RADIOIMMUNOASSAY FOR ANTIBODIES TO RUBELLA VIRUS AND ITS RIBONUCLEOPROTEIN COMPONENT

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IN PREGNANCY, the serological differentiation of recent from remote rubella infection is important, and affects the management of the patient. Currently, this depends on the interpretation of serological responses of the IgM and IgG immunoglobulin classes to the surface antigens of rubella virus, (Best, Banatvala and Watson, 1969). By analogy with influenza, in which recent infection is characterised by an antibody response to the internal ribonucleoprotein component of the virus (Francis and Maassab, 1965), the detection of antibodies to the ribonucleoprotein component of rubella virus might provide additional evidence of recent rubella infection. We have therefore investigated the antibody response to the ribonucleoprotein and envelope components of rubella virus by a radioimmune precipitation technique.

MATERIALS AND METHODS

Cells. BHK-21 cells, clone 13, were obtained from Flow Laboratories, Irvine, Scotland.

Rubella virus was kindly supplied by Professor J. A. Dudgeon, Institute of Child Health, London.

Culture media. *Growth medium:* minimum essential medium (Glasgow modification) was supplemented with 10% tryptose phosphate broth, 10% foetal calf serum, penicillin 100 IU/ml and streptomycin 100 µg/ml; *maintenance medium* was the same as growth medium except that 4% foetal calf serum previously inactivated at 56°C for 30 min. and double the concentration of sodium bicarbonate were used.

Radioactive isotopes. L-(5-³H) arginine monohydrochloride (8.8 Ci/mmol) and (U-¹⁴C) uridine (481 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, Bucks.

Buffers were 0.015M Tris-0.01M EDTA buffer, pH 7.3; 0.01M Tris-0.001M EDTA-0.1M NaCl buffer, pH 7.1; phosphate-buffered saline (PBS), pH 7.3.

Serum samples were all obtained from the Department of Microbiology, University College Hospital, London. They were inactivated at 56°C for 30 min. before use.

Sheep anti-human IgM, IgG and IgA were obtained from Wellcome Laboratories, Kent.

Preparation of radioactively labelled rubella virus. BHK-21 cells were infected with rubella virus at a multiplicity of infection of 10 plaque-forming units per cell and labelled with ³H-arginine (4 µCi/ml) 24 h later; 72 h after infection, the medium was harvested and the virus concentrated by centrifugation at 50 000 g for 2 h at 4°C. The pellet was resuspended in 0.01M Tris EDTA buffer at pH 7.3 and the virus was purified by centrifugation in a linear 5–45% sucrose gradient at 200 000 g for 2 h at 4°C. Six-drop fractions were collected and dialysed against PBS at 4°C overnight before being tested for virus by haemagglutinating activity and radioactivity. The most active fractions were pooled and provided the rubella virus antigen for use in the tests.

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Separation of internal ribonucleoprotein component. Rubella virus labelled with ^3H -arginine (4 $\mu\text{Ci/ml}$) and with ^{14}C -uridine (1 $\mu\text{Ci/ml}$) was degraded with 1% Triton X-100 for 60 min. at 4°C in the presence of a ribonuclease inhibitor, diethyl-pyrocabonate. The subviral components were then separated on a 15–35% linear sucrose gradient, centrifuged at 200 000 g for 1 h at 4°C . Six-drop fractions were collected, dialysed against PBS overnight at 4°C and their haemagglutinating activity and radioactivity were measured.

Radioimmune-precipitation test. Serum samples, inactivated at 56°C for 30 min., were diluted in PBS or PBS containing 10% pooled human serum with a rubella haemagglutination-inhibiting antibody titre of <8 . From each serum dilution, 0.1 ml was mixed with 0.1 ml of labelled rubella-virus antigen and held overnight at 4°C . Next day an equal volume of sheep anti-human IgM, IgG or IgA serum, suitably diluted to give optimal precipitation (Horwitz and Scharff, 1969), was added and the mixture was held for another 24 h at 4°C . Radioactively labelled rubella antigen-rubella specific human immunoglobulin-sheep anti-human immunoglobulin complexes collected by centrifugation at 1000 g for 15 min. at 4°C were washed twice with 10 ml of cold PBS followed by 10 ml of cold 5% trichloroacetic acid and collected on glass-fibre filters, which were then washed three times in cold 5% trichloroacetic acid before radioactivity was counted for 10 min. in a liquid scintillation counter (Inter technique ABAC SL 40). Five replicate titrations of a control serum—having a rubella haemagglutination-inhibition titre of <8 —were included in each assay.

The highest dilution of a test serum in which the mean radioactivity recovered from the precipitate differed from that of the control serum by four or more standard deviations was taken as the end point of the titration.

Separation of immunoglobulins was as described by Cradock-Watson, Bourne and Vander-velde (1972).

Haemagglutination inhibition titration was as described by Plotkin (1968).

Polyacrylamide-gel electrophoresis was as described by Terry (1977).

RESULTS

Separation of internal ribonucleoprotein component

Fig. 1 shows two subviral components, a faster sedimenting peak I labelled with ^3H -arginine and ^{14}C -uridine but with no haemagglutinating activity and a more slowly sedimenting peak II labelled with ^3H -arginine and only a trace of ^{14}C -uridine but with haemagglutinating activity. From this we conclude that peak I is the ribonucleoprotein component and its analysis by polyacrylamide-gel electrophoresis showed a single polypeptide of molecular weight 32 000 free from contamination with envelope-component polypeptides (fig. 2).

Immunoglobulin assay with rubella virus

The presence of rubella-specific immunoglobulins in the sera from patients with recent or remote infection was determined by the double-antibody radioimmune-precipitation test. Four serum specimens from three patients with recent clinically diagnosed rubella known to contain rubella-specific IgM antibodies and two serum specimens from patients for routine antenatal screening known to contain IgG but not IgM antibodies (remote infection), detected by conventional separation and haemagglutination inhibition techniques, were titrated in duplicate for antibodies in the IgM, IgA and IgG immunoglobulin classes by the radioimmune-precipitation technique. Representative titrations of an early serum specimen drawn 22 days after the

FIG. 1.—Separation of ribonucleoprotein and envelope components of rubella virus by rate zonal centrifugation in sucrose. Direction of centrifugation was from right to left. ●—●=³H-arginine label, ●---●=¹⁴C-uridine label.

appearance of a rubelliform rash, a routine antenatal serum specimen and a control serum specimen are illustrated in fig. 3. The radioactivity counts per minute recovered in the precipitates obtained with serial dilutions of the control serum represents the mean of five replicate determinations from which the standard deviation was calculated. The radioactivity recovered in the precipitates obtained with serial dilutions of test sera represents the mean of duplicate determinations.

Rubella-specific IgM, IgA and IgG immunoglobulins were identified in early sera of all three patients with recent infection whereas only IgA and IgG were identified in the two late sera from patients with remote infection (table I).

Immunoglobulin assay with ribonucleoprotein component

The sera used in the above titrations were also titrated in duplicate for IgM, IgA and IgG antibodies to the ribonucleoprotein component of rubella virus.

FIG. 2.—Disc electrophoresis of ribonucleoprotein component of rubella virus in 10% polyacrylamide gel. Direction of electrophoresis was from left to right. The three marker proteins were bovine serum albumin (BSA) m.w. 67 000, ovalbumin (OA) m.w. 43 000 and light chain of γ -globulin (γ L) m.w. 25 000.

FIG. 3.—Radioimmune precipitation of ^3H -arginine-labelled rubella virus. - - - - = serum from patient 22 days after rubelliform rash, - - - - = routine ante natal serum, ●—● = control serum, $\updownarrow$ = standard deviation.

TABLE I.
Antibody titres against rubella virus and its ribonucleoprotein component

Time of infection	Serum no.	Time of sample	Haemagglutination inhibition test		Radioimmune precipitation titre for					
					virus			ribonucleoprotein		
			serum titre	presence of	IgM	IgG		IgM	IgA	IgG
Recent	{ 1a 1b 2 3	{ 4 11 22 9	128	+	+	+	<10	<10	<10	<10
			512	+	+	+	100	<10	1000	<10
			2560	+	+	+	100	<10	1000	10000
			2560	+	+	+	1000	<10	<10	<10
Remote	{ 1 2	routine antenatal	2560	-	-	+	<10	<10	<10	<10
			2560	-	-	+	<10	<10	<10	<10

FIG. 4.—Radioimmune precipitation of ³H-arginine-labelled rubella ribonucleoprotein component. ----- = serum from a patient 22 days after rubelliform rash, = routine ante-natal serum, ●—● = control serum, † = standard deviation.

The amount of radioactively labelled internal component precipitated by immunoglobulin of the representative sera used in fig. 3 are shown in fig. 4. From table I it can be seen that two of the three patients with recent infection developed antibody to the ribonucleoprotein component. Surprisingly, antibody to the ribonucleoprotein component was not found in the IgM immunoglobulin, but IgA and IgG antibodies were produced. To confirm that antibodies to the ribonucleoprotein component were confined to sera from patients with recent infection, ten more sera from patients for routine antenatal screening were screened at a dilution of 1 in 10 for antibody against the ribonucleoprotein component and these results, included in table II, show that none contained any IgA or IgG antibody against the ribonucleoprotein component.

TABLE II
Antibody titres of late sera against rubella ribonucleoprotein

Serum from	Haemagglutination titre*	Radioimmune-precipitation titre* for	
		IgA	IgG
negative control	< 8	< 10	< 10
positive control	2560	1000	10000
1	256	< 10	< 10
2	256		
3	512		
4	2560		
5	1280		
6	128		
7	256		
8	64		
9	512		
10	256		

* Each titre is the mean from two experiments.

DISCUSSION

In this investigation, a radioimmune-precipitation method was used to measure antibodies against intact virus and its ribonucleoprotein component. The method is highly sensitive and reproducible, and does not require preliminary serum fractionation for the identification of antibodies of different immunoglobulin classes. Moreover, because it does not depend on haemagglutination or complement fixation, the antibody to another specifically labelled virus antigen—the ribonucleoprotein component, not so far detected—may be identified. To ensure maximum sensitivity, rubella-virus antigens of high specific activity and free from host contaminants are required; although this can be achieved by preparation of radioactively labelled intact virus, present techniques do not allow a good yield of radioactively labelled ribonucleoprotein component so that the detection of antibody to this antigen by the technique described here cannot yet be a routine test. The number of sera tested is small, but our results show that antibodies belonging to all three immunoglobulin classes are formed against rubella virus during infection; the IgA and IgG antibodies persist after the initial infection and are found in sera taken from patients at intervals long after infection. In this respect, our results are similar to those of Ogra *et al.* (1971) who detected IgA antibody by radioimmune diffusion for at least a year after rubella infection and do not agree with those of Bürgin-Wolff, Hernandez and Just (1971) and Cradock-Watson *et al.* (1972) whose methods showed that IgA antibody persisted only as long as IgM. The presence of IgA antibody is not therefore an indicator of recent rubella infection. In contrast, IgA and IgG antibodies against the ribonucleoprotein component of rubella virus are detected only in sera of patients after recent rubella; and their absence from sera of patients who were remotely infected suggests that their presence is an indicator of recent rubella infection. It is of interest that one of the early sera (no. 1a) was shown to contain IgM by the conventional separation and haemagglutination-inhibition techniques but only IgA by the radioimmune precipitation test. This discrepancy suggests that sedimentation characteristics alone are not sufficient to exclude the presence of aggregates of rubella specific IgA or IgG, or non-specific inhibitors (Al-Nakib, Best and Banatvala 1974) in fractions containing IgM. Although only a few sera have been investigated for antibody to the virus ribonucleoprotein component, the results indicate that the antibody response to the ribonucleoprotein component is related to recent and not to remote infection. A test for this antibody would be a valuable addition to the laboratory tests used for the accurate diagnosis of recent rubella infection. The difficulties of preparing radioactively labelled ribonucleoprotein component prevent the use of such a test for any large-scale investigation at present, but more efficient methods of preparation are being investigated.

SUMMARY

IgA and IgG antibodies to the ribonucleoprotein component of rubella

virus have been demonstrated by radioimmune precipitation in sera from patients with recent but not remote rubella infection. This observation suggests that a test for antibodies to the ribonucleoprotein component may provide additional evidence in the diagnosis of recent rubella infection.

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