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IgA antibody response in acute rubella determined by solid-phase radioimmunoassay

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SUMMARY

A solid-phase radioimmunoassay (RIA) for detecting rubella virus IgA serum antibodies was developed. Purified rubella virus grown in roller cultures of Vero cells was adsorbed onto polystyrene beads. The coated beads were then incubated with dilutions of serum, and rubella IgA antibodies which attached to the virus antigen on the solid-phase were subsequently detected with ^{125}I-labelled anti-human-alpha antibodies. The specificity of the iodinated anti-human immunoglobulins was confirmed by RIA analysis of fractions obtained by chromatography of an early convalescent serum on an agarose column. A complete separation of IgM, IgA, and IgG was observed.

A total of 144 serial serum specimens from 31 adult patients with an acute rubella infection were tested for rubella IgA antibodies, and the results were compared with the RIA IgG and IgM titres reported earlier from the same specimens. The RIA IgA response was detected in each of the 31 patients and the IgA antibodies appeared almost simultaneously with the IgG and IgM antibodies. The maximum titres, which were lower than the IgG and IgM titres, were reached in about 1 week after the onset of rash. In 6 patients out of 31 the IgA antibody response was transient and persisted approximately two months, while in the remaining 25 patients the IgA antibodies persisted throughout the study period of more than 5 months. The results obtained indicate that the presence of rubella IgA antibodies in serum is not an indication for a recent rubella infection.

INTRODUCTION

Conflicting results have been reported on serum IgA antibody response following a rubella infection, particularly on the persistence of the IgA antibodies. These reports indicate a transient rubella IgA antibody response resembling the IgM response (Bürgin-Wolff, Hernandez & Just, 1971; Cradock-Watson, Bourne & Vandervelde, 1972), a more persistent IgA response (Al-Nakib, Best & Banatvala, 1975), only an occasional IgA response (Ogra *et al.* 1971), and a consistent response but variable persistence in individual rubella patients (Hornsleth *et al.* 1975). The

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differences in the reports may be partially explained by the sensitivity of the techniques used in the rubella IgA assays.

Highly sensitive solid-phase radioimmunoassay (RIA) procedures designed to detect viral antibodies, including rubella IgG and IgM, have been developed in our laboratory (Kalimo *et al.* 1976; Meurman, Viljanen & Granfors, 1977). The same principle was recently adapted for the detection of respiratory syncytial virus and adenovirus serum IgA antibodies (Halonen *et al.* 1979). The present report describes the further adaptation of the technique for the assay of rubella IgA serum antibodies, and the IgA antibody responses detected by the developed technique in 144 serial serum specimens of 31 adult patients with an acute rubella infection.

MATERIALS AND METHODS

Sera

Altogether 144 serial serum specimens from 31 patients with acute rubella infection were tested. The rubella haemagglutination-inhibition (HI), RIA IgG, and RIA IgM antibody responses of these patients have been reported earlier (Meurman, 1978).

Labelled anti-human immunoglobulins

The preparation and purification of the anti-human-alpha immunoglobulins will be reported in detail elsewhere (Halonen *et al.* 1979). Briefly, rabbits were immunized with purified human IgA. The hyperimmune antiserum obtained was first cycled through IgG and IgM immunosorbent columns to remove cross-reacting antibodies, followed by immunosorption chromatography on an IgA column. The specific anti-human-alpha antibodies were eluted with 0·1 M glycine HCl, pH 3·0, containing 0·5 M-NaCl, and iodinated with ^{125}I (Amersham, England) using the method of Hunter & Greenwood (1962). The specific activity of the anti-human-alpha immunoglobulins ranged from 6 to 10 μCi/μg.

The specific anti-human-gamma and -mu antibodies were isolated by immunosorption chromatography from sera obtained from Orion Diagnostica (Helsinki, Finland), and labelled with ^{125}I as above.

Antigen preparation

The details of the virus harvest and the purification of the antigen have been reported elsewhere (Meurman & Ziola, 1978). The Therien strain of rubella virus was grown in roller cultures of Vero cells. Daily harvests of the virus were pooled, concentrated by ultrafiltration, and purified by pelleting through 15% sucrose onto a cushion of 60% sucrose. After dialysis the antigen was diluted with phosphate buffered saline (PBS), pH 7·4, to a concentration of 25 μg/ml for coating of the polystyrene beads (Precision Plastic Ball Co., Chicago, Il).

Radioimmunoassay procedure

Our standard RIA procedure was employed (Kalimo *et al.* 1976; Meurman *et al.* 1977), except that PBS containing 20% normal pig serum and 2% Tween 20 was used as diluent for both serum specimens and iodinated anti-human-alpha.

Samples (200 μl) of serial serum dilutions were incubated in disposable plastic tubes with antigen coated beads for 1 h at 37 °C. After washing, 200 μl of iodinated anti-human-alpha was added to each tube, followed by incubation at 37 °C for 1 h. After a final wash the beads were rolled into clean tubes and bound radioactivity assayed in a Wallac LKB gamma counter.

The assay was standardized by diluting the iodinated anti-human-alpha antibodies to a concentration which gave 10000 counts/min(c.p.m.) bound when incubated with a bead adsorbed with 2 μg of purified human IgA. Buffer blanks and a rubella positive and a negative reference serum were included in each assay.

The RIA results were expressed as serum titres. In calculating the end-point titres, the cut-off point used was three times the c.p.m. value of the negative reference serum at the same dilution, with the proviso that the cut-off value should be at least 150 c.p.m. Before calculating the end-point titres, appropriate buffer blank corrections were always made. If a test serum had a shallow dilution versus c.p.m. curve, only the linearly declining part or its extension was used.

Serum fractionation

An early convalescent rubella serum and a rubella negative serum were fractioned by chromatography on Bio-Gel A-5m, 200-400 mesh (BioRad, Richmond, CA) column (Pattison & Mace, 1975). Sixty μl of serum specimen was layered on top of a 21 × 0·8 cm column equilibrated with PBS containing 0·5% Tween 20 and 0·05% NaN_3. The eluant was collected as 400 μl fractions at a rate of 5–6 ml/h.

RESULTS

The specificity of the iodinated anti-human immunoglobulins was confirmed by RIA analysis of the fractions of the convalescent serum specimen (Fig. 1). A complete separation of the radioactivity peaks was obtained. The fractions nos. 12–13 and 22–23, which contained large amounts of IgM and IgG antibodies, respectively, gave low c.p.m. values with the iodinated anti-human-alpha immunoglobulin, and therefore it is obvious that the overlapping observed was mostly caused by incomplete separation of serum immunoglobulins and not by cross-reactivity of the iodinated anti-human immunoglobulins. No radioactivity above the buffer blanks was detected in the fractions collected from the rubella negative serum.

The IgA antibody titre of four representative patients are shown in Table 1, where the HI, RIA, IgG and RIA IgM titres are also indicated for comparison. The appearance and persistence of the IgA antibodies in the serum specimens of each of the 31 patients are presented in Fig. 2. The IgA antibodies appeared almost

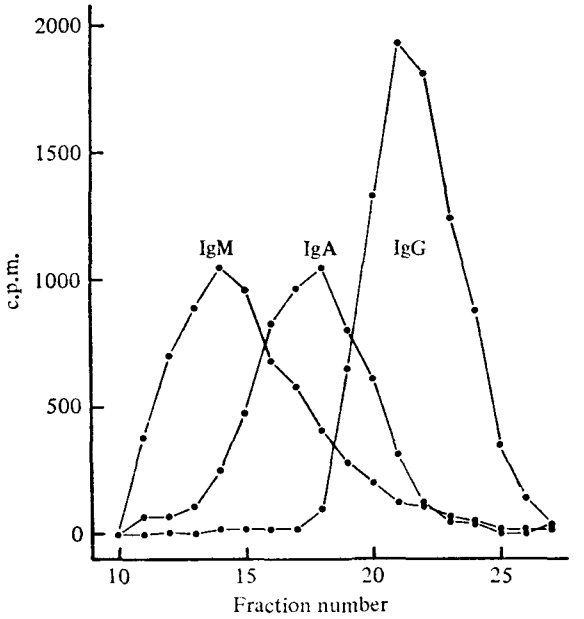

Fig. 1. Radioactivity levels obtained from agarose gel (Bio-Gel A-5m) chromatography fractions of an early convalescent rubella serum with iodinated anti-human-mu (IgM, -alpha (IgA), and -gamma (IgG) immunoglobulins.

Table 1. *Rubella HI, RIA IgG, RIA IgA, and RIA IgM antibody titres in a series of serum specimens taken from four rubella patients*

Patient	Days after onset of rash	Titre			
		HI	RIA IgG	RIA IgA	RIA IgM
J.O.	0	16	< 15.6	125	62.5
	7	256	8000	1000	16000
	15	256	8000	500	4000
	30	256	8000	500	500
	56	256	16000	250	< 15.6
	176	128	4000	250	< 15·6
K.V.	2	128	< 15.6	125	16000
	8	512	16000	2000	64000
	15	256	16000	1000	32000
	33	256	16000	250	8000
	58	256	16000	250	250
	171	128	4000	250	< 15.6
N.H.	2	16	< 15.6	62·5	1000
	8	512	4000	500	32000
	16	512	8000	250	32000
	29	256	8000	250	8000
	162	64	2000	250	< 15·6
H.J.	1	16	15·6	< 15·6	< 15·6
	7	256	16000	1000	8000
	14	256	16000	250	2000
	28	256	16000	62·5	250
	63	512	16000	< 15.6	< 15·6
	189	128	4000	< 15·6	< 15·6

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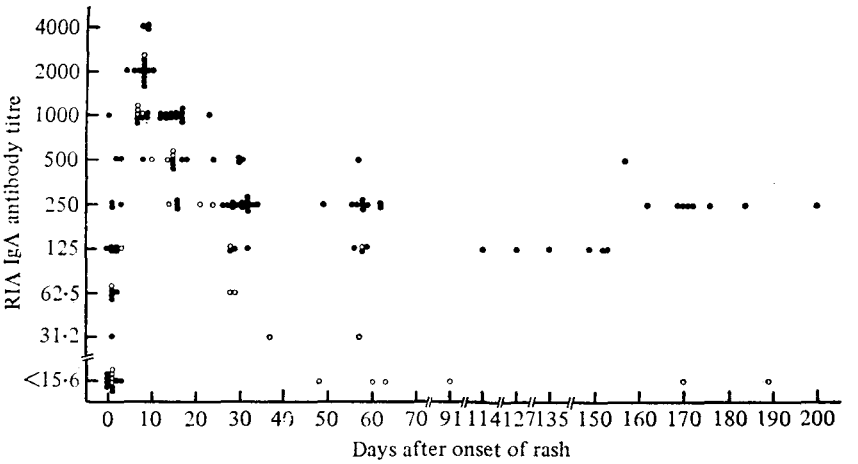

Fig. 2. Rubella RIA-IgA titres obtained from 144 serial serum specimens from 31 patients with acute rubella infection. ●, Patients with a persistent IgA response; ○, patients with a transient IgA response.

simultaneously with the IgM and IgG antibodies and were detectable in each patient within four days after the onset of rash. The maximum titres were reached approximately 1 week after the onset of rash; thereafter a decline in titres was seen. The persistence of the IgA antibodies was found to be variable. In 6 patients out of 31 the IgA antibody response was transient and the antibodies persisted about two months, while in the remaining 25 patients the IgA antibodies persisted throughout the study period. In these latter cases the IgA titres also showed on an average an eightfold decrease from the detected peak titre, but reached a constant level approximately one month after the onset of rash; thereafter no further decrease in the antibody titres was observed. The observed peak IgA antibody titres were about eight times lower than the corresponding IgG and IgM antibody titres.

DISCUSSION

In the present study an IgA antibody response following an acute postnatal rubella infection was detected in each of the 31 adult patients studied. The IgA antibodies appeared almost simultaneously with the IgG and IgM antibodies, but the serum IgA titres observed were lower than the IgG and IgM titres of the same patients (Meurman, 1978). In some patients the IgA antibody response was transient, resembling the IgM antibody response; however, in most of the patients the IgA antibodies persisted at least several months. These results disagree with those obtained by Bürgin-Wolff *et al.* (1971) and Cradock-Watson *et al.* (1972), who found the IgA antibodies to persist only 1–2 months after the onset of rash, but strongly support the results of Hornsleth *et al.* (1975) who detected considerable individual variation in the persistence of the IgA antibodies, the longest persistence time recorded being over 4 years.

According to our results it is evident that the presence of the specific IgA antibodies in serum is not an indication for a recent rubella infection. Moreover, if

rubella IgM antibodies are assayed by a method which simultaneously detects IgA antibodies, e.g. by the HI test following serum absorption with staphylococcal protein A (Ankerst *et al.* 1974) or following removal of serum IgG by precipitation with anti-human-gamma serum (Schmitz *et al.* 1975), a control procedure such as treatment with 2-mercaptoethanol (Roggendorf, Schneweis & Wolff, 1976) should be included.

Although not practical for diagnostic purposes, the determination of the IgA antibodies with the present IgA RIA technique may have applications in evaluating rubella vaccine-induced antibody responses both in serum and in nasopharyngeal secretions (Al-Nakib *et al.* 1975; Ogra *et al.* 1971).

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