometric mean titres (GMT) at different times after vaccination are given in Table 1. The results in Table 1 include spontaneous increases in titre of fourfold or more, which were assumed to be due to natural reinfection, but exclude any increases in titre following revaccination. Two to four months after the administration of RA27/3 vaccine the mean titre was slightly higher after subcutaneous than after intranasal vaccination. After the first year the position appeared to be reversed. The differences, however, are not significant and do not reveal any serological advantage in either method of administration. We have therefore combined the results from all the recipients of RA27/3 in order to compare them with the results which followed the administration of Cendehill. This comparison is shown in Table 2, which also includes the mean titres of neutralizing antibody.

In Table 2 the mean antibody titres after the administration of each vaccine are given in two columns. Rises in titre due to reinfection are included in the first column but excluded from the second. Both columns exclude any increases following revaccination. Two to four months after vaccination the mean HI titre was significantly higher after RA27/3 than after Cendehill ($P < 0{\cdot}001$). During the next 5 years, if reinfections are included, the difference diminished and became insignificant, although mean titres after Cendehill vaccine never actually exceeded those in the RA27/3 group. In both groups the mean titres at first declined, then remained steady and appeared to increase again in the final years of the survey. Reinfections, however, were commoner after Cendehill than after RA27/3 (see below) and if they are excluded then mean titres in the Cendehill group 4–6 years after vaccination remained significantly lower than in those who had received RA27/3.

Individual falls in HI titre of fourfold or more (excluding those which followed

Table 1. *HI antibody titres after administration of Cendehill and RA27/3 rubella vaccines*

Time after vaccination	No. with antibody/no. tested	Range of titres	GMT
	Cendehill subcutaneously		
2–4 months	197/200	< 20–⩾ 1280	159*
1–2 years	158/158	20–⩾ 1280	155
2–3 years	122/122	20–⩾ 1280	118
3–4 years	91/92	< 20–⩾ 1280	122
4–5 years	73/73	20–⩾ 1280	108
5–6 years	41/41	30–⩾ 1280	128
6–8 years	55/55	20–⩾ 1280	160
	RA27/3 subcutaneously		
2–4 months	96/96	40–⩾ 1280	225
1–2 years	51/51	40–320	151
2–3 years	36/36	20–640	137
3–4 years	30/30	30–⩾ 1280	121
4–5 years	23/23	40–480	140
5–6 years	17/17	80–320	163
6–8 years	n.t.	.	.
	RA27/3 intranasally		
2–4 months	30/31	< 20–⩾ 1280	192*
1–2 years	17/17	40–960	213
2–3 years	14/14	40–480	172
3–4 years	12/12	80–320	147
4–5 years	n.t.	.	.
5–6 years	n.t.	.	.
6–8 years	n.t.	.	.

GMT, Geometric mean titre.
n.t., None tested.
* Excluding subjects who did not respond.

reinfection or revaccination) were observed in 56 women who had received Cendehill vaccine and in 25 who had been given RA27/3. Titres which ranged from 80 to ⩾ 1280 fell 4- to 16-fold to reduced values which ranged from < 20 to 240. In five subjects the fall in titre was followed by a significant rise, presumably due to natural reinfection. The incidence of fourfold falls was similar in both groups of subjects since the number of Cendehill vaccinees who were followed up was about twice the number of those who had received RA27/3. The proportion of women with very low HI titres, however, was greater in the Cendehill group: titres fell to 30 in six subjects, to 20 in thirteen, to < 20 in two, and failed to rise above 30 in another four. In the RA27/3 group only one woman had a titre in this range.

During the first year after vaccination HI antibody was accompanied by neutralizing antibody, except in three women who had received Cendehill vaccine. After the first year neutralizing antibody became undetectable in six other women who had been given Cendehill, but persisted in all the recipients of RA27/3. The mean neutralizing titres, given in Table 2, were significantly higher after RA27/3

Table 2. *Geometric mean haemagglutination-inhibition (HI) and neutralizing (N) antibody titres after administration of Cendehill and RA27/3 rubella vaccines*

Time after vaccination		Cendehill		RA27/3	
		Including reinfections*	Excluding reinfections	Including reinfections	Excluding reinfections
2–4 months	HI	159	159	217	217
	N	9	9	15·4	15·4
1–2 years	HI	155	148	163	159
	N	9·7	9·5	13·6	13·6
2–3 years	HI	118	108	146	143
	N	9·6	9·3	16	16
3–4 years	HI	122	94	128	121
	N	9·8	8·7	14	13·2
4–5 years	HI	108	86	140	132
	N	12	10·9	12·8	12·3
5–6 years	HI	128	87	163	157
	N	9·9	9	.	.
6–8 years	HI	160	108	.	.
	N	.	.	.	.

* Reinfection was defined as an increase in HI titre of at least fourfold from the previous estimation. All columns exclude the results of revaccination.

than after Cendehill for 3 years after vaccination, whether reinfections are included or not ($P < 0{\cdot}001$). The difference diminished during the fourth year and became insignificant during the fifth, after which the test was discontinued.

Natural reinfection in women who had received rubella vaccine

Twenty-three symptomless rises in HI titre of fourfold or more, which were assumed to be due to reinfection, were detected in 22 women who had received Cendehill vaccine (Table 3). One woman was apparently reinfected twice – once during the second and again after the sixth year. Some reinfections were detected each year, at an annual rate which varied from 2·5 to 7·3 %. As the survey progressed, the percentage of subjects tested in whom reinfection had occurred increased from 2·5 % in the second year to 25·5 % in the 6–8 year period.

In women who had received RA27/3 vaccine only two reinfections were observed – one after subcutaneous and the other after intranasal administration.

Thirteen of the 24 women who were reinfected were aware of contact with cases of rubella during the previous year.

Reinfections occurred in women whose HI titres ranged from 30 to 320 (GMT = 106) and whose neutralizing titres ranged from 4 to 16. Only three of these women had HI titres of 40 or less. Increases of 4- to 32-fold were observed, yielding HI titres which ranged from 320 to $\geqslant$ 1280 (GMT = 997). The antibody titres after reinfection were thus similar to those which occur after primary natural infection (Tobin, 1974). Further specimens were examined from 18 women who had been reinfected. In ten the elevated titres were maintained for periods of

Table 3. *Number of symptomless reinfections (fourfold rises in HI titre) occurring yearly after administration of Cendehill rubella vaccine*

Years after vaccination	No. of subjects tested	No. in whom reinfection had occurred	No. of new reinfections in each period
1–2	158	4 (2·5)*	4 (2·5)*
2–3	122	7 (5·7)	4 (3·3)
3–4	92	10 (10·9)	6 (6·5)
4–5	73	11 (15·1)	2 (2·7)
5–6	41	7 (17·1)	2 (4·9)
6–8	55	14 (25·5)	4 (7·3)

* Percentages in parentheses.

2–5 years; in the other eight subjects titres gradually declined fourfold or more towards, or sometimes below, their original values.

Two women were pregnant when they experienced subclinical reinfection from close contact with cases of acute rubella. In one woman who had been given RA27/3 vaccine the HI titre rose from 80 to $\geqslant$ 1280 during the eighth month of pregnancy, and a trace of IgM antibody appeared in the blood. The infant was evidently not infected since no specific IgM was found in the cord serum and no HI or IgG antibody was detected 1 year later. Two attempts to isolate rubella virus soon after birth were unsuccessful. The baby showed no abnormalities at birth and appeared to be developing normally at the age of 2 years. The second woman had received Cendehill vaccine and was in the seventh month of pregnancy when her HI titre rose from 240 to $\geqslant$ 1280. Serum was not available from this woman or her newborn baby for an IgM test, but the child appeared to be normal at the age of 5 years, at which time no HI or IgG antibody could be detected.

Effect of revaccination

Twenty-six women whose HI titres after primary vaccination did not rise above 40, or fell to 40 or less, were revaccinated with RA27/3 vaccine of either normal or high titre. Twenty-one of these women had originally received Cendehill vaccine, two had received RA27/3 and three (not included in the previous Tables) had received HPV77 DE5. RA27/3 was given subcutaneously to 19 subjects and intranasally to 7, but no serological advantage in either route of administration was observed. The numbers of women who showed changes in HI titre 3 weeks to 3 months after revaccination are given in Table 4. RA27/3 vaccine of normal titre elicited rises of fourfold or more in 11 out of 16 women (69%) and raised the mean titre to 121. High titre RA27/3 elicited fourfold rises in seven out of ten women (70%) and raised the mean titre to 219 – a value similar to that achieved by RA27/3 of normal potency when used as a primary vaccine. HI titres of 1280 or more were reached in three subjects after revaccination but in general the elevated titres were lower than those which followed natural reinfection. Part of the gain in antibody titres appeared to be only temporary, because they subsequently

Table 4. *Effect of revaccination of women whose HI titres after primary vaccination failed to rise above* 40 *or fell to* ⩽ 40

(Numbers of women showing changes in HI titre after revaccination with RA27/3 of either normal or high titre, given subcutaneously (S-C) or intranasally (I-N).)

	Vaccine used for challenge			
	Normal titre RA27/3		High titre RA27/3	
Fold rise in HI titre	S-C	I-N	S-C	I-N
64	1	0	0	0
32	0	1	3	0
16	0	0	1	1
8	2	1	1	0
4	5	1	0	1
2	3	0	2	1
No change	1	1	0	0
Fall	0	0	0	0
Total number revaccinated	12	4	7	3
GMT after revaccination	121		219	

GMT, Geometric mean titre.

fell 2- to 32-fold over a period of 1–4 years in 12 out of 19 subjects who were followed up.

We expected that IgM responses, if elicited at all by revaccination, would be transient and would be more likely to appear after the use of high titre vaccine. We therefore obtained three specimens of blood from each subject 8–30 days after challenge with high titre RA27/3 and tested these for HI, IgG, IgA and IgM antibodies. Significant rises in HI titre were accompanied by increases in IgG titre ranging from 4- to 32-fold and were detected 16 or more days after revaccination, but not earlier. The interval between challenge and response was therefore similar to that which has been found to follow primary vaccination (Cradock-Watson *et al.* 1974). IgA antibody was found in post-challenge, but not in pre-challenge, sera from 2 women, but IgM antibody was not detected in any subject.

Antibody responses after vaccination of women with low titres of HI antibody resulting from natural infection

Rubella vaccine of normal titre was given to 105 women who had HI titres of 40 or less from previous natural infection. Cendehill vaccine was given to 19 women; RA27/3 was given subcutaneously to 36 women and intranasally to 50. Thirty-one women with similar initial HI titres were left unvaccinated. The numbers of women who showed changes in HI titre 5 weeks to 5 months after challenge are given in Table 5. Cendehill vaccine elicited increases in titre of fourfold or more in 7 out of 19 women (37 %). RA27/3 elicited fourfold rises in 13 out of 36 (36 %) when given subcutaneously and in 11 out of 50 (22 %) when given intranasally. If the results from the standard vaccines are combined, then

Table 5. *Effect of vaccination of* 115 *women with HI titres of* 40 *or less resulting from natural infection*

(Numbers of women showing changes in HI titre after challenge with three different vaccines given subcutaneously (S-C) or intranasally (I-N).)

		Vaccine used for challenge			
Fold rise in HI titre	Un-vaccinated controls	Cendehill S-C	Normal titre RA27/3 S-C	Normal titre RA27/3 I-N	High titre RA27/3 S-C or I-N
32	0	0	0	1	0
16	0	0	0	1	2
8	0	1	6	3	2
4	0	6	7	6	4
2	2	4	14	15	2
No change	29	8	9	24	0
Fall	0	0	0	0	0
Total number challenged	31	19	36	50	10
GMT after challenge		67	76	70	171

GMT, Geometric mean titre.

the mean titre after challenge was 71 – an increase of about twofold. No significant changes in titre occurred among the unvaccinated controls.

High titre RA27/3 vaccine was given subcutaneously to six and intranasally to four women who had initial HI titres of 40 or less. Two to four blood specimens were obtained from each subject 8–42 days after challenge. These results are also given in Table 5. High potency vaccine raised the mean titre to 171 and elicited fourfold rises in eight women (80%), six of whom had been challenged subcutaneously and two intranasally. Significant rises in HI titre were accompanied by increases in IgG titre ranging from 2- to 16-fold. In one woman these changes were detected 9 days after challenge, and a trace of IgM antibody was observed at 17 days. Among the other women rises in HI and IgG titres were detected 16 or more days after challenge, but not earlier. IgA antibody was not detected in any of these subjects.

Transmission of vaccine virus to susceptible infants

No rubella antibody was detected in the babies of 39 women who had been given RA27/3 vaccine *post partum*, nor in 50 out of 51 whose mothers had received Cendehill. One healthy baby in the latter group had an HI titre of 60 and a neutralizing titre of 8 at the age of 14 weeks, which may have been due to transmission of vaccine virus.

DISCUSSION

Mean HI titres in women who received RA27/3 vaccine were initially significantly higher than in those who were given Cendehill. After the first year this difference diminished, and during the remainder of the survey antibody titres

were well maintained in both groups. If mean titres alone are considered, then there appears to be little to choose between the two vaccines and no indication for regular revaccination. More prolonged study, however, is desirable because immunity may be needed for 20 years or more.

Increases in HI titre due to presumed reinfection were commoner after Cendehill than after RA27/3. This observation, made on vaccinees in the general community, supports those of numerous other observers who have studied vaccinees in institutions (reviewed by Banatvala, 1977). Low HI titres, also, were commoner after Cendehill, but the relation between reinfection and the HI titre is not straightforward since the titres in our 22 subjects before reinfection ranged from 30 to 320 (median = 80) and in only three cases were they 40 or less. Greater resistance to reinfection after RA27/3 may be due to a broader immune response, including higher titres of neutralizing antibody (Plotkin, Farquhar & Ogra, 1973; Grillner, 1975). If reinfections are disregarded, then a relatively large difference in mean HI titre is found to persist between the two groups of subjects. The maintenance of mean antibody titres among women who are given Cendehill vaccine may therefore depend to some extent upon reinfection with wild virus. This can occur in the United Kingdom, where vaccination is confined to females aged 11 years or more; it is less likely to occur in the United States, where young children of both sexes are vaccinated with the object of reducing the circulation of virus.

In adults the choice between RA27/3 and Cendehill vaccines may be affected by the incidence of side-effects. Some of these have been found to be commoner after RA27/3 and are particularly disturbing for women who are vaccinated *post partum* (Best, Banatvala & Bowen, 1974; Sharp & MacDonald, 1973). In schoolgirls the choice should depend solely upon the immunogenic properties of the vaccine, because side-effects at this age are mild and infrequent (Zealley, 1974; Freestone, Reynolds, McKinnon & Prydie, 1975; Böttiger & Heller, 1976). If reinfection is a risk to the fetus, then RA27/3 appears to be preferable. The extent of this risk, however, is unknown, because the outcome of reinfection during early pregnancy in vaccinated women has not been described. There was no evidence of fetal infection in the two cases described here, but the mothers were in the seventh and eighth months of pregnancy when reinfection occurred.

Revaccination produced significant increases in HI and IgG antibody titres in about 70% of women. Although revaccination is not indicated in the United Kingdom as a regular procedure, it might occasionally be valuable for women such as schoolteachers who are likely to come into repeated contact with rubella. The increase in antibody should be greater with the high titre vaccine than with the standard product and it might be justifiable to keep special supplies of this material for purposes of this kind. We found that the intranasal and subcutaneous methods of revaccination were equally satisfactory, in contrast to Plotkin *et al.* (1973) who found the intranasal route to be superior.

Challenge with vaccine elicited weaker responses in women who had experienced natural infection than in those whose antibody was vaccine-induced. The more solid immunity which follows the natural disease not only restricts the response to challenge but also diminishes the chance of natural reinfection (Horstmann *et al.*

1970; Davis *et al.* 1971). The risk to the fetus when this occurs in early pregnancy is difficult to assess because few cases have been described. An example of possible fetal infection in these circumstances was given by Eilard & Strannegård (1974); on the other hand Boué, Nicolas & Montagnon (1971), who studied three women with subclinical reinfection, found no evidence of intrauterine infection in the infants, all of whom appeared normal. Studies now in progress in the Manchester laboratory have so far confirmed these negative findings. The risk to the fetus from subclinical reinfection is probably remote, and deliberate vaccination of women with low titres of naturally acquired antibody is rarely likely to be useful. In practice we recommend immunization for women with HI titres of 30 or less (about 50 i.u./ml) despite the fact that recent work by the Public Health Laboratory Service (in preparation) has shown that the minimum immune titre is about 24 units. We recognize that for the majority of women with titres of 20 or 30 this procedure is unnecessary and unlikely to reinforce immunity, but we wish to ensure that those few women whose HI activity is due to non-specific inhibitors do not escape vaccination.

Our studies of the specific immunoglobulin responses after revaccination have shown that the increase in HI titre consists mainly of IgG antibody. We detected traces of specific IgM in one vaccinee who had recently experienced natural reinfection and in one woman with naturally acquired antibody who had been challenged with high titre RA27/3 vaccine. IgM responses in subclinical reinfection, however, are probably weak and infrequent, and whether they indicate an increased risk to the fetus is not known.

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