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Specific immunoglobulin responses in serum and nasal secretions after the administration of attenuated rubella vaccine

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SUMMARY

The indirect immunofluorescent technique has been used to study the specific immunoglobulin responses in the sera of 63 non-immune adult women who received either Cendehill rubella vaccine subcutaneously, RA27/3 rubella vaccine subcutaneously, or RA27/3 vaccine intranasally. IgG, IgA and IgM antibodies increased virtually simultaneously, starting about 2 weeks after vaccination. IgG antibody appeared in all subjects and reached maximum titres 4–6 weeks after vaccination. The mean IgG titres elicited by the three different methods of vaccination did not differ significantly. IgA and IgM antibodies reached their highest titres between 21 and 28 days after vaccination and then declined to low or undetectable titres within about 9 weeks. The maximum IgA titres observed after intranasal administration of RA27/3 vaccine were significantly higher than those which occurred when the same vaccine was given subcutaneously, but no significant difference in IgM titres was observed. When unfractionated sera were examined IgA antibody was detected in 57 cases (91 %) and IgM in 51 (81 %). Fluorescent examination of fractions obtained by centrifugation on sucrose density gradients frequently revealed small amounts of IgA and IgM antibody which could not be detected by staining unfractionated serum, and with the inclusion of these results IgA antibody was detected in 61 cases (97 %) and IgM in 59 (94 %).

When 39 adults with pre-existing serum antibody were challenged with vaccine a definite IgA response was detected in only one subject and in no case was there any evidence of the appearance of IgM antibody.

Nasal antibody, consisting of IgG or IgA or both, was detected in 17 out of 23 non-immune subjects (74 %) who received RA27/3 vaccine, either subcutaneously or intranasally. Titres were much lower than those which occur in the

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natural disease and there was no evidence that nasal antibody was elicited more readily by intranasal than by subcutaneous vaccination.

INTRODUCTION

In acute rubella IgG and IgM antibodies develop rapidly in the blood within a few days of the onset of the rash and reach high titres within two weeks. IgG antibody persists but IgM declines to undetectable titres in a few weeks. IgA antibody also appears in the blood, and secretory IgA appears in the nasal secretions. Bürgin-Wolff, Hernandez & Just (1971) showed that serum IgA antibody reached a peak 5–18 days after the onset of the rash and then declined to low or undetectable titres within three months. Cradock-Watson, Bourne & Vandervelde (1972) and Cradock-Watson, Ridehalgh, Bourne & Vandervelde (1973) also found that IgA antibody followed a transient course, both in the blood and in nasal secretions. However, Ogra *et al.* (1971) found that serum and secretory IgA appeared between one and two months after the rash and persisted for at least a year.

After the administration of attenuated rubella vaccine IgM antibody follows a transient course similar to that which occurs in the natural disease, but the titres attained are lower and IgM may be correspondingly difficult to detect in some cases (Brown & O'Leary, 1970; Ogra *et al.* 1971; Vesikari, Vaheri & Leinikki, 1971; Gupta, Peterson & Murphy, 1972). The appearance of IgA antibody in the serum and nasal secretions after vaccination was studied by Ogra and his colleagues, who compared RA27/3 vaccine given intranasally with HPV77 DK12 vaccine given subcutaneously. After intranasal vaccination the serum and secretory IgA responses were similar to those which had been found to occur in the natural disease, but titres were about twofold lower. After the injection of HPV77 DK12 vaccine IgA antibody was detected in the serum in only 5 out of 30 children but it persisted in these for at least a year; in nasal secretions IgA antibody made only a transient appearance in three cases. Unpublished studies by other workers (quoted by Plotkin, Farquhar & Ogra, 1973) have shown that nasal antibody appears in about 80 % of persons who receive RA27/3 vaccine intranasally and in about 40 % of those who receive the same vaccine subcutaneously, but does not develop after the injection of Cendehill or HPV77 vaccines.

We have used the indirect immunofluorescent technique to study the appearance of specific immunoglobulins in the serum and nasal secretions of adult volunteers receiving attenuated rubella vaccine, either subcutaneously or intranasally, in order to compare the response which follows vaccination with that which occurs in the natural disease and to observe whether the former is affected by the route of administration of the vaccine. In some cases we have centrifuged sera on sucrose density gradients and examined the fractions by immunofluorescence to see if this procedure would improve the detection of low titres of IgA or IgM antibody. This method proved to be very sensitive and we therefore applied it to sera taken after the administration of vaccine to subjects with low initial titres of serum antibody in order to observe whether challenge by vaccine virus was followed by an IgA or IgM response.

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MATERIALS AND METHODS

Adults with no pre-existing antibody to rubella

Rubella vaccine was administered to 63 female students, aged 18–22 years, who had no serological evidence of immunity to rubella. Initial haemagglutination-inhibition (HAI) titres were less than 20 in every case and no specific IgG, IgA or IgM antibodies were detected at a dilution of 1/8 by immunofluorescence. These volunteers were divided into three groups for vaccination.

Group 1. 15 volunteers received Cendehill* vaccine subcutaneously in a dose of $10^{3\cdot35}$ TCD 50.

Group 2. 20 volunteers received RA27/3† vaccine subcutaneously.

Group 3. 28 volunteers received RA27/3 vaccine intranasally. The dose of RA27/3 vaccine which was given intranasally was similar to that which was given by injection and was either $10^{3\cdot49}$ or $10^{3\cdot6}$ TCD 50. For intranasal administration the vaccine was reconstituted in 0·5 ml. of distilled water and then dropped slowly into both nostrils while the patient lay supine with the head extended.

Serial blood samples were taken from as many individuals as possible 2, 3, 4 and 6 weeks after vaccination. These specimens were tested for HAI antibody and were examined by the indirect immunofluorescent technique for the presence of IgG, IgA and IgM antibodies. Twenty selected sera taken between 21 and 28 days after vaccination were also centrifuged on sucrose density gradients and the antibody titres in the fractions were measured by the HAI test and by immunofluorescence.

Nasal washings were collected from 9 volunteers in Group 2 and 14 in Group 3 by methods previously described (Cradock-Watson *et al.* 1973). A preliminary washing was taken before the administration of vaccine and from five to eight serial specimens were taken between 1 and 6 weeks after vaccination. Nasal washings were inoculated into cultures of RK13 cells for isolation of rubella virus and were examined by immunofluorescence for the presence of antibody. The total concentrations of IgA were measured by single radial diffusion in commercial immuno-plates,‡ using solutions of 7S IgA as standards.

Adults with pre-existing serum antibody due to natural infection with rubella in the past

In an attempt to discover whether the administration of attenuated virus to persons with pre-existing antibody would be followed by any increase in the titres of immune globulins or by a reappearance of nasal antibody we administered rubella vaccine to 10 male and 13 female volunteers (laboratory staff and students), aged 20–53 years, who already possessed HAI antibody in titres ranging from 20 to 1280 and IgG antibody in titres from 64 to 2048. Five received Cendehill vaccine subcutaneously, 6 received RA27/3 vaccine subcutaneously and 12 received RA27/3 vaccine intranasally. A preliminary nasal washing was taken

* 'Cendevax', manufactured by Smith Kline and French Limited.

† 'Almevax', manufactured by Burroughs Wellcome & Company.

‡ Obtained from Hyland Division of Travenol Laboratories Limited, Thetford, Norfolk.

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before the administration of vaccine and serial specimens of serum and nasal washings were taken 2, 3 and 4 weeks afterwards. These specimens were examined by immunofluorescence for the presence of IgG, IgA and IgM antibodies. Nasal washings taken after challenge were also inoculated into RK13 cells for isolation of rubella virus. During the course of this work it appeared that the most sensitive method for detecting small amounts of IgM antibody was to examine density gradient fractions by immunofluorescence; we therefore fractionated sera taken 14, 21 and 28 days after challenge from four of these volunteers whose initial HAI titres were low (20–40) and tested the fractions for specific IgA and IgM as well as for HAI antibody.

We expected that any increase in the titres of immune globulins would be most likely to occur in subjects with low HAI titres. However, only four such volunteers were available among the staff for the prospective collection of serial specimens. We therefore selected, retrospectively, 16 additional patients with low HAI titres (10–80) who had been given rubella vaccine and from each of whom a second specimen of blood had been taken 21–45 days later. Sera taken from these patients after challenge were examined by the HAI test, by immunofluorescence and by centrifugation on sucrose density gradients. Five of these patients had been challenged with Cendehill vaccine, seven with RA27/3 vaccine subcutaneously and four with RA27/3 intranasally.

Immunofluorescent technique

Titres of rubella-specific immunoglobulins were determined by the indirect immunofluorescent technique, using the methods described in our previous work. Cover-slip cultures of BHK21 (clone 13) cells infected with the Judith strain of rubella virus were treated with dilutions of serum, nasal washings, or density gradient fractions and were then stained with fluorescein-conjugated globulins prepared against human IgG, IgA or IgM (Wellcome Reagents Limited). The stained cover-slips were examined in a Reichert microscope, using quartz-halogen dark-ground illumination and an interference exciter filter.

Sucrose density gradient centrifugation

Specimens for centrifugation on sucrose density gradients received prior absorption with chick red cells for at least 1 hr. at 4° C. A volume of 0·5 ml. of a 1/2 dilution of serum, or 0·5 ml. of concentrated nasal washings, was layered on top of a gradient extending from 12·5 to 37·5 % (w/v) which was then centrifuged at 40,000 rev./min. for 17 hr. About 12 fractions were collected after piercing the bottom of the tube. The presence of separate classes of immunoglobulin in serum fractions was detected by double diffusion in agar, using antisera specific for human IgG, IgA and IgM (Wellcome Reagents Limited). The total IgA concentrations in fractions from nasal washings were measured in Hyland immunoplates. Rubella-specific immunoglobulins in the fractions were titrated by the immunofluorescent technique and HAI activity was titrated in microtitre trays.

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Table 1. *Serum immunoglobulin responses in 15 adult females after the administration of Cendehill rubella vaccine subcutaneously*

	IgG			IgA			IgM		
Days after vaccination	No. with antibody/no. tested	Range of titres	Median titre	No. with antibody/no. tested	Range of titres	Median titre	No. with antibody/no. tested	Range of titres	Median titre
14	2/13	< 8–64	< 8	4/13	< 8–32	< 8	3/13	< 8–32	< 8
21	14/14	8–1024	181	14/14	16–256	64	13/14	< 8–256	64
28	13/13	8–1024	256	12/13	< 8–256	32	12/13	< 8–128	32
33–45	13/13	16–1024	256	7/13	< 8–128	8	6/13	< 8–32	8
46–53	4/4	128–1024	362	2/4	< 8–32	8	1/4	< 8–16	< 8
54–63	12/12	64–1024	256	*5/12	< 8–32	< 8	†5/12	< 8–32	< 8

* Four months after vaccination IgA antibody was detected in two subjects in titres of 16 and 32, but was no longer detectable in the other three.

† Four months after vaccination IgM antibody was no longer detectable in these five subjects.

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Haemagglutination-inhibition titrations

Sera were inactivated at 56° C for ½ hr., absorbed with kaolin, and titrated in WHO plastic trays by the method in routine use in the Manchester Public Health Laboratory (Thompson & Tobin, 1970).

RESULTS

Serum immunoglobulin responses in non-immune subjects

The immunoglobulin responses in Groups 1, 2 and 3 after the administration of three different forms of rubella vaccine are summarized in Tables 1, 2 and 3 respectively. The general pattern of antibody response was similar in the three groups. All three classes of antibody increased virtually simultaneously, starting about 2 weeks after vaccination. IgG antibody appeared in all individuals, increased rapidly in titre during the third week and reached maximum titres 4–6 weeks after vaccination. HAI antibody followed a similar course. The mean final IgG and HAI titres which were attained in the three groups are shown in Table 4 and were four- to eightfold lower than those which develop in the natural disease. The IgG and HAI titres which followed the injection of RA27/3 vaccine were similar to those which followed intranasal administration and appeared to be higher than those elicited by Cendehill vaccine. Differences of this order in the HAI response have been noted in several other studies (see Plotkin *et al.* 1973) but in our groups the differences between the IgG and HAI titres were not statistically significant.

IgA antibody was detected by fluorescent examination of unfractionated sera in 57 subjects (91 %) and IgM in 51 (81 %). In five subjects neither IgA nor IgM was detected. IgA and IgM antibodies increased rapidly in titre during the third week after vaccination and followed similar courses in the three groups. In the majority of individuals the highest IgA and IgM titres were observed about 21 days after vaccination, but in some cases the highest titres occurred a week later and in others the titres at 21 and 28 days were the same. The geometric means of the highest observed titres of specific IgA and IgM are shown in Table 4. The maximum IgA titres elicited by intranasal RA27/3 vaccine were significantly higher than those which were observed when the same vaccine was given subcutaneously ($P < 0{\cdot}05$). The differences between the IgM titres are not statistically significant. After the fourth week IgA and IgM antibodies declined and were detected in only about a third of the subjects 6 weeks after vaccination. Eight or 9 weeks after vaccination further specimens were taken from 28 (45 %) of the subjects, including all those who had shown IgA or IgM antibody at the previous examination. IgA antibody was still present in low titres in nine subjects and low titres of IgM were also present in nine. From the majority of volunteers who still had IgA or IgM antibody at this time further specimens were taken 4 months after vaccination when it was found that IgM antibody had disappeared but that IgA antibody was still present in three cases in titres of 8, 16 and 32.

We sought other evidence for the possible persistence of specific IgA or IgM by staining serum specimens from 43 other women who had been successfully

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Table 2. *Serum immunoglobulin responses in* 20 *adult females after the administration of RA*27/3 *rubella vaccine subcutaneously*

	IgG			IgA			IgM		
Days after vaccination	No. with antibody/no. tested	Range of titres	Median titre	No. with antibody/no. tested	Range of titres	Median titre	No. with antibody/no. tested	Range of titres	Median titre
14	2/11	< 8–32	< 8	2/11	< 8–32	< 8	3/11	< 8–16	< 8
21–22	16/17	< 8–2048	128	14/17	< 8–1024	64	14/17	< 8–512	32
25–31	14/14	64–2048	256	10/14	< 8–512	16	8/14	< 8–256	32
33–45	16/16	128–1024	724	5/16	< 8–64	< 8	5/16	< 8–64	< 8
46–53	4/4	128–1024	256	2/4	< 8–64	8	2/4	< 8–32	8
54–63	9/9	256–2048	1024	*3/9	< 8–32	< 8	†4/9	< 8–16	< 8

* Four months after vaccination one subject still had an IgA titre of 8. No further specimens were available from the other two.

† Four months after vaccination IgM antibody was no longer detectable in three subjects. No further specimen was available from the fourth.

Table 3. *Serum immunoglobulin responses in* 28 *adult females after the administration of RA*27/3 *rubella vaccine intranasally*

	IgG			IgA			IgM		
Days after vaccination	No. with antibody/no. tested	Range of titres	Median titre	No. with antibody/no. tested	Range of titres	Median titre	No. with antibody/no. tested	Range of titres	Median titre
14	2/16	< 8–16	< 8	2/16	< 8–32	< 8	3/16	< 8–32	< 8
19–23	25/25	32–2048	256	24/25	< 8–1024	128	21/25	< 8–256	32
25–31	18/18	64–1024	512	15/18	< 8–256	45	13/18	< 8–256	32
33–45	16/16	128–1024	512	6/16	< 8–64	< 8	4/16	< 8—16	< 8
46–53	10/10	128–2048	362	3/10	< 8–32	< 8	3/10	< 8–32	< 8
54–63	7/7	128–2048	1024	*1/7	< 8–8	< 8	0/7	< 8	< 8

* No further specimens were available from this subject.

Table 4. *Geometric mean titres of serum antibodies to rubella in three groups of adults who received different forms of rubella vaccine*

	Group 1 Cendehill vaccine subcutaneously	Group 2 RA27/3 vaccine subcutaneously	Group 3 RA27/3 vaccine intranasally
Mean final HAI titre 42–63 days after vaccination	131	211	188
Mean final IgG titre 42–63 days after vaccination	487	751	724
Mean of highest observed IgA titres*	99	60	141
Mean of highest observed IgM titres*	86	41	50

* Titres of < 8 were regarded as = 4 for the purpose of calculating geometric means.

Table 5. *Rubella antibodies in fractions obtained by centrifuging serum on a sucrose density gradient*

(23 days after intranasal vaccination)

Fraction no.	HAI titre	Immunoglobulin detected by gel diffusion			Immunofluorescent titre of rubella-specific immunoglobulin		
		IgG	IgA	IgM	IgG	IgA	IgM
1	< 2	–	–	–	< 1	< 1	< 1
2	4	–	–	tr	< 1	< 1	4
3	16	–	–	+	< 1	1	16
4	8	–	–	+	< 1	4	4
5	8	tr	–	–	< 1	16	< 1
6	16	+	+	–	16	32	< 1
7	32	+	+	–	16	16	< 1
8	64	+	+	–	64	16	< 1
9	8	+	+	–	16	4	< 1
10	8	+	–	–	2	< 1	< 1
11	4	+	–	–	2	< 1	< 1
12	4	–	–	–	< 1	< 1	< 1
Total units of antibody	.	.	.	.	116	89	24
Titre before fractionation	240	.	.	.	512	256	16

tr = trace.

vaccinated with RA27/3 vaccine 3–6 months previously, either subcutaneously (28 cases) or intranasally (15 cases). Low titres of specific IgA (8–16) were found in three women and an IgM titre of 16 was found in a fourth, but in the remaining 39 cases there was no evidence of persistent IgA or IgM antibody.

In previous work on the specific antibody response in acute rubella it was found that the fluorescent method sometimes failed to stain IgM antibody in dilutions of whole serum but demonstrated it quite clearly in the heavy fractions obtained after centrifuging the same serum on a sucrose density gradient (Cradock-Watson *et al.* 1972). This finding was attributed to competition between specific IgG and

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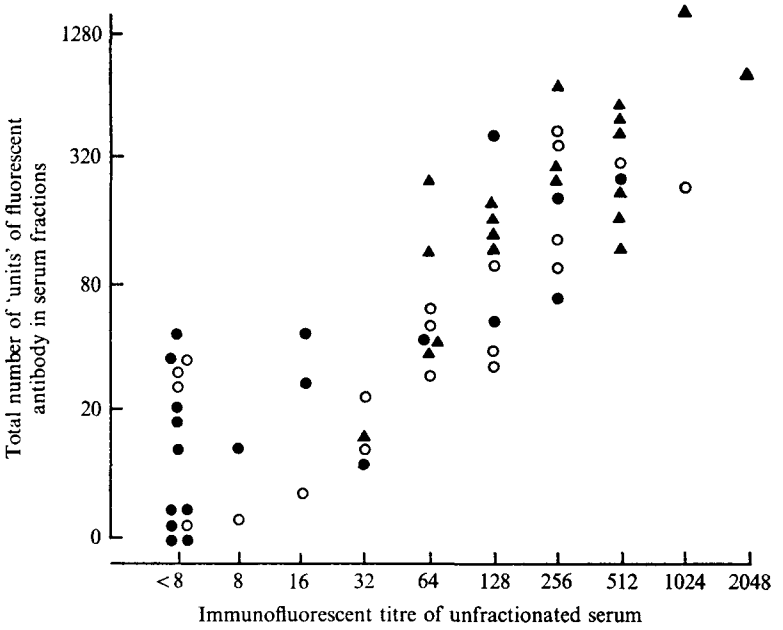

Fig. 1. IgG (▲), IgA (○) and IgM (●) antibody titres of 20 sera taken 21–28 days after the administration of Cendehill vaccine subcutaneously (1 case), RA27/3 vaccine subcutaneously (8 cases) or RA27/3 vaccine intranasally (11 cases). Abscissa = immunofluorescent titre of specific immunoglobulin in unfractionated serum. Ordinate = total number of 'units' of specific immunoglobulin in fractions obtained by centrifugation on a sucrose density gradient (see text).

IgM for the same antigenic sites during the first stage of staining. Because of the possibility that similar competition might affect the detection of IgM antibody in vaccinees we centrifuged a selection of 20 sera taken 21–28 days after vaccination and measured the fluorescent titres of IgG, IgA and IgM antibodies in the fractions. These sera consisted of specimens with a wide range of fluorescent IgM titres and included ten of the twelve sera in which IgM antibody had not been detected when unfractionated material was examined. In each fraction we regarded the reciprocal of the fluorescent titre as indicating the number of arbitrary 'units' of specific antibody. A figure for the total number of units of specific immunoglobulin in a serum was then obtained by adding together the titres in those fractions in which that class of antibody occurred. An example of the results from one serum is shown in Table 5. In Fig. 1 the total number of units of each class of antibody in each serum has been plotted against the fluorescent titre of the unfractionated material. IgG antibody was present in all twenty sera and there was an approximately linear relationship between the fluorescent titre and the total number of units of this antibody in the fractions. In ten sera IgM antibody was not detected when unfractionated material was examined but in eight of these it was found in one or more of the heavy fractions in quantities ranging from 3 to 48 units. In four of these ten sera IgA antibody also appeared to be absent when whole serum was examined but was found in the appropriate fractions in quantities ranging from 2 to 34 units.

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Table 6. *Maximum fluorescent titres of rubella antibody in nasal washings from 23 adults who received RA27/3 rubella vaccine subcutaneously or intranasally*

	Number of subjects with the indicated maximum titre of antibody			
	RA27/3 vaccine subcutaneously (9 subjects)		RA27/3 vaccine intranasally (14 subjects)	
Titre	IgG	IgA	IgG	IgA
32	0	0	0	1
16	0	0	0	0
8	0	1	0	0
4	0	2	1	3
2	1	1	3	2
1	4	2	5	2
< 1	4	3	5	6
Median titre	1	1	1	1

In order to assess the relative sensitivity of the HAI test and the immunofluorescent technique for detecting IgM antibody in the heavy fractions we compared the HAI and the fluorescent IgM titres in 45 fractions which contained IgM antibody but not IgG or IgA. In eight fractions the HAI titres were twofold greater than the fluorescent titres and in seven fractions the HAI and fluorescent titres were the same. In the remaining 30 fractions the fluorescent method was more sensitive than the HAI. In 24 of these the fluorescent titres were 2- to 16-fold greater than the HAI titres and in 6 fractions the fluorescent method gave titres ranging from 1 to 4 in the absence of any definite HAI activity.

Nasal immunoglobulin responses in non-immune subjects

Antibody consisting of IgG or IgA, or both, was detected in one or more nasal washings from 17 out of 23 subjects (74%) who received RA27/3 vaccine. IgG antibody was detected between 21 and 42 days after vaccination and IgA between 18 and 36 days, but the two types of antibody were not always present together and were detected erratically in some specimens and not in others. The highest titres which were detected following subcutaneous or intranasal administration are shown in Table 6. An IgA titre of 32 was found in a single nasal washing from one volunteer who had received intranasal vaccine but apart from this instance there was no evidence to suggest that nasal antibody was elicited more readily by intranasal than by subcutaneous vaccination.

The total IgA content of nasal washings ranged from < 3·8 to 10·7 mg./100 ml. In view of these relatively low concentrations it is possible that small amounts of IgA antibody may have escaped detection in some specimens. Low titres of antibody may also have been obscured by nonspecific fluorescence which sometimes occurred when undiluted specimens were stained for IgA. Previous experience with cases of acute rubella, however, had shown that concentrating the specimens

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Table 7. *Rubella antibodies in fractions obtained by centrifuging concentrated pooled nasal washings on a sucrose density gradient*

(24 pooled washings from 9 subjects, 21–34 days after intranasal vaccination)

Fraction no.	HAI titre	Immunoglobulin detected by gel diffusion*		Immunofluorescent titre of rubella-specific immunoglobulin	
		IgG	IgA mg./100 ml.	IgG	IgA
1	< 2	–	< 4	< 1	< 1
2	2	–	< 4	< 1	< 1
3	4	–	6·2	< 1	2
4	8	–	10	< 1	8
5	16	–	24	< 1	16
6	8	–	12	< 1	4
7	2	+	4·5	2	< 1
8	2	+	4·5	4	< 1
9	2	–	< 4	< 1	< 1
10	< 2	–	< 4	< 1	< 1
11	< 2	–	< 4	< 1	< 1
12	< 2	–	< 4	< 1	< 1
Titre before fractionation	.	.	44	4	16

* IgG was detected by double diffusion in agar. IgA concentration was measured in Hyland immuno-plates.

aggravated the nonspecific staining and therefore we did not attempt to detect low titres of antibody by concentrating individual washings before testing.

Nasal washings which were found to contain antibody were pooled and the globulins were concentrated by precipitation with half saturated ammonium sulphate. The precipitate from 25 ml. of washings was redissolved in 1 ml. of PBS and 0·5 ml. of this material was centrifuged on a sucrose density gradient. The distribution of rubella antibody in the fractions from pooled washings obtained after intranasal administration of RA27/3 vaccine is shown in Table 7. IgA sedimented more rapidly than IgG and there was no overlap between these antibodies. Although no markers were used the results suggest that nasal IgA antibody was predominantly in the 11S dimeric form and was therefore probably locally produced. When pooled nasal washings obtained after the subcutaneous injection of RA27/3 vaccine were centrifuged the sedimentation pattern was qualitatively similar.

Rubella virus was isolated from the nasal washings of 4 out of 9 persons who received RA27/3 vaccine by injection and 9 out of 14 who received the same vaccine intranasally. Isolations were made between 7 and 25 days after vaccination, from one, two or three specimens per patient. In general, virus tended to appear earlier than antibody in nasal washings but there was some overlap between these events and in four subjects virus and antibody were present in the same specimens. However, there was no definite association between virus isolation

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and the presence or subsequent appearance of antibody and in three subjects from whom virus was isolated no antibody was detected.

Responses in subjects with pre-existing serum antibody

Among the 23 staff and students who were challenged with three different forms of rubella vaccine no variation in serum HAI or IgG titres of more than twofold occurred during the period of observation, even in the 4 whose initial HAI titres were low (20–40). Four volunteers had initial serum IgA titres of 8 (1 case), 32 (2 cases) and 64 (1 case) which did not alter after challenge. No serum IgM antibody was detected by fluorescence in any of these subjects at the time of challenge nor did any appear during the four subsequent weeks. Traces of IgG antibody, but not IgA, were detected in one or more nasal washings from five subjects but showed no definite relation to the time of challenge. No virus was isolated from nasal washings from any of these individuals 2, 3 or 4 weeks after the administration of vaccine. There was therefore no evidence of reinfection of the upper respiratory tract in any of these subjects.

From 20 persons with low initial HAI titres (10–80) 28 sera taken between 14 and 45 days after challenge were examined by centrifugation on sucrose density gradients as well as by indirect immunofluorescence in an attempt to detect an IgA or IgM response. Four subjects were from the previously mentioned group of staff and students, and from each of these we examined three sera taken 14, 21 and 28 days after challenge. Sixteen were patients, from each of whom a second specimen of blood had been taken between 21 and 45 days after challenge. Eleven of these patients showed rises of at least fourfold in the titres of HAI or IgG antibody, or both. Six of the twenty subjects had been challenged with Cendehill vaccine (3 rises), eight with RA27/3 subcutaneously (5 rises) and six with RA27/3 intranasally (3 rises). None had any pre-existing IgA or IgM antibody. A definite IgA response occurred in only one case. This was a patient who had been given Cendehill vaccine 35 days previously and who showed a rise in HAI titre from 10 to 120 and in IgG titre from 16 to 32. IgA antibody was detected by fluorescence in the second serum in a titre of 16 and was present in three fractions from this serum in a total quantity of 20 units. It was not detected in fractions from serum taken on the day before vaccination. In one staff volunteer who showed only a twofold rise in HAI titre after subcutaneous challenge with RA27/3, a trace of IgA antibody (2 units) was found 14 and 21 days after challenge, in only a single fraction on each occasion. None was detected in fractions from sera taken before vaccination or 28 days thereafter. However, the significance of such a small amount of IgA is uncertain because it was detected at the threshold of sensitivity of the technique. In no case was there any evidence of an IgM response after challenge with rubella vaccine.

DISCUSSION

The general pattern of serum immunoglobulin response which followed each of the three methods of rubella vaccination was similar to that which we have

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found in the natural disease except that titres were about four- to eightfold lower. All three classes of antibody developed simultaneously after a delay of about 14 days which may be regarded as the incubation period of vaccine-induced infection and was the same whether vaccine was given subcutaneously or intranasally.

Serum IgA antibody after vaccination followed a transient course similar to that which we have found in acute rubella. The maximum titres appeared to be significantly higher after intranasal than after subcutaneous administration of RA27/3 vaccine but it is uncertain whether this in fact indicates a greater humoral response. Comparison of the titres attained by temporary antibodies are valid only if samples are taken at peak times and the inevitable failure to do this may tend to enlarge or diminish the differences between groups. Traces of IgA antibody lingered in a few cases but it is probable that this also occurs after the natural disease because we have occasionally detected low titres of IgA in adults who possessed antibody from infection in the distant past.

We detected nasal antibody in the majority of subjects who received RA27/3 vaccine, whether subcutaneously or intranasally, and the sedimentation results suggested that the IgA component was locally produced. The titres of nasal antibody, however, were much lower than those which we obtained by identical methods in acute rubella and in the majority of specimens antibody was detected only in undiluted material. This difference invites a similar comparison between the reinfection rates in the face of natural challenge which are higher among vaccinees than among those with naturally acquired immunity (Hortsmann *et al.* 1970; Davis *et al.* 1971). In previous work, however, we showed that nasal antibody in acute rubella was transient, and in the work described here we found no evidence of recall of nasal IgA when subjects with naturally acquired immunity were challenged with vaccine. In view of these two findings it seems doubtful if the more solid immunity which follows the natural disease can be attributed to greater production of nasal antibody. Immunity probably also depends on cell-mediated mechanisms which in rubella have received very little study.

IgM antibody in vaccinees also followed a transient course similar to that which occurs in the natural disease. Fluorescent staining of unfractionated sera showed persistent IgM in only one case, and although more instances might have been revealed if late sera had been fractionated, persistence of IgM after about 9 weeks is probably uncommon. Live virus occasionally lingers in the body for several weeks after vaccination and might be a risk to the fetus if a woman were to become pregnant during this time, but to what extent persistence of virus is accompanied by prolongation of the IgM response is unknown.

Comparison of fluorescent IgM titres in vaccinees with those obtained in cases of acute rubella is difficult because there is evidence that in sera from cases of natural infection the presence of a high titre of IgG antibody may block the attachment of IgM to antigen during staining and so prevent the detection of quite large amounts of IgM antibody. We have the general impression, however, that considerably less IgM antibody is formed after vaccination and this conclusion agrees with the findings of other workers (Ogra *et al.* 1971; Vesikari *et al.* 1971). Although IgM titres were relatively low we were able to detect this class

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of antibody in 51 out of 63 vaccinees by fluorescent staining of unfractionated serum. In eight out of ten sera in which fluorescence had failed to detect IgM we were still able to detect this antibody in the heavy fractions obtained after centrifugation on density gradients. However, the amounts of IgM revealed in this way were small and although blocking may have helped to prevent their detection in whole serum there was no evidence from the results shown in Fig. 1 that blocking was preventing the detection of larger amounts of IgM antibody. Possibly the IgG antibody in vaccinees competes with IgM less avidly than the IgG which develops in higher titre after natural infection. If the cases in which IgA and IgM antibodies were detected only by fluorescent staining of gradient fractions are added to those in which these antibodies were detected by staining whole serum then IgA antibody was detected in 61 cases (97 %) and IgM in 59 (94 %). Allowing for the possibility that sampling may not always have occurred at optimum times it seems reasonable to conclude that both classes of antibody are formed in most, if not all, patients who receive Cendehill or RA27/3 vaccine. In some cases the amounts are likely to be small and will only be detected by the most sensitive methods.

When 39 subjects with pre-existing serum antibody were challenged with vaccine there was no evidence of an IgM response although 20 of these subjects had low initial HAI titres and eleven of these 20 showed fourfold rises in the titres of HAI or IgG antibody, or both. In only one case was a definite IgA response detected. It is possible that abbreviated IgM responses occurred which did not correspond with the times when blood samples were taken but even so it seems likely that IgM responses in patients with pre-existing antibody are rare and that the chances of detecting them are small. Rubella vaccine is occasionally administered inadvertently to women in the early stages of pregnancy whose immune status is unknown (Mair & Buchan, 1972; Wyll & Herrmann, 1973). The detection of IgM antibody in such a case during the fourth week after vaccination would probably indicate that the patient was not previously immune and that there was a risk of fetal infection.

Our results show clearly the value of fluorescent staining of density gradient fractions for detecting low titres of specific IgM. Fluorescent titres were usually higher than HAI titres and fluorescence sometimes revealed traces of IgM antibody in fractions which had no definite HAI activity. When only traces of IgM are present most of the antibody from 0·25 ml. of serum is contained in one or two fractions, each of 0·4 ml., which are thus equivalent to a dilution of about 1/2 or 1/4 of the original serum. In practice the fluorescent detection of such low titres of IgM in whole serum is unreliable and the fluorescent examination of fractions provides a gain in sensitivity because nonspecific staining is less and competition between IgG and IgM cannot occur.

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