

## **Rubella-specific IgM reactivity in sera from cases of infectious mononucleosis**

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### SUMMARY

Eight sera from 125 cases of infectious mononucleosis (IM) were reactive for rubella-specific IgM in an M-antibody capture radioimmunoassay. The reactivity of individual sera varied depending upon the source of the rubella antigen used in the assay. One serum gave strongly positive results with some rubella haem-agglutinating antigens but negative results with others and may have contained an IgM antibody which was capable of distinguishing between strains of rubella virus.

If the diagnosis of rubella is based solely on detection in solid-phase immunoassay of rubella-specific IgM, IM should be excluded.

### INTRODUCTION

Infectious mononucleosis (IM) is an infrequent consequence of primary infection with Epstein-Barr virus (EBV). This virus infects B lymphocytes and stimulates their proliferation. This proliferation is regulated by a T lymphocyte response to the infected B lymphocytes (Epstein & Achong, 1977). During the course of IM a number of IgM antibodies develop (Henle & Henle, 1979) which may be directed against antigens apparently unrelated to EBV. This response is the basis of heterophil-antibody tests used for diagnosis of IM such as the Paul Bunnell test or slide equivalents (e.g. Monospot<sup>®</sup>, Ortho Diagnostics Ltd., U.S.A.) which detect antibodies directed against sheep or horse erythrocytes. It has been suggested that these antibodies appear as a result of the EBV stimulation of B lymphocytes already committed to antibody production (Henle & Henle, 1979). Although it is well established that non-specific production of antibodies in IM may result in a positive Wasserman reaction (Carter, 1966), there is scant information about IgM antibody directed against viral antigens other than those associated with the Epstein-Barr virus.

The diagnosis of virus infections by the detection in serum of virus-specific IgM is now well accepted and has become particularly important in the diagnosis of primary rubella. In the UK the established diagnostic method for this has usually included serum fractionation and subsequent testing of IgM-rich serum fractions

for haemagglutination-inhibiting (HI) antibody (Pattison & Dane, 1975). These assays are labour intensive and alternative solid-phase immunoassays have been sought (Mortimer *et al.*, 1981). During the evaluation of a solid-phase IgM antibody capture radioimmunoassay (MACRIA) for rubella-specific IgM, the discovery of the serum from a patient with IM which reacted strongly in the rubella MACRIA prompted an investigation into the prevalence of rubella-specific IgM detected by MACRIA in patients with IM.

#### PATIENTS AND METHODS

##### *Patients*

The original patient (Patient A, Table 1) was a 34-year-old male admitted with a sore throat, lymphadenopathy, splenomegaly and jaundice. Lymphocytosis with atypical mononuclear cells, a positive test for heterophil antibody (Monospot®) and the detection of EBV-specific IgM confirmed the diagnosis of IM. Rheumatoid factor was not detected. Total serum IgM was 4 g/l (normal 0.4–2.0 g/l). A serum obtained 4 months later had a normal IgM level and was Monospot® negative.

Paul Bunnell or Monospot® positive sera from a further 124 patients submitted for tests for heterophil antibody on clinical grounds were examined for the presence of rubella-specific IgM.

##### *Methods*

Rubella-specific IgM was detected by MACRIA, the method being adapted from that described by Mortimer *et al.* (1981). Briefly, polystyrene beads coated with rabbit-anti- $\mu$  (Dako Ltd., Copenhagen, Denmark) were incubated in a 1:1000 dilution of serum. After washing, the beads were incubated in a dilution of rubella haemagglutinating antigen (HA) before washing again and adding  $^{125}\text{I}$ -labelled monoclonal anti-rubella antibody (Tedder, Yao & Anderson, 1982). After further incubation the beads were washed and bound radioactivity measured in a  $\gamma$ -counter. In each assay a series of control sera containing 40, 10, 3.3 and 1 arbitrary units of rubella-specific IgM antibody (Tedder, Yao & Anderson, 1982) were included and a standard calibration curve obtained. The reactivity of test sera was expressed in units of rubella-specific IgM by comparison with the calibration curve. This assay has previously proved to be specific for rubella when values above 3.3 units are obtained which usually corresponds to a test-negative ratio of about 6 to 1. Levels between 1 and 3.3 units occur with sera from approximately 3% of patients in whom there is no supporting evidence for recent rubella (unpublished data). There is no evidence that rheumatoid factor interferes with the assay (Mortimer *et al.* 1981).

The rubella HA routinely used is supplied by the Division of Microbiological Reagents and Quality Control, Public Health Laboratory Service (PHLS), Colindale, UK. Initial results obtained with serum A, (Table 1) led to the investigation of six other commercially available rubella HA antigens and a control antigen in the MACRIA. All the antigens are prepared in baby hamster kidney (BHK) cells and undergo Tween/ether treatment, some of tissue culture supernatant virus (snv) only, others of the cell-associated (cav) and supernatant virus. The strains of rubella virus used differ. The concentration of the rubella HA antigen used in

the MACRIA was adjusted so that equivalent binding of the  $^{125}\text{I}$  anti-rubella antibody was obtained with the 40 unit control-positive standard serum. The adjusted concentration bore no relation to the rubella HA titre when determined using day-old chick erythrocytes. In addition certain selected sera were tested by a commercially available indirect immunoassay (Rubazyme-M<sup>®</sup>, Abbott Ltd., UK) in accordance with the manufacturers' instructions.

Rubella-specific IgM-HI antibody was detected in IgM-rich fractions separated by gel filtration on Sephadex G200 or Sephacryl S300 using either PHLs or Flow Laboratories rubella antigen (Pattison & Dane, 1975; Morgan-Capner, Davies & Pattison, 1980). Immunofluorescence for rubella-specific IgM was performed on IgM-containing fractions, separated by sucrose density gradient centrifugation, using BHK cells infected with the Judith strain of rubella virus as substrate (Cradock-Watson, Bourne & Vandervelde, 1972) by Dr Cradock-Watson, PHLs, Manchester.

Evidence of prior infection with rubella was determined by radial haemolysis (RH), (Kurtz *et al.*, 1980).

Heterophil antibody was demonstrated by the Monospot<sup>®</sup> test and by the Paul Bunnell test. EBV-specific IgM was detected with standard fluorescence techniques by Dr Joan Edwards, Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London.

## RESULTS

Eight of the 125 heterophil antibody-positive sera evaluated using the PHLs rubella HA antigen in MACRIA contained rubella-specific IgM at a level equal or greater than 3.3 units. A further nine sera gave levels between 1 and 3.3 units. All sera contained detectable levels of EBV-specific IgM and rubella-specific IgG antibody by RH. Of the remaining 108 sera, 67 were tested by RH and 54 contained rubella-specific IgG.

The eight more strongly reactive sera were evaluated using six other rubella HA antigens and the uninfected cell culture control antigen (Table 1). Serum A was strongly reactive with four antigens, equivocal with one, and unreactive with two rubella HA antigens. The serum obtained four months later from this patient contained no detectable rubella-specific IgM and no heterophil antibody. The other seven sera also gave positive results and the level of reactivity varied with the rubella HA antigen used. Similar reactivities for all sera were obtained with the two rubella HA antigens prepared from the Gilchrist strain (Flow Laboratories and Microbiological Associates antigens). However, there was no apparent relationship between the results obtained and whether the rubella HA antigen was prepared from snv or snv/cav. No sera gave significant reactivity with the control antigen. Seven of the more highly reactive sera were also tested in an indirect enzyme immunoassay kit (Rubazyme-M<sup>®</sup>, phase 1). Six were reactive at diagnostic levels. In a subsequent experiment with modified kits (phase 2), which used an improved formulation of solid-phase antigen, four were still reactive at diagnostic levels.

Serum A was fractionated by gel filtration during the initial investigation and no rubella-specific IgM HI antibody was detected using Flow HA antigen for the haemagglutination inhibition tests. Unfortunately, the fractions were discarded

Table 1. Investigation of eight sera in the rubella MACRIA and in the indirect ELISA using different strains of virus

Sera	IgM-capture RIA (MACRIA)											Indirect ELISA	
	Judith* cav (PHLS)	Gilchrist snv (Flow)	Gilchrist cav & snv (MA) <sup>†</sup>	RA27/3 mod cav & snv (Behring)	RA27/3 cav (Orion)	Baylor cav & snv (Gibco)	Thomas cav & snv (Wellcome)	Control BHK antigen (Wellcome)	Phase 1	Phase 2			
A <sup>f</sup>	20**	< 1	< 1	> 40	> 40	2.2	34	< 1	+	+			
B <sup>f</sup>	3.3	> 40	> 40	18	9.0	31	27	< 1	+	+			
C <sup>f</sup>	6.0	3.3	5.8	7.7	5.2	3.0	2.2	< 1	-	-			
D	3.5	6.5	8.0	1.9	9.8	5.0	11.5	< 1	+	+			
E	3.8	7.8	7.8	6.4	8.4	8.1	9.5	< 1	+	-			
F	6.0	9.0	8.4	9.2	7.3	4.5	7.4	< 1	+	+			
G <sup>f</sup>	4.2	24	27	18	17	13	15	< 1	+	+			
H	4.1	3.9	4.6	4.8	10	4.5	8.6	< 1	NT	NT			

NT, Not tested.

f, Also tested for IgM HI antibodies by serum fractionation.

\* Strain of virus, source from tissue culture (cav = cell-associated virus, snv = supernatant fluid virus) and commercial supplier in parentheses.

† Microbiological Associates.

‡ Rubella-specific IgM in arbitrary units per ml.

and a lack of sufficient serum precluded re-fractionation and evaluation using the other rubella HA antigens; however, this serum contained rubella-specific IgM antibodies detected by immunofluorescence.

Three of the remaining seven sera were fractionated by gel filtration, and the IgM containing fractions assayed for HI antibody using Flow and PHLs HA antigens. In one serum (B) nearly 20% of the total rubella HAI activity (titre 320) was present in the IgM fractions when tested with both the PHLs and Flow HA antigens and this reactivity was sensitive to reduction by 2-mercaptoethanol. IgM-rich fractions from two other sera (C and G) did not contain rubella HI antibody of the IgM class when tested using either HA antigen.

#### DISCUSSION

Significant reactivity ( $\geq 3.3$  u/ml) in the MACRIA for rubella-specific IgM was detected in eight (6%) of 125 sera from patients being investigated for possible IM. Varying degrees of reactivity were observed in individual sera using different rubella HA antigens but the reactivities using the Judith strain of antigen were low ( $< 10$  units) except for the index case (serum A). In appropriately timed sera from cases of acute primary rubella (1–4 weeks after onset of clinical illness), the levels of reactivity are usually higher than seen in seven of the eight cases of IM and such levels are more appropriate to sera collected some weeks or months after the illness. Therefore, low levels of rubella-specific IgM reactivity in sera collected a short time after the onset of the illness (say 1–4 weeks) should be treated with suspicion. All sera contained heterophil antibody and EBV-specific IgM. Although it is possible that some of these patients had had rubella during the preceding months, the frequency of detection of rubella-specific IgM suggests that this could not be the explanation in all cases. For instance, Urquhart & Carson (1982) state that they could only detect one case of recent rubella for every 10000 antenatal sera received if sera with HI titres  $\geq 256$  were examined for the presence of rubella-specific IgM. In addition to the eight sera giving significant reactivity, nine sera (7%) reacted at levels of doubtful significance (1–3.3 u/ml). Again, this rate is higher than the rate of 3% seen with random diagnostic sera (unpublished observations).

As the symptomatology of IM and rubella may be similar (rash, lymphadenopathy, sore throat) these findings must be taken into account when a diagnosis of rubella is being made based on the detection of rubella-specific IgM by solid-phase immunoassay. Detection of such antibody is of critical importance during the early months of pregnancy when termination may be advised if recent primary rubella is diagnosed. We would therefore recommend that screening tests for heterophil antibody are performed on all sera showing reactivity in solid-phase immunoassay unless there is confirmatory evidence of recent rubella. Unless a seroconversion has been demonstrated it would seem prudent at present to confirm the diagnosis of acute rubella during pregnancy by demonstrating the presence of anti-rubella IgM HAI antibody until such time as the true prevalence of false reactivity in MACRIA tests is known.

All 17 MACRIA-reactive patients had had a previous rubella infection as their sera contained rubella antibody detectable by radial haemolysis. This finding is

compatible with the suggestion that production of various IgM antibodies in IM is a result of EBV induced stimulation of B lymphocytes already committed by prior antigenic stimulation. In this case anti-rubella IgM antibodies might be produced in response to EBV infection. Alternatively it is possible that the IgM antibodies detected were directed against cellular antigens which could have been expressed as a result of virus infection in culture and which are tightly incorporated into the lipoprotein envelope of the rubella virus (Rott *et al.*, 1966). The interaction in any solid-phase immunoassay between these antigens and the IgM antibodies would be indistinguishable from the reaction between specific anti-rubella IgM and rubella HA antigen.

The results obtained with serum A are of interest because of the differences seen with the various rubella HA antigens. The only apparent important difference between the antigens is the strain of rubella virus used. Serum A gave a positive result with antigen preparations using the Judith strain of rubella (SDG/immuno-fluorescence and MACRIA with PHL5 rubella HA antigen), RA 27/3 derived antigen (MACRIA with Behring and Orion rubella HA antigens) but a negative result with the Gilchrist strain (MACRIA with Flow and Microbiological Associates rubella HA antigens and fractionation using Flow HA antigen for HI tests). These results support the hypothesis that the IgM is directed against a virus specified antigen. The explanation would be that the reactive IgM produced by patient A is selecting an antigen present in some strains of rubella virus but not present in other strains. However, it does not exclude that such an antigen may be a cellular antigen incorporated into the virus lipoprotein envelope (Rott *et al.* 1966) since although all the rubella HA antigens are produced in BHK cells, it is possible that, after multiple passage in various laboratories, lines of BHK cells are not antigenically identical. Additionally, adapted tissue culture strains might incorporate host antigens preferentially. IgM HI antibody was only detected in one of the three sera examined with a range of HA antigens. Had the MACRIA reactivity in all of these sera been due to HA specific antibody it should have been detected in the IgM fractions following gel filtration.

We would suggest that when tests for microbial-specific IgM are being evaluated numbers of sera from cases of IM are tested to estimate the frequency with which epidemiologically unrelated positive results occur. If the IgM reactivity is directed solely against cellular antigens, positive results should be obtained only with preparations that contain such antigens; alternatively if positive reactions are related to microbial antigens we would predict that their prevalence would be proportional to the prevalence of prior infection with the agent. Irrespective of the mechanisms, the significance of reactivity in any solid-phase immunoassay for virus specific IgM of sera from cases of acute EBV infection will need careful interpretation.

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