J . Hyg., Camb. (1973), **71**, 603
With 1 plate
Printed in Great Britain

Nasal immunoglobulin responses in acute rubella determined by the immunofluorescent technique

By J. E. CRADOCK-WATSON and MARGARET K. S. RIDEHALGH
Public Health Laboratory, Withington Hospital, Manchester M20 8LR

M. S. BOURNE
University of Salford Health Service

and ELISE M. VANDERVELDE
Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

(*Received* 23 *February* 1973)

SUMMARY

The indirect immunofluorescent technique has been used to study the specific immunoglobulin responses in nasal secretions from ten adults with acute rubella. Titres of IgA antibody in nasal washings usually exceeded those of IgG, but both types of antibody were detected in all patients. They appeared a few days after the rash, reached maximum titres during the second week and then declined. IgA antibody was no longer detectable after 47 days and was not detected at all in nasal washings from adults who had experienced rubella in the past. Low titres of IgG antibody persisted in some patients for longer than IgA and traces of IgG were found in nasal washings from a minority of adults with a past history of rubella. Nasal antibodies in acute rubella are therefore transient and unlikely to take part in resistance to reinfection.

In sucrose-density gradients nasal IgA antibody sedimented more rapidly than IgG and there was little overlap between these two types of antibody. IgA antibody in serum was more heterogeneous; it was found in nearly all the fractions which contained IgG antibody and in many of those which contained IgM.

INTRODUCTION

In many viral infections antibody appears at mucous surfaces and may be found in nasal and other external secretions (Artenstein, Bellanti & Buescher, 1964). Studies in which nasal washings have been fractionated by gel filtration and by centrifugation on density gradients have shown that secretory antibodies consist principally of IgA, often with lesser amounts of IgG, and that both components may have antiviral activity (Bellanti, Artenstein & Buescher, 1965; Rossen *et al.* 1966; Alford, Rossen, Butler & Kasel, 1967). Secretory IgA is predominantly in the 11 S dimeric form and is produced locally by plasma cells in the lamina propria underlying the mucosa of the respiratory tract and intestine (Butler, Rossen & Waldmann, 1967; Rossen *et al.* 1967; Tourville, Adler, Bienenstock & Tomasi, 1969).

In infections with rhinoviruses and parainfluenza viruses, in which only superficial replication occurs, resistance to reinfection is related more closely to the presence of secretory antibody than to the titre of antibody in the serum (Cate *et al.* 1966; Smith, Purcell, Bellanti & Chanock, 1966; Perkins *et al.* 1969).

In infections with viruses such as measles and poliovirus, in which viraemia occurs, circulating antibody plays a part in protection from clinical disease, but in the latter infection secretory antibody produced in the intestine and nasopharynx may also help by preventing the virus from establishing itself at the primary site of infection (Ogra & Karzon, 1971).

In rubella the mechanism of resistance is not clear. The acute attack is normally followed by the appearance of circulating antibody and by lifelong immunity, but the latter is not related solely to serum antibody titres which in immune persons cover a very wide range. Secretory antibody in rubella has received little study except by Ogra *et al.* (1971), who demonstrated specific IgA and IgG antibodies in nasal washings from children with acute rubella by means of the radio-immunodiffusion technique. We have used the indirect immunofluorescent technique to study specific immunoglobulin antibodies in nasal washings from adults with acute rubella, patients with maculo-papular rashes not caused by rubella, and healthy volunteers who had experienced rubella in the past.

MATERIALS AND METHODS

Patients with acute rubella

Specimens were obtained from one male and nine female patients, aged 21–33 years, who were suffering from acute rubella. Six of the nine females were pregnant. Altogether 49 specimens of nasal washings and 35 specimens of serum were obtained from patients in this group at various times after the onset of the rash. The diagnosis was confirmed by a rise of at least fourfold in the haemagglutination-inhibition (HAI) titre in all cases and by isolation of rubella virus from the nasal washings in nine cases.

Adults with serum antibodies due to past infection with rubella

Single specimens of nasal washings were taken from 11 male and 11 female healthy adult volunteers aged 18–45 years, all of whom possessed rubella HAI antibody. Four of the males had suffered from serologically confirmed rubella about 1 year previously. The other volunteers gave no recent history of the disease and were presumed to have experienced it in the more distant past.

Patients with rashes not due to rubella

Eleven patients, aged 13–27 years, were studied because they had clinical syndromes consisting of a rubelliform rash, accompanied in some cases by enlargement of the cervical or occipital lymph nodes. Three patients were male. All the eight females were pregnant. Paired sera were obtained from all patients in this group. Paired specimens of nasal washings were obtained from eight patients, but

only a single washing was available from each of the other three (at 6, 8 and 12 days after the onset of the rash).

Collection and treatment of nasal washings

While the patient sat upright with the head extended, 5 ml. of phosphate-buffered saline (PBS) was instilled into one side of the nasal cavity through an infant feeding tube which was passed about 2 cm. into the nose. The patient then leaned forward and forcibly expelled the washings into a glass jar. This procedure was repeated on the opposite side and the washings from both sides were pooled. The sample was emulsified in a blender and volumes of 0·2 ml. were inoculated into cultures of RK13, secondary rhesus monkey kidney, HEp2 and WI38 cells for virus isolation. The remainder was left overnight at 4° C. to allow all globulin to be fully eluted from any residual shreds of mucus. The specimen was then centrifuged at 2000 rev./min. for 20 min. and the supernatant inactivated at 56° C. for $\frac{1}{2}$ hr. Gentamicin (100 μg./ml.) was added to the final material, which was then stored at $-20°$ C. until required.

Nasal washings were tested for rubella HAI antibody and were examined by the indirect immunofluorescent technique for the presence of specific IgG, IgA and IgM antibodies. In the detection of antibodies to rubella the washings were used to stain cover-slip preparations of BHK21 cells infected with rubella virus. Specimens from five patients with acute rubella were also tested for antibodies to respiratory syncytial (RS) virus, for which purpose cover-slip preparations of the same cells infected with RS virus were used (see below).

The total IgA concentration was measured by single radial diffusion in commercial immuno-plates designed for the measurement of low concentrations of serum IgA,* using solutions of 7 S IgA as standards. Assuming that the IgA in nasal washings has a sedimentation constant of 11 S, this method could be expected to give results which would be about 70 % of the true values (Hobbs, 1970). In fact many of the precipitation rings were too narrow for accurate measurement and have been recorded as '< 4 mg./100 ml.'. For this reason we have not tried to adjust the readings to give a truer index of 11 S IgA concentration, nor have we altered the specific IgA titres to allow for differences in the total amount of IgA. The results are nevertheless of some value as a guide to the degree of variation in the concentration of IgA. The albumen content was also measured in Hyland immuno-plates.

Concentration of the globulins in selected nasal washings was carried out before centrifugation on sucrose-density gradients. We initially attempted to concentrate individual specimens by dialysis against 'Carbowax' and by treatment with 'Lyphogel', but the results were inconstant and the increase in total IgA concentration was usually less than proportional to the reduction in volume. Better results were obtained by precipitating the globulins with ammonium sulphate and then redissolving in a smaller volume of PBS. Before precipitation the washings were absorbed with chick red cells for 1 hr. at 4° C. in order to remove non-specific agglutinins. One volume (7 ml.) of saturated ammonium sulphate at pH 7·0 was

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

then added slowly to an equal volume of washings while the latter was agitated continuously by a magnetic stirrer. The mixture was allowed to stand overnight at 4° C. and the resulting fine precipitate was deposited in an ultracentrifuge at about 25,000*g*. The supernatant was removed and the deposit was resuspended in PBS to one eighth of the original volume. No reprecipitation or dialysis was carried out. The resulting solution was clarified by light centrifugation and 0·5 ml. of the supernatant was layered on top of a sucrose gradient. Comparisons of specific IgA titres showed that an increase in concentration of about fourfold was produced by this means.

Cover-slip preparations for fluorescent staining

Cover-slip cultures of BHK21 (clone 13) cells infected with the Judith strain of rubella virus were prepared by the method described in previous work (Cradock-Watson, Bourne & Vandervelde, 1972). For the preparation of cultures of the same cells infected with RS virus the method was modified in the following manner. Pieces of cover-slip 5 mm. in width were placed in $4 \times \frac{1}{2}$ in. tissue culture tubes to which 1 ml. volumes of growth medium containing between 200,000 and 250,000 cells were then added. The tubes were incubated at 37° C. in a sloped position for 24 hr. when they were infected by replacing the growth medium with a suspension of the Long strain of RS virus in maintenance medium. The virus was stored in liquid nitrogen as a stock of known titre and was diluted for use so that it had a final titre of about $10^{3 \cdot 5}$ TDC50 per ml. when titrated in HEp2 cells. The infected tubes were reincubated at 35° C. for 48 hr., after which the cover-slips were removed, rinsed in PBS, fixed in acetone and finally allowed to dry in air. Uninfected cultures were prepared for use as controls.

Fluorescent staining

Cover-slip preparations were stained by the indirect immunofluorescent technique as previously described (Cradock-Watson *et al.* 1972). Each serum received two preliminary absorptions at a dilution of 1/8 with washed BHK21 cells in order to reduce non-specific fluorescence. Nasal washings were not absorbed with these cells before staining. After treatment with serum or with nasal washings the cover-slips were washed in PBS and then stained with fluorescein-conjugated globulins prepared against human IgG, IgA or IgM (Wellcome Reagents Limited). Anti-IgG conjugate was not absorbed and was used at a dilution of 1/120. Conjugates prepared against IgA and IgM were absorbed once at a dilution of 1/8 with BHK21 cells and were used at dilutions of 1/32 and 1/48 respectively. A single batch of each type of conjugate was used throughout. The stained cover-slips were washed in PBS, mounted in glycerol and examined by quartz-halogen illumination, using an interference filter of the type described by Rygaard & Olsen (1969).

Haemagglutination-inhibition titrations

Sera were inactivated at 56° C. for $\frac{1}{2}$ 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). Nasal washings were not treated with kaolin but were absorbed with chick red cells and were tested in a similar manner.

Sucrose-density gradient centrifugation

A volume of 0·5 ml. of nasal washings, or 0·5 ml. of a 1/2 dilution of serum, was layered on top of a sucrose gradient extending from 12·5 to 37·5 % (w/v) which was then centrifuged at 35,000 rev./min. for 17 hr. About 12 fractions were collected after piercing the bottom of the tube. The presence of IgG and IgM in the fractions was detected by double diffusion in agar, using antisera specific for human IgG and IgM (Wellcome Reagents Limited). The concentration of IgA in each fraction was measured in Hyland immuno-plates, using solutions of 7S IgA as standards. Rubella-specific immunoglobulins in the fractions were titrated by the indirect immunofluorescent technique, and HAI activity was titrated in microtitre trays.

RESULTS

Microscopic appearances of fluorescent staining

The appearance of rubella-infected cells stained with nasal washings was similar to that produced by staining with serum. Fine fluorescent granules were seen in the cytoplasm of a proportion of cells in the preparation. Fluorescence was often brightest near the nucleus, but no nuclear staining was seen. In titrations of nasal washings and sera the numbers and brightness of individual fluorescent cells progressively diminished, and the end-point was taken as the last dilution at which specific fluorescence could clearly be seen. Some nasal washings, when tested undiluted for IgA, showed non-specific staining which could not be removed by prior absorption with BHK21 cells. This disappeared with increasing dilution and seldom interfered with the measurement of specific IgA. Non-specific staining in specimens stained for IgG was negligible. Apart from this, no consistent difference was observed between IgG and IgA staining. An example of fluorescent staining for rubella IgA antibody in a nasal washing is shown in Plate 1, fig. 1.

In BKH21 cells infected with RS virus the fluorescent material was cytoplasmic and consisted of particles ranging from relatively large round or oval bodies down to small granules and fine threads. No nuclear fluorescence was seen. An example of fluorescent staining for IgG antibody to RS virus in human serum is shown in Plate 1, fig. 2.

HAI antibody in nasal washings from patients with acute rubella

HAI antibody appeared in nasal washings from all patients in titres ranging from 8 to 64 (Table 1). It was first detected (in titres > 1) 3 days after the onset of the rash and reached its highest titres in 7–15 days. It then rapidly declined and only low levels of HAI activity persisted in those patients (cases 1, 2, 3 and 10) in whom follow-up was possible.

Nasal immunoglobulin responses in patients with acute rubella

Immunofluorescent staining showed that IgA was the predominant type of rubella antibody in nasal secretions taken in early convalescence (Table 1). IgA antibody was found in all patients. It was first detected 4 days after the onset of

Table 1. *Haemagglutination-inhibition and immunoglobulin antibody titres in nasal washings from ten patients with acute rubella*

Case no.	Days after onset of rash	Albumen concentration (mg./100 ml.)	Total IgA concentration (mg./100 ml.)	HAI titre	Immunoglobulin titre obtained by immunofluorescence: Rubella IgG	Rubella IgA	Respiratory syncytial virus IgG
1	1*	< 10	4·6	< 1	< 1	< 1	< 1
	3*	13	4·6	1	< 1	< 1	1
	8	15	5·9	32	16	64	2
	10*	13	6·4	16	2	32	2
	15	15	5·9	8	2	8	2
	17	< 10	4·6	1	1	< 1	< 1
	24	< 10	< 4	1	< 1	NSF†	< 1
	42	< 10	< 4	1	< 1	< 1	< 1
2	8*	29	10	64	32	256	2
	10*	12	< 4	16	8	128	< 1
	12*	12·5	4·1	16	2	16	< 1
	15	10·5	< 4	4	1	16	< 1
	20	11	4·1	4	1	4	.
	47	12	5·6	4	4	2	< 1
	168	< 10	< 4	1	2	< 1	< 1
3	1*	12	3·7	< 1	< 1	< 1	< 1
	10*	18	4·6	8	8	16	2
	21	< 10	3·7	2	1	2	< 1
	28	< 10	3·7	1	1	2	1
	56	< 10	4·3	1	1	< 1	< 1
4	0*	< 10	7·6	< 1	< 1	< 1	.
	7	< 10	8·8	8	2	8	.
	10	< 10	7·2	4	2	2	.
	14	10·5	5·2	1	1	< 1	.
	21	10·5	5·2	2	2	NSF	.
5	4*	11	5·2	8	< 1	4	1
	8	11	5·2	64	8	128	1
	11*	15	17	64	16	128	2
	16	21	34	32	16	32	2
	22	21	6·2	16	32	2	4
	30	11	5·2	4	2	< 1	1
6	1*	10	4·6	< 1	< 1	< 1	.
	5	11	4·6	2	< 1	8	.
	8	< 10	4·6	8	< 1	8	.
	15	< 10	5·5	16	2	32	.
7	3*	50	17	8	< 1	NSF	.
	7	11	5·2	16	1	16	.
	11	< 10	4·6	2	< 1	2	.
	24	< 10	4·9	2	< 1	< 1	.
8	1*	17	10·5	1	< 1	<1	.
	8*	21	13	16	2	2	.
9	5*	11	6·2	4	< 1	4	.
	8	10·5	7·7	32	1	32	.
	12	13	7·2	8	2	8	.
10	10	52	34	64	8	32	< 1
	15	11	4·9	8	< 1	4	< 1
	28	10	4·9	1	1	< 1	< 1
	35	10	4·9	< 1	< 1	< 1	< 1
	83	< 10	10	2	2	< 1	< 1

* Rubella virus isolated. † NSF = non-specific fluorescence interfered with reading.

https://doi.org/10.1017/S002217240004660X Published online by Cambridge University Press

Table 2. *Haemagglutination-inhibition and immunoglobulin antibody titres in sera from ten patients with acute rubella*

Case no.	Days after onset of rash	HAI titre	Immunoglobulin titre obtained by immunofluorescence: Rubella IgG	Rubella IgA	Rubella IgM	Respiratory syncytial virus IgG
1	1	< 20	< 8	< 8	< 8	2048
	3	60	64	64	16	.
	8	1280	1024	1024	1024	1024
	10	960	1024	512	256	.
	17	960	4096	512	64	1024
	24	640	1024	16	< 8	.
	42	480	2048	16	< 8	.
	143	320	2048	16	< 8	2048
2	1	< 20	< 8	32	< 8	1024
	8	1280	1024	512	< 8	.
	12	2560	4096	2048	< 8	1024
	15	2560	8200	1024	< 8	.
	47	1280	4096	< 8	< 8	512
3	1	< 20	< 8	< 8	< 8	2048
	10	640	1024	256	64	2048
4	0	< 20	< 8	< 8	< 8	.
	7	1280	4096	256	< 8	.
5	4	240	64	512	128	1024
	8	2560	1024	512	128	512
6	1	< 20	16	< 8	< 8	.
	5	480	128	1024	128	.
7	3	160	32	128	256	.
	7	1280	2048	1024	1024	.
	11	1280	2048	512	512	.
	24	1280	4096	128	128	.
	108	640	4096	< 8	< 8	.
8	1	< 20	< 8	< 8	< 8	.
	8	160	128	128	32	.
9	5	160	256	128	64	.
	8	640	1024	512	128	.
	12	1280	2048	512	128	.
10	2	< 20	8	128	< 8	1024
	10	1280	1024	4096	64	1024
	28	640	1024	128	< 8	.
	35	480	2048	< 8	< 8	2048

the rash, reached titres ranging from 2 to 256 in 7–15 days, and then rapidly declined. It was not detected after 47 days, but in cases 6, 8 and 9 the duration of IgA antibody was uncertain because of insufficient follow-up. Attempts to detect very low levels of IgA antibody by staining concentrated specimens proved unsatisfactory because of non-specific staining. The total IgA concentration showed only slight variation, except in a few individual instances (Table 1). Attempts to

Table 3. *Rubella antibodies in fractions obtained by centrifuging neat nasal washings on a sucrose-density gradient*

(Case no. 2, pooled washings 8 and 10 days after rash.)

Fraction no.	HAI titre	Immunoglobulin detected by gel diffusion*		Immunofluorescence titre of rubella-specific immunoglobulin	
		IgG	IgA mg./100 ml.	IgG	IgA
1	< 1	–	< 4	< 1	< 1
2	< 1	–	< 4	< 1	2
3	4	–	< 4	< 1	16
4	8	–	5·8	< 1	64
5	8	–	7·4	< 1	128
6	4	–	< 4	< 1	8
7	4	tr	< 4	32	2
8	4	tr	< 4	32	1
9	2	–	< 4	2	1
10	< 1	–	< 4	< 1	1
11	< 1	–	< 4	< 1	1
Titre before fractionation	32	.	.	32	256

tr = trace.

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

adjust the specific IgA titres to allow for differences in the total amount of IgA would not have altered the general pattern of IgA response.

IgG antibody was found in nasal washings from all patients in titres which were usually lower than those of IgA. It was first detected 7 days after the onset of the rash, reached titres ranging from 1 to 32 within 7–22 days, and then declined. A little was still detectable at 30 days in case 5, at 56 days in case 3, at 83 days in case 10, and at 168 days in case 2. In case 1, the only other patient in whom follow-up was possible, IgG antibody was no longer detectable at 24 days. In five patients (cases 1, 2, 3, 5 and 10) evidence of an approximate relationship between the titre of IgG antibody and the concentration of albumen suggested the possibility that nasal IgG might be derived from the serum and that its concentration in nasal washings might reflect either the quality of the specimen or the presence of inflammatory exudation. We sought other evidence for the transfer of serum IgG to the nasal secretions by examining washings from these five cases for the presence of specific IgG antibody to RS virus. However, although the serum titres of this antibody were of the same order as those of IgG antibody to rubella (Table 2) the nasal titres were less than those of rubella IgG and showed much less variation.

No nasal IgM antibody to rubella virus was detected in any patient in this group.

Nasal antibody in adults with serum antibody due to past infection

When undiluted washings were examined for IgA antibody non-specific fluorescence interfered with detection in three cases. No IgA antibody was detected in the other 19.

IgG antibody was detected in undiluted washings from one male volunteer who had experienced an attack of confirmed rubella one year previously. When concentrated washings were examined traces of IgG antibody were detected in four more males and one female, none of whom gave any recent history of the disease.

No IgM antibody was detected in any volunteer in this group.

HAI activity was detected in undiluted washings from three male volunteers, and traces of activity were detected in seven others, but no activity was detected in dilutions of 1/2 or greater. Three of the washings with HAI activity also contained traces of IgG antibody when examined after concentration.

Nasal antibody in patients with rashes not due to rubella

Nine patients in this group had stationary serum HAI titres in acute and convalescent sera, indicating rubella infection in the past. Two patients had no serum antibody and did not develop any during their illnesses. In one patient with serum HAI antibody a trace of specific IgG was detected in the first nasal washing (day 1) which also had an HAI titre of 4, but not in the second washing (day 8) which showed no HAI activity. No rubella-specific immunoglobulin was detected in nasal washings from any other patient in this group. One other patient had an HAI titre of 4 in a single nasal washing taken 12 days after the rash, but in all other patients in this group HAI activity was either absent or detectable only in undiluted washings.

No virus was isolated in cell culture from any of the patients in this group. Specimens from four patients were inoculated intracerebrally into suckling mice with negative results. No serological evidence of infectious mononucleosis, cytomegalovirus infection or toxoplasmosis was obtained, and the causes of the rashes in these eleven patients remained undiscovered.

Centrifugation of nasal washings on sucrose-density gradients

In three patients (cases 1, 2 and 5) high titres of specific IgG and IgA antibodies were found in nasal washings and we attempted to characterize these immunoglobulins further by centrifuging the specimens on sucrose-density gradients. Pooled nasal washings from case 2 (days 8 and 10) were examined before and after concentration. The results are shown in Tables 3 and 4 respectively. IgA sedimented more rapidly than IgG, and in unconcentrated washings almost no overlap was detected between these globulins. The effect of concentrating the washings was to produce an increase of at least fourfold in the titres of antibody. There was consequently some broadening of the zones in which each class of antibody was detected and some increase in the area of overlap between IgG and IgA, but no evidence that precipitation with ammonium sulphate caused any qualitative change in the sedimentation pattern.

Table 4. *Rubella antibodies in fractions obtained by centrifuging concentrated nasal washings on a sucrose-density gradient*

(Case no. 2, pooled washings 8 and 10 days after rash.)

Fraction no.	HAI titre	Immunoglobulin detected by gel diffusion* IgG	Immunoglobulin detected by gel diffusion* IgA mg./100 ml.	Immunofluorescent titre of rubella-specific immunoglobulin IgG	Immunofluorescent titre of rubella-specific immunoglobulin IgA
1	4	–	< 4	< 1	< 1
2	8	–	6·6	< 1	< 1
3	16	–	7·4	< 1	32
4	16	–	14	< 1	128
5	⩾ 64	–	35	< 1	1024
6	⩾ 64	–	29	< 1	512
7	16	+	8·4	16	32
8	16	+	7·4	128	2
9	8	+	6·6	64	2
10	4	–	< 4	2	< 1
11	2	–	< 4	< 1	< 1
Titre before fractionation	2048	.	.	128	1024

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

Table 5. *Rubella antibodies in fractions obtained by centrifuging concentrated nasal washings on a sucrose-density gradient*

(Case no. 5, pooled washings 8 and 11 days after rash.)

Fraction no.	HAI titre	Immunoglobulin detected by gel diffusion* IgG	Immunoglobulin detected by gel diffusion* IgA mg./100 ml.	Immunofluorescent titre of rubella-specific immunoglobulin IgG	Immunofluorescent titre of rubella-specific immunoglobulin IgA
1	< 1	–	< 4	< 1	< 1
2	< 1	–	< 4	< 1	< 1
3	< 1	–	< 4	< 1	< 1
4	2	–	4·1	< 1	< 1
5	2	–	7·4	< 1	16
6	4	–	16	< 1	64
7	8	–	29	< 1	128
8	4	–	14	< 1	64
9	2	tr	5·8	8	< 1
10	4	tr	9·2	64	1
11	2	tr	< 4	16	< 1
12	< 1	–	< 4	< 1	< 1
13	< 1	–	< 4	< 1	< 1
Titre before fractionation	16	.	.	64	256

tr = trace

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

https://doi.org/10.1017/S002217240004660X Published online by Cambridge University Press

Table 6. *Rubella antibodies in serum fractions obtained by centrifugation on a sucrose-density gradient*

(Case no. 2, 12 days after rash.)

Fraction no.	HAI titre	Immunoglobulin detected by gel diffusion*			Immunofluorescent titre of rubella-specific immunoglobulin		
		IgG	IgA mg./100 ml.	IgM	IgG	IgA	IgM
1	2	–	< 4	–	< 1	< 1	32
2	4	–	< 4	+	< 1	4	128
3	4	–	< 4	+	< 1	64	64
4	8	tr	8·4	–	16	512	< 1
5	16	+	22	–	1024	2048	< 1
6	⩾ 64	+	33	–	1024	512	< 1
7	⩾ 64	+	29	–	1024	32	< 1
8	8	+	7·4	–	512	16	< 1
9	4	tr	< 4	–	32	8	< 1
10	16	tr	< 4	–	4	< 1	< 1
Titre before fractionation	2048	.			4096	2048	< 8

tr = trace.

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

Table 7. *Rubella antibodies in serum fractions obtained by centrifugation on a sucrose-density gradient*

(Case no. 5, 8 days after rash.)

Fraction no.	HAI titre	Immunoglobulin detected by gel diffusion*			Immunofluorescent titre of rubella-specific immunoglobulin		
		IgG	IgA mg./100 ml.	IgM	IgG	IgA	IgM
1	4	–	< 4	–	< 1	4	32
2	8	–	< 4	+	< 1	8	64
3	4	–	6·6	+	< 1	16	64
4	4	–	8·4	tr	< 1	64	16
5	8	tr	9·2	–	8	512	< 1
6	32	+	25	–	512	256	< 1
7	⩾ 64	+	43	–	4096	256	< 1
8	⩾ 64	+	47	–	4096	32	< 1
9	32	+	23	–	1024	32	< 1
10	4	+	5·8	–	32	2	< 1
11	4	–	< 4	–	8	2	< 1
12	16	–	< 4	–	4	1	< 1
Titre before fractionation	4096	.	.	.	1024	512	128

tr = trace.

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

https://doi.org/10.1017/S002217240004660X Published online by Cambridge University Press

Satisfactory results with specimens from cases 1 and 5 were obtained only after previous concentration, which again raised the antibody titre fourfold. In both cases the IgA antibody sedimented more rapidly than the IgG, with only slight overlap. The results from case 5 are shown in Table 5. The distribution of specific antibody determined by fluorescence closely matched the concentration of IgA determined in immuno-plates. Although no markers were used the results suggest that nasal IgA antibody in these cases was predominantly in the 11S dimeric form. HAI activity was associated with both types of antibody.

Serum immunoglobulin responses in patients with acute rubella

The titres of rubella antibody in the IgG, IgA and IgM immunoglobulin classes are shown in Table 2. Antibodies in all three classes increased virtually simultaneously. IgG antibody followed the same course as HAI antibody and reached titres ranging from 128 to 8200. IgA antibody was detected in all patients and reached titres ranging from 128 to 4096. IgM antibody was detected by fluorescence in eight patients and reached titres ranging from 32 to 1024. It was not detected by fluorescence in cases 2 and 4 when whole serum was examined but was detected by fluorescence in the heavy fractions of serum taken from case 2 on the twelfth day after the rash (Table 6). It was impossible to obtain serial samples from all patients but the results agree with previous findings that IgA and IgM antibodies are transient in acute rubella and that the presence of either may be an indication of recent infection (Bürgin-Wolff, Hernandez & Just, 1971; Cradock-Watson *et al.* 1972).

Centrifugation of sera on sucrose density gradients

Sera from case 1 (day 8), case 2 (day 12) and case 5 (day 8) all contained high titres of IgA antibody. We therefore centrifuged these sera on density gradients in order to compare the sedimentation behaviour of the IgA in the serum with that of the IgA in the nasal washings taken at about the same time. The sedimentation patterns of the three sera were similar and resembled those obtained in our previous work. The results from cases 2 and 5 are shown in Tables 6 and 7 and correspond in time to the nasal washings shown in Tables 4 and 5 respectively.

A large proportion of serum antibody detectable by fluorescence consisted of IgG, the distribution of which corresponded well with the presence of IgG as detected by gel diffusion.

IgA antibody detected by fluorescence showed a wider distribution in the fractions from serum than in those from nasal washings. Nearly all the serum fractions containing IgG antibody also contained IgA. The distribution of IgA antibody detectable in serum by fluorescence did not exactly match the total IgA concentrations but showed a shift towards the heavier fractions and extended into the IgM zone. The results suggest that the freshly formed serum IgA antibody in these patients was heterogeneous in composition, and may have contained dimeric and polymeric forms in addition to monomers.

IgM antibody was detected by fluorescence in the heavy fractions from all three

patients, although in case 2 it had not been detected by fluorescence when whole serum was examined.

DISCUSSION

Our results show that antibodies to rubella virus appear in the nasal secretions in adults with acute rubella but not in patients with rubelliform rashes due to other causes. The appearance of nasal antibody was not associated with rhinorhoea, the symptoms of which had disappeared from all patients several days before the antibody responses reached their peaks.

Nasal antibody was predominantly IgA which appeared a few days after the rash, reached a peak during the second week and then rapidly declined. IgA antibody was no longer found in acute cases after 47 days and none was found in specimens from normal adults who had experienced rubella in the past. Our results differ from those obtained by Ogra *et al.* (1971), who found that nasal antibody developed between one and two months after the rash and persisted with very little fall for at least a year.

The observation that nasal and serum IgA antibodies follow similar courses is consistent with the possibility that nasal IgA in rubella is derived from the serum. However, the difference in the sedimentation behaviour of nasal and serum IgA is more suggestive of the generally accepted view that most of the secretory IgA is in the form of an 11S dimer produced locally.

The origin of IgA in the serum in man is uncertain, but consideration of the relative numbers of IgG- and IgA-producing cells in peripheral lymphoid tissue has led to the suggestion that a significant amount of IgA produced in the mucosa in the form of 7S monomers and 10S dimers may diffuse via the lymphatics into the blood (see Tomasi, 1972). The results described here are consistent with this view because comparison of the distribution of virus-specific IgA with total IgA in density gradients suggests that a disproportionate amount of freshly formed IgA antibody sediments relatively heavily and may therefore be in dimeric or polymeric form.

Titres of serum IgG antibody rose rapidly and remained high whereas nasal IgG antibody, which appeared at about the same time, tended to decline to low levels or disappear altogether. The observation that nasal and serum IgG antibodies followed different courses suggests that most of the nasal IgG in acute rubella is locally produced. This conclusion is supported by the observation that nasal titres of IgG antibody to RS virus were low and showed comparatively little variation although the titres of IgG antibody to this virus in the serum were comparable with the titres to rubella.

HAI activity to influenza A2 virus was demonstrated in nasal washings by Alford *et al.* (1967) following experimental infection in human volunteers. The activity varied according to the total amounts of IgA and protein in the specimens and was considered to be a non-specific effect due to the presence of mucoproteins. In our work slight rubella HAI activity, which may well have been non-specific, was detected in nasal washings from a few individuals in the control groups. However, in the cases of acute rubella HAI activity and fluorescent antibody levels in

nasal washings followed similar courses. Moreover in density gradient fractions the HAI activity varied with the titres of specific IgG and IgA and it therefore seems likely that most of the HAI activity in nasal washings from acute cases was specific and attributable to both these types of antibody.

By comparison with some other viral infections nasal antibody in rubella appears to be short-lived. Smith *et al.* (1966) found that titres of nasal antibody to parainfluenza virus type 1 remained high for 5 weeks and that antibody was still detectable in some cases after 8 months. Cate *et al.* (1966) found that rhinovirus antibodies were still present in nasal secretions after 56 days with little evidence of fall in titre. Although reinfection with both these agents can occur, resistance to reinfection is probably closely related to the nasal antibody titre. In the cases of rubella described here nasal IgA antibody rapidly disappeared and only traces of IgG remained in a few patients. It seems unlikely, therefore, that nasal antibody helps to prevent reinfection. It is possible that it could take part in recovery from the acute infection but its efficiency is uncertain because in four of the patients described here rubella virus was isolated from the washings after nasal antibody had reached, or even passed, its peak.

Immunofluorescence has certain advantages in the study of secretory antibodies. It is sensitive and specific and allows the detection of immunoglobulin antibodies in secretions from individual patients without the need for fractionation. The method is independent of phenomena such as complement fixation, neutralization and agglutination, and free from the limitations which tests for these functions impose. It seems a promising technique for the study of secretory antibodies from other sites and in other infections.

We are grateful to Mr K. M. Thompson, F.I.M.L.T., and his staff for carrying out the serum HAI titrations and to the patients and volunteers who agreed to take part in this study.

REFERENCES

ALFORD, R. H., ROSSEN, R. D., BUTLER, W. T. & KASEL, J. A. (1967). Neutralizing and hemagglutination-inhibiting activity of nasal secretions following experimental human infection with A2 influenza virus. *Journal of Immunology* **98**, 724.

ARTENSTEIN, M. S., BELLANTI, J. A. & BUESCHER, E. L. (1964). Identification of the antiviral substances in nasal secretions. *Proceedings of the Society for Experimental Biology and Medicine* **117**, 558.

BELLANTI, J. A., ARTENSTEIN, M. S. & BUESCHER, E. L. (1965). Characterization of virus neutralizing antibodies in human serum and nasal secretions. *Journal of Immunology* **94**, 344.

BÜRGIN-WOLF, A., HERNANDEZ, R. & JUST, M. (1971). Separation of rubella IgM, IgA and IgG antibodies by gel filtration on agarose. *Lancet* ii, 1278.

BUTLER, W. T., ROSSEN, R. D. & WALDMANN, T. A. (1967). The mechanism of appearance of immunoglobulin A in nasal secretions in man. *Journal of Clinical Investigation* **46**, 1883.

CATE, T. R., ROSSEN, R. D., DOUGLAS, R. G. JR., BUTLER, W. R. & COUCH, R. B. (1966). The role of nasal secretion and serum antibody in the rhinovirus common cold. *American Journal of Epidemiology* **84**, 352.

CRADOCK-WATSON, J. E., BOURNE, M. S. & VANDERVELDE, E. M. (1972). IgG, IgA and IgM responses in acute rubella determined by the immunofluorescent technique. *Journal of Hygiene* **70**, 473.

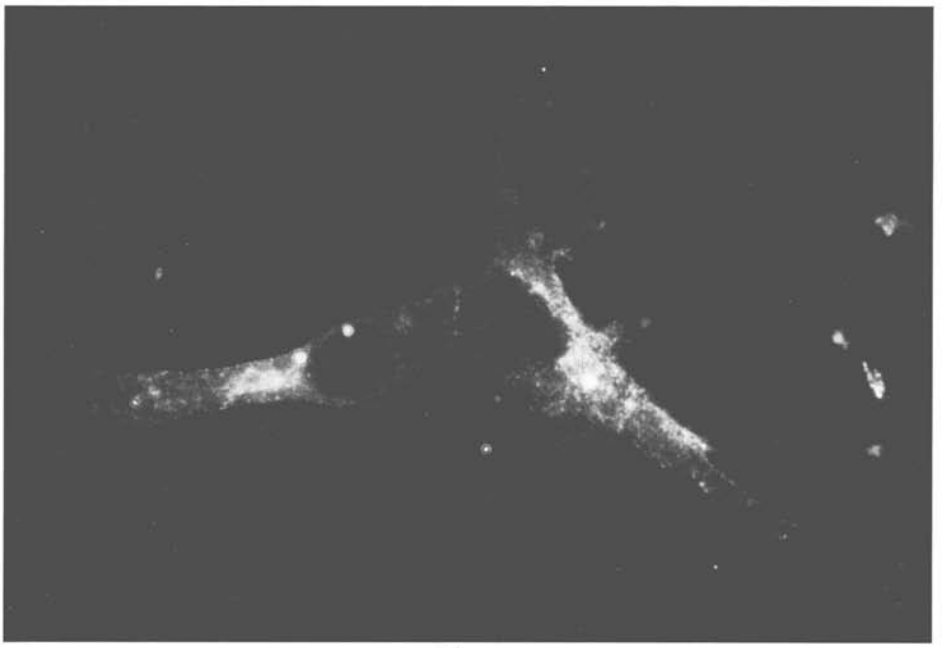

Fig. 1

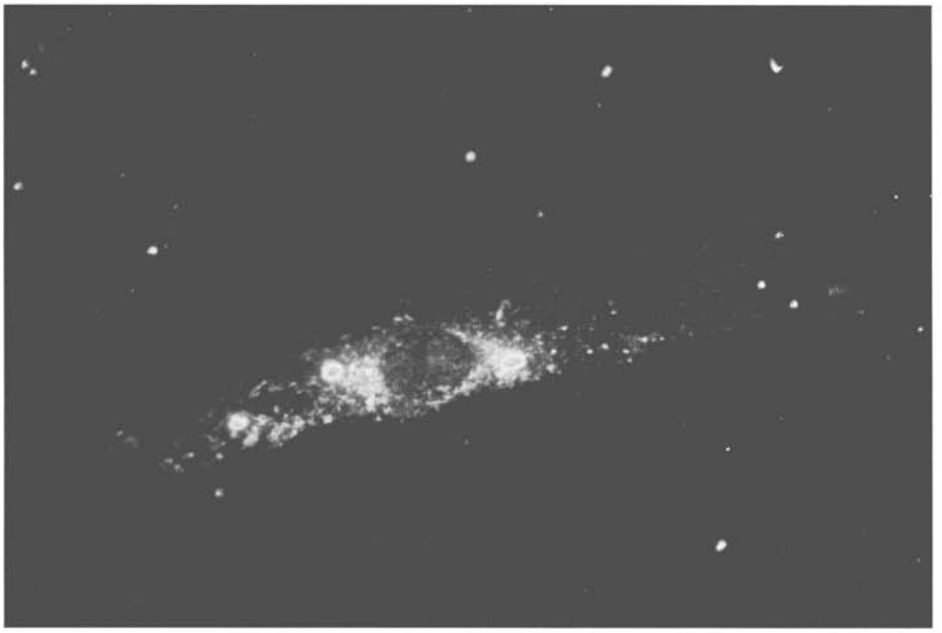

Fig. 2

https://doi.org/10.1017/S002217240004660X Published online by Cambridge University Press

Hobbs, J. R. (1970). Immune globulins in some diseases. *British Journal of Hospital Medicine* **3**, 669.

Ogra, P. L. & Karzon, D. T. (1971). Formation and function of poliovirus antibody in different tissues. *Progress in Medical Virology* **13**, 156.

Ogra, P. L., Kerr-Grant, D., Umana, G., Dzierba, J. & Weintraub, D. (1971). Antibody response in serum and nasopharynx after naturally acquired and vaccine-induced infection with rubella virus. *New England Journal of Medicine* **285**, 1333.

Perkins, J. C., Tucker, D. N., Knopf, H. L. S., Wenzel, R. P., Kapikian, A. Z. & Chanock, R. M. (1969). Comparison of protective effect of neutralizing antibody in serum and nasal secretions in experimental rhinovirus type 13 illness. *American Journal of Epidemiology* **90**, 519.

Rossen, R. D., Douglas, R. G. Jr., Cate, T. R., Couch, R. B. & Butler, W. T. (1966). The sedimentation behaviour of rhinovirus neutralizing activity in nasal secretion and serum following the rhinovirus common cold. *Journal of Immunology* **97**, 532.

Rossen, R. D., Morgan, C., Hsu, K. C., Butler, W. T. & Rose, H. M. (1967). Localization of 11S external secretory IgA by immunofluorescence in tissues lining oral and respiratory passages in man. *Journal of Immunology* **100**, 706.

Rygaard, J. & Olsen, W. (1969). Interference filters for improved immunofluorescence microscopy. *Acta Pathologica et Microbiologica Scandinavica* **76**, 146.

Smith, C. B., Purcell, R. H., Bellanti, J. A. & Chanock, R. M. (1966). Protective effect of antibody to parainfluenza type 1 virus. *New England Journal of Medicine* **275**, 1145.

Thompson, K. M. & Tobin, J. O'H. (1970). Isolation of rubella virus from abortion material. *British Medical Journal* ii, 264.

Tomasi, T. B. (1972). Secretory immunoglobulins. *New England Journal of Medicine* **287**, 500.

Tourville, D. R., Adler, R. H., Bienenstock, J. & Tomasi, T. B. (1969). The human secretory immunoglobulin system: immunohistological localization of A, secretory 'piece', and lactoferrin in normal human tissues. *Journal of Experimental Medicine* **129**, 411.

EXPLANATION OF PLATE

Fig. 1. Immunofluorescent staining of nasal IgA antibody to rubella. × 700. Nasal washings from case 2, 8 days after the rash, at a dilution of 1/16.

Fig. 2. Immunofluorescent staining of serum IgG antibody to respiratory syncytial virus. × 420. Serum dilution 1/40.