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Rubella-specific IgG subclass concentrations in sera using an enzyme-linked immunosorbent assay (ELISA): the effect of different sources of rubella antigen

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SUMMARY

Five rubella antigens were evaluated in an antiglobulin enzyme-linked immunosorbent assay for rubella-specific IgG subclass antibody. One monoclonal anti-human IgG subclass antibody was used for each of IgG_1, IgG_2 and IgG_4, but two were compared for IgG_3. A total of 101 sera were tested from cases of rubella in the distant past and from cases of primary rubella, reinfection and following immunization. Only one serum gave a discrepant result for specific IgG_1, being positive with only one rubella antigen, a commercially prepared antigen coated on to microtitre wells (Enzygnost; Behringwerke). No sera contained detectable specific IgG_2. Only four sera contained specific IgG_4, and this was detectable only with Enzygnost antigen. For specific IgG_3 little difference was observed between the two monoclonal anti-human IgG_3 subclass antibodies; only two very weakly positive sera gave discrepant results. However, varying results were obtained for specific IgG_3 with the different antigens. Enzygnost gave more positive results for specific IgG_3 with most categories of sera.

It is concluded that the differences between various reports of the rubella-specific IgG subclass profile cannot be explained entirely by the use of different rubella antigens.

INTRODUCTION

There have now been several reports on the specific IgG subclass response to rubella, but there has been little or no uniformity in the methods used, and the results have differed. Some workers have used serum fractionation (Beck, 1981), but most studies have been done using an indirect solid-phase antigen enzyme-linked immunosorbent assay (ELISA). Different kit components and sources of rubella antigen have been used. Linde (1985) used the antigen-coated beads of Rubazyme G (Abbott Laboratories); Skvaril (1983) and Skvaril & Schilt (1984) used antigen-coated wells produced by Behringwerke Ltd; and Stokes, Mims & Grahame (1986) coated wells themselves with a commercially available rubella haemagglutinating antigen (HA) (Wellcome Reagents Ltd). Sarnesto *et al.* (1985) coated cuvettes with rubella HA they had grown themselves.

Therefore, a study was undertaken to examine the possible influence of rubella

antigen on the specific IgG subclass profile of various groups of sera. These comprised sera from cases of rubella in the distant past and from cases of primary rubella, reinfection and immunization.

MATERIALS AND METHODS

Sera

The following sera were tested against a panel of four different rubella antigens.

(*a*) Nineteen sera with no rubella antibody detectable by radial haemolysis (RH) (Kurtz *et al.* 1980) and latex agglutination (LA) (Rubalex; Orion Diagnostica, Finland).

(*b*) Twenty-one sera from people who had had rubella in the distant past and which had rubella-specific IgG detectable at a concentration of > 15 international units by RH.

(*c*) Thirty-three sera from cases of recent symptomatic primary rubella, which had been confirmed by the detection of elevated concentrations of specific IgM and either seroconversion or rising concentrations of specific IgG.

(*d*) Fifteen sera from cases of rubella reinfection diagnosed on the serological profile obtained and consideration of previous rubella antibody testing and/or immunization.

(*e*) Nine sera from seronegative people immunized with rubella vaccine 6–8 weeks before (3, Cendehill; 3, Almevax; 3, unknown) and four sera from people immunized 24–28 months earlier (3, Cendehill; 1, unknown).

Fifty-four of the above sera (11, seronegative; 9, rubella in distant past; 15, primary rubella; 8, reinfection; 11, recent immunization) were also tested against a fifth source of rubella antigen (Enzygnost, Behringwerke, Marburg) supplied pre-coated on microtitre plates.

ELISA

Rubella antigens

The following rubella antigens were used with optimum concentration having been determined by chessboard titration with sera positive and negative for rubella-specific IgG.

(1) Rubella HA and control antigen (Wellcome Reagents Ltd, Kent), used at a dilution of 1 in 200.

(2) Rubella HA produced from infected cell culture supernatant only (Col-S), and its corresponding control antigen (Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, London (DMRQC)) used at a dilution of 1 in 200.

(3) Rubella HA produced from infected cells and supernatant (Col-C), and its corresponding control antigen (DMRQC) used at a dilution of 1 in 200.

(4) Rubella antigen for complement fixation tests (Col-CF) (DMRQC) used at a dilution of 1 in 50. The control antigen for Col-S was used at 1 in 50 in conjunction with Col-CF.

(5) Rubella antigen- and control antigen-coated polystyrene microtitre wells (Enzygnost, Behringwerke AG, Postfach 1140, Marburg 1, D-3550).

Assay

The antiglobulin ELISA used, including the monoclonal anti IgG subclass antibodies, has been detailed in Thomas & Morgan-Capner (1988). In addition, another anti-IgG_3 monoclonal (HP6047) was also used, at a dilution of 1 in 1000. This antibody was not used for assessment of the Enzygnost rubella antigen-coated plates. Standard curves were prepared for specific IgG_1 and IgG_3, and sera were considered positive if concentrations > 3 arbitrary units (a.u.) were found (Thomas & Morgan-Capner, 1988). For specific IgG_2 and IgG_4, sera were considered positive if they gave an optical density more than the mean plus three standard deviations of a minimum of five negative sera.

RESULTS

The optical density readings for the control antigens were lowest with Enzygnost, followed closely by Wellcome and then the Col-S control antigen. No sera reacted significantly with the control antigens.

None of the sera lacking detectable rubella antibody by RH or LA was reactive for any specific IgG with any of the rubella antigens.

Rubella-specific IgG_1

Apart from one serum, specific IgG_1 was detected in all the remaining sera with all antigens. The one serum which gave a discrepant result was collected 6–8 weeks after immunization and gave a positive result (19 a.u.) only with Enzygnost.

Rubella-specific IgG_2

Specific IgG_2 reactivity was not observed with any serum or antigen preparation.

Rubella-specific IgG_3

The results for specific IgG_3 with the monoclone SJ33 are given in Table 1. Only two sera gave results discrepant between the two monoclonal antibodies SJ33 and HP6047. One serum from a case of recent primary rubella gave a negative result (< 1 a.u.) with SJ33 and Wellcome and Col-C antigens, but was weakly positive with HP6047 (4 a.u. and 3 a.u. respectively; serum 6, Table 2). The other serum was from a case of reinfection and was weakly positive (6 a.u.) with SJ33 but negative (2 a.u.) with HP6047 and Col-CF antigen (serum 13, Table 2).

A number of discrepancies were observed, however, for specific IgG_3 using the different rubella antigens (Table 2). For 15 sera a positive result was only obtained with Enzygnost plates. In 12 the concentration was low (< 10 a.u.). Col-CF antigen failed to detect specific IgG_3 in two sera which gave positive results with the other antigens used (sera 1 and 5, Table 2). One serum was positive with only Col-S and Enzygnost (serum 6, Table 2) and another was negative only with Col-C (serum 8, Table 2). A further serum (serum 11, Table 2) was negative with two (Col-S, Col-C) but positive with two antigens (Wellcome, Col-CF).

Table 1. *Results of testing for specific IgG_3 with monoclonal antibody SJ33*

Category	Total tested	Number of sera positive (> 3 a.u.)/number tested				
		Enzygnost	Wellcome	Col-S	Col-C	Col-CF
Seronegative	19	0/11	0/19	0/19	0/19	0/19
Rubella in distant past	21	4/9	3/21	3/21	3/21	0/21
Primary rubella	33	14/15	30/33	31/33	29/33	30/33
Rubella reinfection	15	6/8	11/15	10/15	10/15	11/15
Post-immunization (6–8 weeks)	9	5/7	1/9	1/9	1/9	1/9
Post-immunization (24–28 months)	4	4/4	0/4	0/4	0/4	0/4

Table 2. *Rubella-specific IgG_3 (arbitrary units) for sera giving discrepant results using monoclonal antibody SJ33*

Category	Serum no.	Rubella antigen				
		Wellcome	Col-S	Col-C	Col-CF	Enzygnost
Rubella in the past	1	18	20	15	< 1	ND
	2	< 1	< 1	< 1	< 1	7
	3	< 1	< 1	< 1	< 1	7
	4	< 1	< 1	< 1	< 1	5
	5	10	12	8	< 1	ND
Primary rubella	6	< 1	4	< 1	< 1	9
	7	< 1	< 1	< 1	< 1	12
	8	20	6	< 1	6	ND
Rubella reinfection	9	< 1	< 1	< 1	< 1	8
	10	< 1	< 1	< 1	< 1	5
	11	20	< 1	< 1	11	ND
	12	24	2·5	3	13	ND
	13	14	11	10	6	ND
Post-immunization (6–8 weeks)	14	< 1	< 1	< 1	< 1	8
	15	< 1	< 1	< 1	< 1	7
	16	< 1	< 1	< 1	< 1	9
	17	< 1	< 1	< 1	< 1	6
	18	< 1	< 1	< 1	< 1	31
Post-immunization (24–28 months)	19	< 1	< 1	< 1	< 1	21
	20	< 1	< 1	< 1	< 1	4
	21	< 1	< 1	< 1	< 1	6
	22	< 1	< 1	< 1	< 1	3

ND, not done.

Rubella-specific IgG_4

Only 4 of the 101 sera gave a positive result for specific IgG_4. These were 2 sera from cases of primary rubella and 2 from cases of rubella in the distant past. However, the reactivity was low and seen only with the Enzygnost plates. Repeated attempts to detect specific IgG_4 in these sera with the other antigens were unsuccessful.

DISCUSSION

We considered it important to investigate various types and sources of rubella antigen in the IgG subclass ELISA, as previous authors have reported widely divergent results. All authors have reported the detection of rubella-specific IgG_1 in various categories of rubella infection and, except for Beck (1981), that this is the predominant IgG subclass. This had been irrespective of the source and type of rubella antigen and the monoclonal anti-human IgG subclass antibody used.

Using Enzygnost pre-coated plates Doerr, Fleischer & Wiesman (1984) detected specific IgG_3 in sera from cases of primary rubella but gave no further detail. Skvaril (1983) and Skvaril & Schilt (1984) also used Enzygnost, but apparently only examined a small number of sera, which were from cases of rubella in the distant past. They, not surprisingly considering our results, failed to detect specific IgG_3 but did report 2 of 11 sera with low concentrations of specific IgG_4. It is interesting that the only positive results for specific IgG_4 that we obtained were with Enzygnost, but were only seen with 4 of the 101 sera we examined. Skvaril (1983) also reported low concentrations of specific IgG_2, but we failed to confirm this. Indeed, specific IgG_2 has been reported in only one of the other studies and in a single serum only (Linde, 1985).

Stokes, Mims & Grahame (1986) failed to detect specific IgG_3 even in cases of recent rubella. They used Wellcome antigen and their results disagree with ours, as we detected specific IgG_3 in many sera with a similar antigen. They did use a different monoclonal anti-human IgG_3 subclass antibody (ZG4], but we have used this reagent previously (unpublished observations) and detected specific IgG_3, although the standard curves were not reproducible. They also detected specific IgG_4 in 2 of 21 sera from cases of remote rubella, and we were unable to confirm their report.

Linde (1985) used the rubella antigen-coated beads of Abbott Laboratories as the solid phase, and reported the predominance of specific IgG_1 and IgG_3 that we report here. However, she also detected specific IgG_4 in a significant proportion (7 of 35) of sera from cases of remote rubella, unlike our results, whichever antigen was used.

Two studies (Sarnesto *et al.* 1985; Lehtinen, 1987) have used purified rubella antigen prepared by the authors. These authors reported the detection of specific IgG_1 and IgG_3 only. Thus consideration of previously published reports and the results we present suggests that the differences observed have not simply been a reflection of the different types and sources of rubella antigens used.

Overall we obtained good correlation between the antigens we used, with little difference between the specific IgG_3 results using the monoclonal anti-human IgG_3 reagents SJ33 and HP6047. The differences that were observed between the five different antigens suggest that the Enzygnost pre-coated wells provided a more sensitive solid phase for detecting specific IgG_3. This is particularly apparent when testing sera collected 6–8 weeks after rubella immunization when, by comparison with sera from cases of primary rubella, specific IgG_3 should be detectable. This would agree with Lehtinen (1987), who reported specific IgG_3 in all cases following immunization. The discrepancies in specific IgG_3 results both in

this group and other groups were manifest mainly with sera that showed low concentrations. Consideration of the results obtained with the other four antigens suggests that Col-CF is less sensitive at detecting specific IgG_3. There was little difference between the other three antigens, all of which were satisfactory.

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REFERENCES

BECK, O. E. (1981). Distribution of virus antibody activity among human IgG subclasses. *Clinical and Experimental Immunology* **43**, 626–632.

DOERR, H. W., FLEISCHER, G. & WIESMAN, M. (1984). Detection of rubella specific antibodies in different immunoglobulin-(sub)classes by ELISA. Abstract. Thirty-ninth meeting of the Deutsche Gesellschaft, Hygiene and Microbiology.

KURTZ, J. B., MORTIMER, P. P., MORTIMER, P. R., MORGAN-CAPNER, P., SHAFI, M. S. & WHITE, G. B. B. (1980). Rubella antibody measured by radial haemolysis. Characterization and performance of a simple screening method for use in diagnostic laboratories. *Journal of Hygiene* **84**, 213–222.

LEHTINEN, M. (1987). Affinity and subclass distribution of IgG and subclass antibodies following vaccination with a live rubella vaccine. *Vaccine* **5**, 88–89.

LINDE, G. A. (1985). Subclass distribution of rubella virus-specific immunoglobulin G. *Journal of Clinical Microbiology* **21**, 117–121.

SARNESTO, A., RANTA, S., VAANAREA, P. & MAKELA, O. (1985). Proportions of IgG classes and subclasses in rubella antibodies. *Scandinavian Journal of Immunology* **2**, 275–282.

SKVARIL, F. (1983). Human IgG subclasses in anti-viral antibodies determined with monoclonal antibodies in ELISA. *Immunoenzymatic Techniques* (ed. S. Avrameas), pp. 287–290. Amsterdam: Elsevier.

SKVARIL, F. & SCHILT, U. (1984). Characterization of subclasses and light chain types of IgG antibodies to rubella. *Clinical and Experimental Immunology* **55**, 672–676.

STOKES, A., MIMS, C. A. & GRAHAME, R. (1986). Subclass distribution of IgG and IgA responses to rubella virus in man. *Journal of Medical Microbiology* **21**, 283–285.

THOMAS, H. I. J. & MORGAN-CAPNER, P. (1988). Specific IgG subclass antibody in rubella virus infections. *Epidemiology and Infection* **100**, 443–454.