Rubella-specific IgG subclass avidity ELISA and its role in the differentiation between primary rubella and rubella reinfection

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SUMMARY

An antiglobulin enzyme-linked immunosorbent assay for rubella-specific IgG_1 and IgG_3 was adapted to measure antibody avidity by incorporating a mild protein denaturant, diethylamine (DEA), into the serum diluent. Sera were tested at varying dilutions, both with and without DEA, if they contained sufficient specific IgG_1 or IgG_3. The optical density (OD) was measured and curves were plotted. The highest OD (V) was noted and halved ($V/2$). The distance between the OD curves at $V/2$ was measured as the DEA shift value.

Sera were examined from people whose sera contained rubella-specific antibodies as a consequence of infection or vaccination in the distant past (24 sera), recent primary rubella (66 sera), symptomatic reinfection (11 sera) or asymptomatic reinfection (64 sera). For specific IgG_1 the DEA shift value was < 0.6 for cases of rubella in the distant past, compared with > 0.8 for the first month after primary infection. The maximum DEA shift value for the sera from cases of reinfection was 0.65.

No serum from cases of rubella in the distant past contained sufficient specific IgG_3 to estimate avidity. The sera collected within 1 month of onset of primary rubella gave DEA shift values > 0.7 compared with sera from reinfections, which gave DEA shift values < 0.6, except for two sera from a case of symptomatic reinfection.

Thus the assessment of specific IgG subclass avidity is of value in differentiating serologically primary rubella from reinfection.

INTRODUCTION

Rubella reinfection is usually subclinical, and is diagnosed when it is concluded from consideration of results of previous testing, vaccination history and serological findings that the antibody response demonstrated occurred in a person who had previously been infected with rubella as a consequence of natural infection or immunization. As reinfection in pregnancy is considered currently to present minimal risk to the fetus (Morgan-Capner, 1986), distinction from asymptomatic primary rubella, which carries a significant risk to the fetus (Cradock-Watson *et al.* 1981), is of critical importance.

As specific IgM can be detected both in primary rubella and rubella reinfection, the serological differentiation can no longer be based solely on its detection

(Morgan-Capner *et al.* 1985). We have previously investigated the rubella-specific IgG subclass profile in primary rubella and rubella reinfection (Thomas & Morgan-Capner, 1988). Although such testing may provide helpful information as specific IgG_3 may not be detectable in reinfection, it cannot be used reliably to distinguish the two. We have investigated, therefore, a further serological approach. the functional affinity (avidity) of rubella-specific IgG_1 and IgG_3.

The sum of the attractive and repulsive forces between a monovalent antibody and a monovalent antigen or hapten is the *affinity* of the reaction. Each IgG molecule has two antigen-binding sites, and viruses (or viruses crudely prepared as antigens) are multivalent antigens. The multivalent binding is termed the functional affinity or *avidity* (Roitt, Brostoff & Male, 1986). The avidity is thus the function which applies to an antibody population directed against a virus. An antibody population produced in response to an antigen contains antibodies of many avidities. It has been shown that antibodies produced early after infection or antigenic challenge are generally of lower avidity than those produced later (Eisen & Siskind, 1964; Siskind & Benecerraf, 1969; Rodkey & Freeman, 1970; Devey *et al.* 1988). Therefore it should be possible to distinguish between primary rubella and rubella reinfection on the basis of specific antibody avidity.

Inouye *et al.* (1984) and Rousseau & Hedman (1988) showed that differences could be demonstrated for avidity of rubella-specific IgG between patients with primary rubella, a small number of cases with rubella reinfection and those who had had rubella in the distant past. They used protein denaturants, guanidine hydrochloride and urea respectively, to demonstrate differences in avidity. and this is essentially the same approach used by Devey *et al.* (1988), who used diethylamine instead to look at the development of avidity of antibodies produced against tetanus toxoid. We have used the latter method, and report the results on large groups of sera from cases of primary rubella and rubella reinfection.

MATERIALS AND METHODS

Sera

The following sera were tested.

(*a*) Twenty-four sera from cases of rubella in the distant past. All contained rubella-specific IgG detectable by radial haemolysis (Kurtz *et al.* 1980). All contained rubella-specific IgG_1 at a concentration > 30 arbitrary units (a.u.) (Thomas & Morgan-Capner, 1988).

(*b*) Sixty-six sera from 53 cases of primary rubella. The date of onset was known for 35 cases from whom 42 sera were obtained. Fifty-nine aof the 66 sera contained sufficient rubella-specific IgG_1 (> 30 a.u.) for the avidity studies to be performed. and 48 contained sufficient rubella-specific IgG_3 (> 30 a.u.). Seven sera did not contain sufficient IgG_1 for the avidity studies; four were undated sera and three were taken soon after onset of the illness (< 21 days). All seven contained low concentrations of specific IgG_1. Eighteen sera from cases of primary rubella had insufficient specific IgG_3 for avidity studies, 4 were sera from children with hereditary spherocytosis and aplastic crisis, 4 were not dated. 5 were taken early and 5 were taken late after onset of symptoms. All sera contained specific IgM except three: two taken 1 day and one taken more than 2 months after onset.

(*c*) Eleven sera taken from six cases of confirmed clinically-apparent rubella reinfection. The date of onset of illness was known for five cases. All sera contained sufficient rubella-specific IgG_1 for the avidity studies, but only two contained sufficient specific IgG_3. Eight of the sera contained specific IgM detectable at a concentration greater than 3 a.u. by IgM antibody-capture radioimmunoassay MACRIA (Mortimer *et al.* 1981).

(*d*) Sixty-four sera from 39 cases of confirmed asymptomatic rubella reinfection. All sera contained sufficient specific IgG_1 for the avidity studies, but only 10 had sufficient specific IgG_3. The date of contact was known for 30 cases, and 21 sera contained detectable specific IgM at a concentration greater than 3 a.u.

Isotype-specific avidity ELISA

Microtitre plates were coated with rubella antigen as described previously (Thomas & Morgan-Capner, 1988). The treatment of serum with the protein denaturant diethylamine (DEA) was an adaptation of the method described by Devey *et al.* (1988). Sera were diluted initially 1 in 10 in phosphate-buffered saline containing 0·05% Tween 20 (PBST) and 2% normal goat serum (2% NGS/PBST). They were then diluted in 50 μl volumes in antigen-coated wells in twofold dilutions from 1 in 20 to 1 in 2560 in parallel in 2% NGS/PBST and in 2% NGS/PBST containing 35 mM diethylamine (Analar) (2% NGS/PBST/DEA). The plates were incubated in a sealed moist box for 1 h at room temperature before washing six times with PBST. The remainder of the assay (monoclonal anti-IgG subclass antibodies, peroxidase-conjugated anti-mouse immunoglobulin and substrate) was carried out as described previously (Thomas & Morgan-Capner, 1988). The reaction was stopped by the addition of $4N-H_2SO_4$ before the maximum optical density (OD) reached 2·00. Optical density at 490 nm was measured using an MR 600 spectrophotometer (Dynatec Laboratories Ltd, Billinghurst, Sussex) and the OD plotted against the $\log_{10}$ of the reciprocal of the serum dilution (Fig. 1). Two titration curves, with and without DEA, were plotted for each serum. The highest OD value (*V*) was noted and halved (*V*/2). At the OD *V*/2 the distance between the two curves was measured. This distance was the DEA shift value and was therefore equal to the $\log_{10}$ of the reciprocal serum dilution in 2% NGS/PBST at *V*/2 minus the $\log_{10}$ of the reciprocal serum dilution in 2% NBS/PBST/DEA at *V*/2 (Fig. 1).

RESULTS

Specific IgG_1 shift values

With sera taken from cases of rubella in the distant past, the mean shift value was 0·45, with a standard deviation of 0·15 and a range of 0·2–0·6 (Table 1).

The mean DEA shift value for the 59 sera from cases of primary rubella was 0·91, with a standard deviation of 0·24 and a range of 0·45–1·3 (Table 1). Fig. 2 shows the relation between DEA shift value and time after onset of symptoms. It shows that within the first 28 days after onset the DEA shift value was always $\geqslant 0·8$ and then started to decline. Three sera taken on day 28 gave lower values (0·75, 0·75, 0·53) and another on day 33 gave a value of 0·48. Amongst the sera of unknown date of illness, 17 had DEA shift values less than 0·9. Four of these sera

Optical density, 490 nm

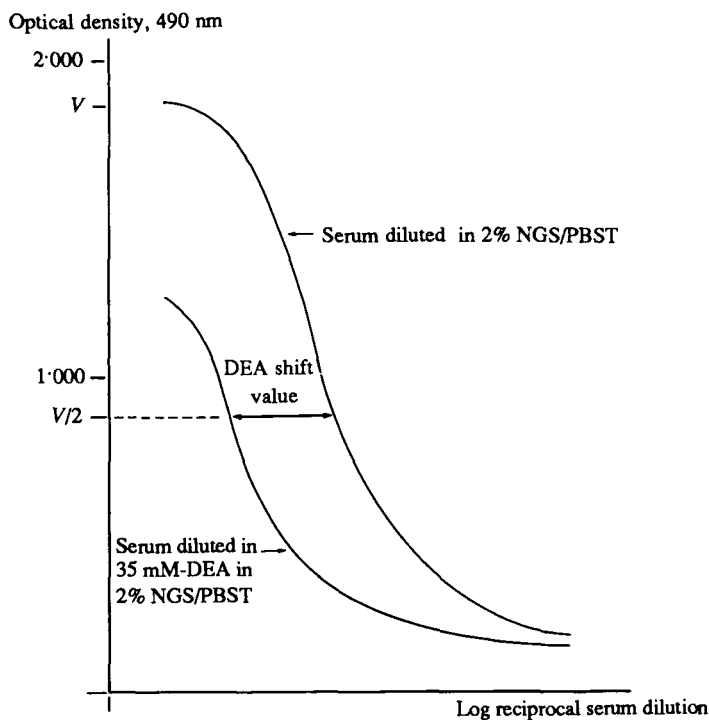

Fig. 1. Calculation of the DEA shift value for sera containing rubella-specific IgG.

Table 1. *Mean, standard deviation and range of DEA shift values*

DEA shift value

	Specific IgG$_1$				Specific IgG$_3$			
Category	No. of tests	Mean	Standard deviation	Range	No. of tests	Mean	Standard deviation	Range
Rubella in the distant past	24	0·45	0·15	0·2–0·6	0	—	—	—
Primary rubella	59	0·91	0·24	0·47–1·3	48	1·05	0·31	0·45–1·6
Rubella reinfection	75	0·41	0·12	0·1–0·65	12	0·48	0·17	0·25–0·8

were from two children with an aplastic crisis and hereditary spherocytosis and were strongly positive for parvovirus B19-specific IgM confirming recent parvovirus B19 infection. All four had low concentrations of rubella-specific IgM, suggesting either primary rubella some while before or cross-reacting IgM with rubella having occurred in the distant past. The serological profile and clinical details of the other 13 sera from 10 patients suggested that they were all collected some while after the rubelliform illness, with most being investigated for persisting polyarthralgia.
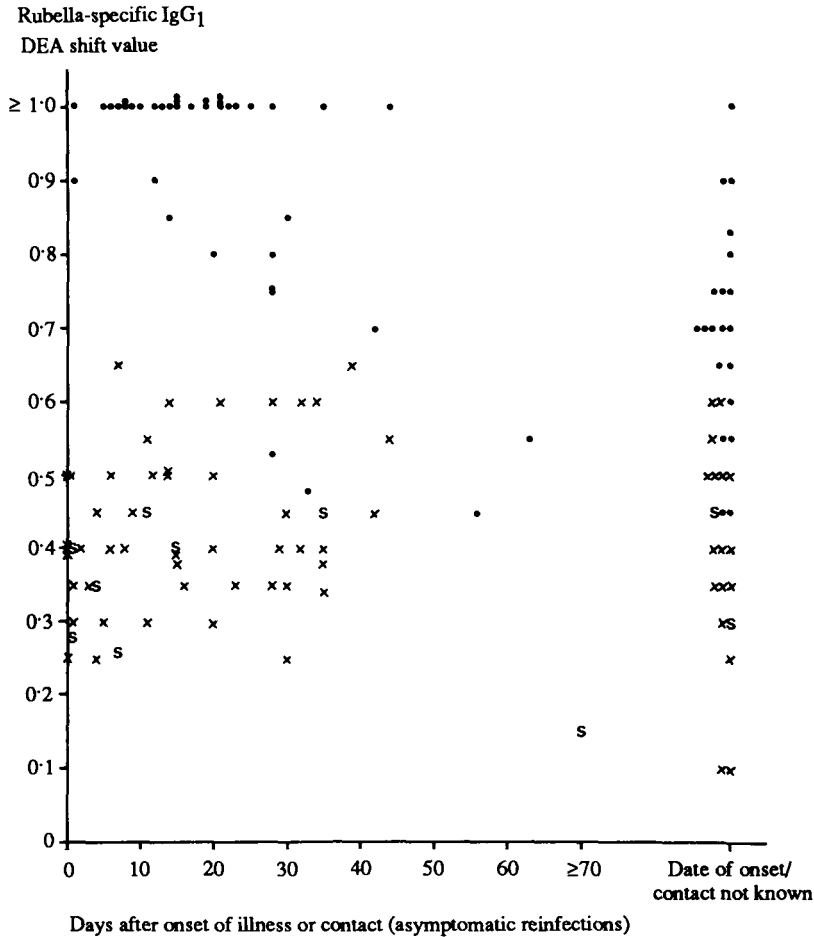
Rubella-specific IgG$_1$
DEA shift value

Days after onset of illness or contact (asymptomatic reinfections)

Fig. 2. DEA shift values for specific IgG$_1$ with sera from primary rubella and rubella reinfection. Sera from cases of rubella and symptomatic reinfection are recorded in relation to days after onset of illness. Sera from cases of asymptomatic reinfection are recorded against days after contact with an index case of rubella. ●, Primary rubella; ×, asymptomatic reinfection; S, symptomatic reinfection.

All 75 sera from the clinically apparent and subclinical reinfections contained sufficient specific IgG$_1$ for avidity studies. The mean DEA shift value was 0·41, with a standard deviation of 0·12 and a range of 0·1–0·65 (Table 1). The relation between DEA shift value and contact (asymptomatic reinfection) or onset of illness (symptomatic reinfection) is shown in Fig. 2. The DEA shift values do not change with time and are lower than those seen in primary rubella.

Specific IgG$_3$ shift values

The mean DEA shift value for the 48 sera from cases of primary rubella was 1·05, with a standard deviation of 0·31 and a range of 0·45–1·6 (Table 1). The relation to days after onset of rash is shown in Fig. 3, with all sera collected within the first 30 days after onset of illness giving DEA shift values ≥ 0·7. Of the 16
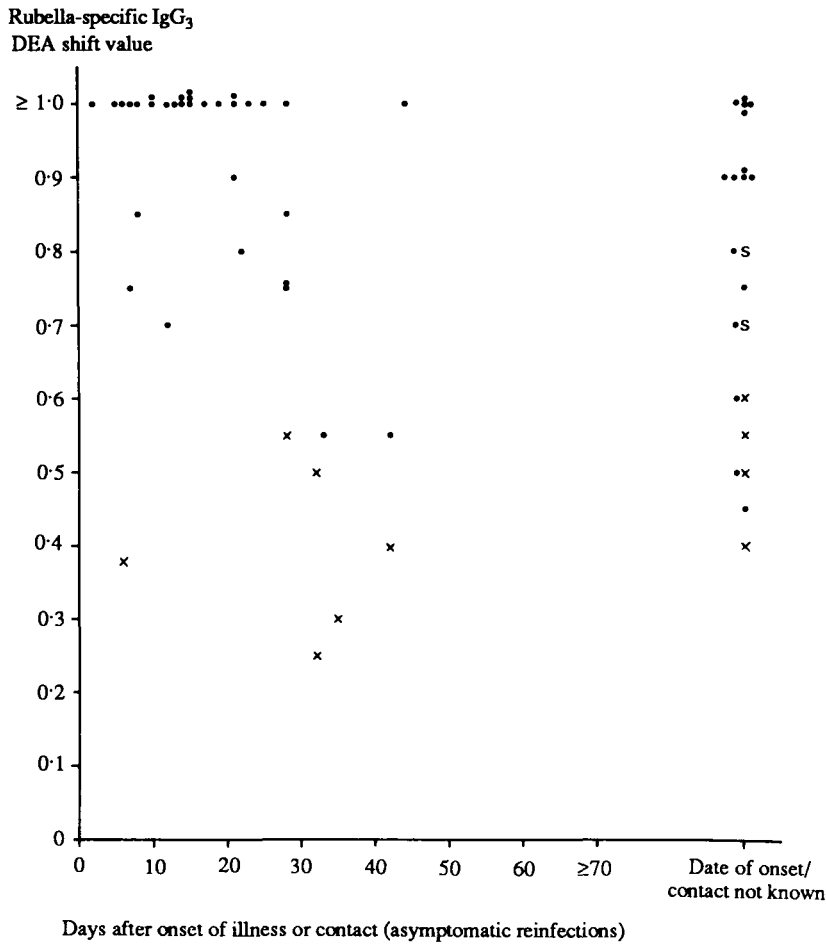
Fig. 3. DEA shift values for specific IgG$_3$ with sera from primary rubella and rubella reinfection. Sera from cases of rubella and symptomatic reinfection are recorded in relation to days after onset of illness. Sera from cases of asymptomatic reinfection are recorded against days after contact with an index case of rubella. ●, Primary rubella: ×, asymptomatic reinfection; S, symptomatic reinfection.

undated sera, 3 gave values < 0·7 and the serological profile and clinical details suggested that these sera were taken some time after the onset of the illness.

Twelve of the 75 sera from cases of reinfection contained sufficient concentrations of specific IgG$_3$ for avidity studies. Six were dated and all had DEA shift values ≤ 0·55 (Fig. 3). The maximum DEA shift value seen with the undated sera from asymptomatic reinfection was 0·6, with two sera from one case of symptomatic reinfection having values of 0·7 and 0·8. This patient gave low DEA shift values, however, for specific IgG$_1$ (0·45 and 0·3), but did have high concentrations of specific IgG$_3$ (> 100 a.u.) for reinfection. The mean DEA shift value of specific IgG$_3$ for cases of reinfection was 0·48, with a standard deviation of 0·17 and a range of 0·25–0·8 (Table 1).

DISCUSSION

The adaptation to measure avidity of the antiglobulin ELISA for rubella-specific IgG subclass antibody was straightforward, using the protein denaturant diethylamine as described by Devey *et al.* (1988). We examined the avidity of specific IgG_1 and IgG_3 separately, as we had recently developed these assays and had demonstrated their validity (Thomas & Morgan-Capner, 1988). However, the method described here should be readily adaptable to an antiglobulin ELISA for total rubella-specific IgG or antibody, and we have confirmed this with a few sera.

In primary rubella the avidity of rubella-specific IgG_1 and IgG_3 increased with time after the onset of illness. Two sera taken 28 and 33 days after the stated date of onset of primary rubella had low DEA shift values. However, consideration of the concentrations of specific IgM and the given history suggests that these sera were possibly taken later after the infection than was stated. All sera taken within 1 month of onset of primary rubella gave DEA shift values of $\geqslant 0.8$ for specific IgG_1 and $\geqslant 0.7$ for specific IgG_3. This contrasted with the values obtained for specific IgG_1 for sera from cases of rubella in the distant past, which all gave a DEA shift value < 0.6. Unfortunately we had no sera from cases of rubella in the distant past which had sufficient IgG_3 to determine avidity.

The avidity of specific IgG_1 and IgG_3 antibody did not increase with time after contact or illness for the cases of reinfection. No serum from a case of reinfection had a DEA shift value > 0.65 for rubella-specific IgG_1, a marked difference from the results for sera known to have been taken soon after onset of primary rubella. From these results, if due account is taken of the date of the serum in relation to illness or contact, the determination of specific IgG_1 subclass avidity is a reliable approach to distinguishing recent primary rubella from rubella reinfection. However, it would not be surprising if occasional exceptions are seen, should further sera be tested by this method. It is unlikely that any approach will always be able to distinguish serologically with certainty between reinfection and primary rubella if only late sera are available.

This study demonstrates that specific IgG_3 also differs in avidity between sera taken soon after primary infection from those taken late after primary infection, that is, following a reinfection. All sera taken within a month of primary infection had DEA shift values of $\geqslant 0.7$ compared with sera from reinfection which, with two exceptions, had DEA shift values $\leqslant 0.6$. The exceptions were two sera from one case of symptomatic reinfection, which had DEA shift values of 0.7 and 0.8. These sera gave low specific IgG_1 DEA shift values, compatible with reinfection, and specific IgM concentrations that were low (6.5, 5.1 a.u.). However, the sera had unusually high concentrations of specific IgG_3 (both $\geqslant 100$ a.u.) for reinfection (Thomas & Morgan-Capner, 1988). No pre-contact or earlier serum was available, but the patient had been immunized against rubella 9 years previously. There was no precise date of contact given.

Lehtonen & Meurman (1982) used a complicated mathematical approach to determine rubella-specific IgG avidity and concluded that high-avidity antibody developed more slowly than total antibody after primary infection. They contrasted this with their findings for sera from seven clinically apparent

reinfections in immunized women, where high-avidity antibody developed at a faster rate. Inoye *et al.* (1984) and Rousseau & Hedman (1988) used more simple approaches but investigated only very few cases of reinfection. Our findings agree with their reports. Inoye *et al.* (1984) also examined and compared sera taken at various times after rotavirus infection and demonstrated changing avidity. Thus this approach may not only be of value in distinguishing primary rubella from reinfection but also is of potential value in the serological diagnosis of other infections.

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