DETECTION OF RUBELLA VIRUS SPECIFIC IgM BY FAST PROTEIN LIQUID CHROMATOGRAPHY AND ENZYME-LINKED IMMUNOSORBENT ASSAY

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SUMMARY. A sensitive and rapid method for the detection of rubella specific IgM by fast protein liquid chromatography and enzyme-linked immunosorbent assay is described.

INTRODUCTION

The differentiation of recent from remote infection is important in the diagnosis of rubella in pregnancy (Rawls, Desmyter and Melnick, 1968; Sever and White, 1968). The demonstration of specific antibodies of the IgM class is often the only proof of recent infection. Current methods of rubella IgM detection usually involve physical separation of the immunoglobulin classes and subsequent testing by haemagglutination inhibition (Best, Banatvala and Watson, 1969; Pattison and Dane, 1975). These methods are time consuming and labour intensive and vary in their sensitivity and specificity. In this report we describe a new approach to detection of rubella-specific IgM antibody which combines a fast method of serum fractionation with the sensitivity, specificity and safety of an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Cells, virus strains and purification methods, culture media and buffers were those previously described by Ho-Terry and Cohen (1980).

Enzyme-linked immunosorbent assay (ELISA) was as described by Vaheri and Salonen (1980).

Fast protein liquid chromatography (FPLC). Serum fractionation by FPLC was as described in Separation News (1.1982), Pharmacia Fine Chemicals. For elution, two buffers were used; these were (1) Buffer A (phosphate buffer 0.025M, pH 6.7) and (2) Buffer B (phosphate buffer 0.3M, pH 6.5). The shape of the eluting gradient was described by the percentage of buffer B used. Elution was first performed with 12 ml of buffer A (0% buffer B) for the collection of the early samples, followed by 10 ml of equal volumes of buffer A and buffer B (50% buffer B) for the collection of further samples, and then 8 ml of buffer B (100% buffer B) for the collection of the remaining samples as shown on the upper border of fig. 1.

RESULTS

Fractionation of IgM. Immunoglobulins of the IgM class from paired serum samples obtained from two patients with rubella were fractionated by FPLC.

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FIG. 1.—Fractionation of immunoglobulins IgM, IgA and IgG from a pooled human serum sample by the MonoQ and FPLC system. The shape of the eluting gradient is indicated by the percentage of buffer B used.

Untreated serum (50 μ l), diluted 1 in 4 in buffer A was filtered through a 0.22- μ m membrane before loading on to a MonoQ column (Pharmacia, Sweden). The chromatogram obtained (fig. 1) shows the separation of serum proteins into four peaks of which peaks I, II and III contained immunoglobulins (table). The distribution pattern of immunoglobulins in each peak indicates that only peak III contained IgM, although this was contaminated with IgA and IgG (table).

TABLE
Distribution of serum immunoglobulins IgM, IgA and IgG in peaks I, II and III

Peak	Percentage of total absorbance* due to		
	IgM	IgA	IgG
I	0	5	91
II	0	65	5
III	100	30	4

O.D. associated with specific class of immunoglobulin in one peak
* Percentage of total absorbance = $\frac{\text{O.D. associated with the same class of immunoglobulin in all peaks}}{\text{O.D. associated with specific class of immunoglobulin in one peak}} \times 100\%$
O.D. associated with the same class of immunoglobulin in all peaks

Results represent the average of four experiments with a direct ELISA assay.

Detection of rubella-specific IgM. Samples (100 μ l) of each fraction collected from the MonoQ column were diluted 1 in 10 in phosphate-buffered saline (pH 7.4) containing Tween 20 0.05% and tested for rubella antibody by the indirect ELISA technique. The samples were incubated for 2 h at 4°C in rubella virus or control (BHK cells) antigen-coated wells in polyvinyl microplates to allow antigen-antibody reactions to occur; anti-human IgM conjugated with alkaline phosphatase was then added and incubation continued for a further 2 h at 4°C. Substrate was added and absorbance at 410 nm was read the next day. The results illustrated in fig. 2 show the presence of rubella-specific IgM in the four serum samples from two patients with rubella. In one case the IgM increased between the fifth and fifteenth days after the

FIG. 2.—Demonstration of virus specificity of fractionated IgM by indirect ELISA; — against purified rubella virus antigen, - - - - - against control (BHK) antigen. All absorbance values represent the results of duplicate experiments and were calculated as (absorbance values of test serum — absorbance value of a control negative serum). Serum samples were from patient A, 5 days (c) and 15 days (d) after the rash and from patient B, 20 days (a) and 10 days (b) after the rash.

rash whereas in the other it decreased between the tenth and twentieth days after the rash.

DISCUSSION

Physical methods of immunoglobulin separation with subsequent testing of the fractions obtained for haemagglutination inhibition for detection of rubella IgM are well established and reliable (Best *et al.*, 1969; Pattison and Dane, 1975). However, the methods have several disadvantages; they are time consuming, the haemagglutination inhibition test is less sensitive than other methods for the detection of rubella antibody, and 2-mercaptoethanol treatment is required to distinguish rubella IgM antibody from non-specific inhibitors, non-specific agglutinins and other contaminating specific immunoglobulins (Best *et al.*, 1969; Al-Nakib, Best and Banatvala, 1974). The advantages of the method described here are that separation may be achieved in 20 min, that only 50 μ l of serum are required and that the ELISA test is very sensitive. Evidence of the presence of rubella-specific IgM can, therefore, be obtained easily in one day. The small volume needed may be of particular advantage in the investigation of sera from infants with congenital rubella.

The use of the ELISA technique to detect rubella IgM antibodies in unfractionated serum is not entirely satisfactory because false positive results due to rheumatoid factor reacting with bound virus specific IgG remain a serious problem (Vejtorp, 1980). Moreover, the sensitivity for IgM is also reduced by competition of specific IgG and IgA for antigen on the solid phase. The preliminary separation of IgM as described here increases the sensitivity and specificity of the ELISA test not only by reducing any interference from rubella IgG and IgA to a minimum but also by allowing binding of rubella specific IgM without IgG to purified virus antigen on the solid phase, thus eliminating false positive results due to rheumatoid factor.

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