Immunoglobulin Responses in Rubella and its Complications

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Summary: Normal responses of rubella-specific IgG and IgM antibody were assessed in eight patients by immunofluorescence. A prolonged rubella-specific IgM response was shown in three patients with complications of rubella infection. Two patients had thrombocytopenic purpura and one had carpal-tunnel compression.

Introduction

The detection of rubella-specific IgG and IgM antibodies by immunofluorescence has been recommended before (Baublis and Brown, 1968; Cohen *et al.*, 1968). In developing the method described below we felt it necessary to make accurate estimations of these antibodies in frequent sera at similar intervals after acquired infection. The range of antibody responses was assessed in serial specimens of sera obtained from eight patients following uncomplicated rubella during an epidemic in Belfast in the spring of 1969.

In the same epidemic two patients presented with thrombocytopenic purpura and a third patient had signs and symptoms of carpal-tunnel compression. Previous observations indicate that immunological mechanisms may be involved in the pathogenesis of complications of rubella (Lee *et al.*, 1960; Myllylä *et al.*, 1969); therefore the titres of virus-specific immunoglobulin fractions of sera from these three patients were compared with those from the patients with uncomplicated infection. The clinical findings will be reported in more detail later.

Methods

Uncomplicated Rubella.—Three sera from each patient were obtained during the first week; further specimens were obtained at the end of the second, third, and seventh weeks, and another about three months after the appearance of the rash. A throat swab for virus isolation was taken from each patient at the first visit. The age of the eight patients ranged from 18 to 38 years. Sera were also obtained from a further 25 patients with uncomplicated rubella at varying times, all more than eight weeks after onset.

Complicated Rubella.—Several sera were obtained from two patients who were admitted to hospital because of severe thrombocytopenic purpura, suspected as being caused by rubella infection. Three samples of sera were available from a patient with signs and symptoms of carpal-tunnel compression which began at the same time as a rubelliform rash. A throat swab for virus isolation was taken from each patient when the first serum was obtained.

All sera were stored at −20°C. and each different antibody test was done on all samples from one patient at the same time.

Conventional Diagnostic Tests.—Virus was isolated from throat swabs on RK13 cells and identified by immunofluorescence (Schmidt *et al.*, 1966; Gispen and Brand-Saathof, 1967). All sera were tested for antihaemagglutinin (H.A.I.), rubella haemagglutinin (Flow Laboratories Ltd.) being used.

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Titration of Rubella-Specific IgG and IgM Antibody

(1) *Antigen.*—BHK21 cells growing on coverslips were infected with the Judith strain of rubella virus. Three days later these were fixed in acetone for 10 minutes at room temperature, air-dried for 30 minutes, and stored in a sealed container with silica gel at −20° C. until required.

(2) *Immunoglobulins.*—Sheep anti-human IgG and anti-human IgM obtained from Burroughs Wellcome Laboratories were tested for purity and specificity by immunoelectrophoresis. The same batch of each antiglobulin was used throughout after being conjugated with fluorescein isothiocyanate (Sigma Chemical Company), Rinderknecht's (1962) method being used.

(3) *Adsorption of Sera and Conjugates.*—Patients' sera were adsorbed overnight at +4°C. with mouse liver powder; the conjugates were adsorbed with BHK21 cells.

(4) *Staining.*—Optimum staining titres were determined and used in the indirect test at 35°C. Staining by IgG antibody showed large cytoplasmic granules and peripheral staining of the cells as described by Cohen *et al.* (1968) (Fig. 1). Staining by rubella-specific IgM antibody showed more diffuse granular cytoplasmic staining (Fig. 2).

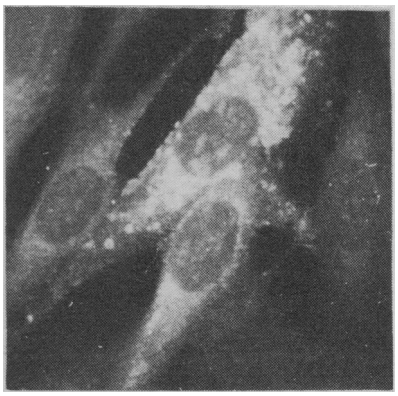

FIG. 1.—Rubella virus antigen in BHK cells stained by the indirect method; patient's serum and anti-human IgG antibody conjugated with fluorescein isothiocyanate were used. Three cells, two infected and one uninfected, are seen. (× 1,200.)

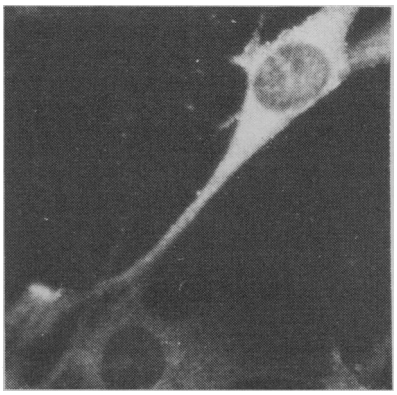

FIG. 2.—Rubella virus antigen in BHK cells stained by the indirect method; patient's serum and anti-human IgM antibody conjugated with fluorescein isothiocyanate were used. Two cells, one infected and one uninfected, are seen. (× 1,200.)

(5) *Titration.*—Doubling dilutions of adsorbed patients' sera were applied separately for one hour to coverslip preparations of infected cells. The coverslips were thoroughly washed and the respective conjugated antiglobulin was applied for one hour at 35°C. Each coverslip was again washed and dipped for 10 seconds in 1 in 150,000 Evans blue, rinsed in distilled water, and mounted in glycerol-saline, *p*H 8·3. Antibody titre was expressed as the highest dilution of the patient's serum giving definite positive staining of rubella antigen. Control titrations of a standard serum were included in each batch of tests.

Results

The geometric mean titres of H.A.I., rubella-specific IgG, and rubella-specific IgM antibodies in serial sera in eight patients with uncomplicated rubella are shown in Fig. 3. This series has been selected for testing because all but the last specimens, which were taken between three and six months after convalescence, were obtained from the patients at similar time intervals. The sustained levels of both H.A.I. and IgG antibodies are noted, but the IgM response is temporary and the titre is reduced to less than 1 in 5 in seven weeks. Rubella virus was isolated from the eight patients.

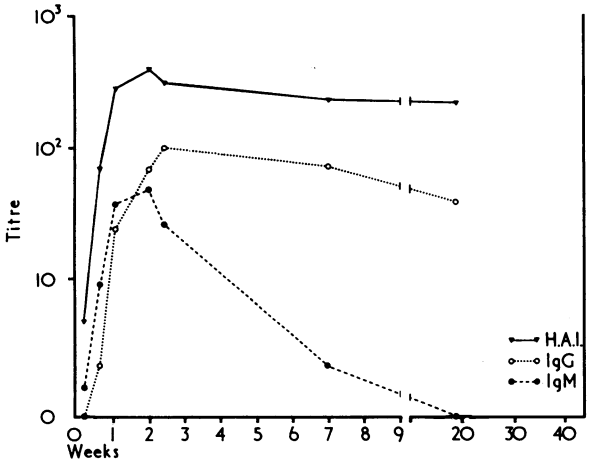

FIG. 3.—Geometric mean titres of rubella H.A.I., rubella-specific IgG, and rubella-specific IgM antibodies in serial sera of eight patients with uncomplicated rubella. Beginning of week 1 = first day of rash.

Similar titrations of convalescent sera from a girl and a boy, both aged 14 years, who were admitted to hospital because of severe thrombocytopenic purpura are shown in Figs. 4 and 5. In both patients a significant rise in titre of rubella-specific IgG between the initial and later specimens was of value in diagnosis. The H.A.I. titre, already raised in the first specimen, was not of diagnostic value. The IgM response was prolonged in both patients, and the customary fall found in cases of uncomplicated infection did not take place during the periods of testing, 38 weeks (Fig. 4) and 36 weeks (Fig. 5) respectively. Rubella virus was isolated from both patients. In each case the platelet counts were about 15,000/cu.mm. when illness was most severe, but in six weeks had reached 100,000/cu.mm.

Three widely spaced sera from a patient with signs and symptoms of carpal-tunnel compression were tested in a similar manner (Fig. 6). Significant rises of titres in all antibodies were present, but the interesting finding, as in the cases with purpura, is the rise in rubella-specific IgM antibody six months after onset; some symptoms were still present at this time. Rubella virus was not isolated from this patient. The sera of 25 other patients, whose infection was confirmed by serological response, taken at varying times from 8 to 37 weeks after the onset did not contain rubella-specific IgM, either at a 1 in 5 or at a 1 in 10 dilution.

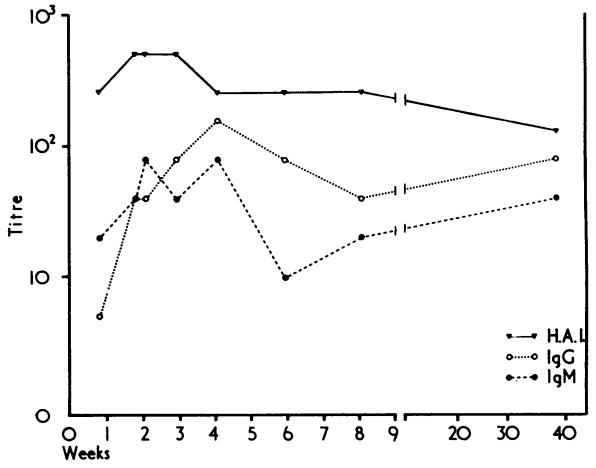

FIG. 4.—Rubella H.A.I., rubella-specific IgG, and rubella-specific IgM antibody response in a girl aged 14 years with severe thrombocytopenic purpura following rubella.

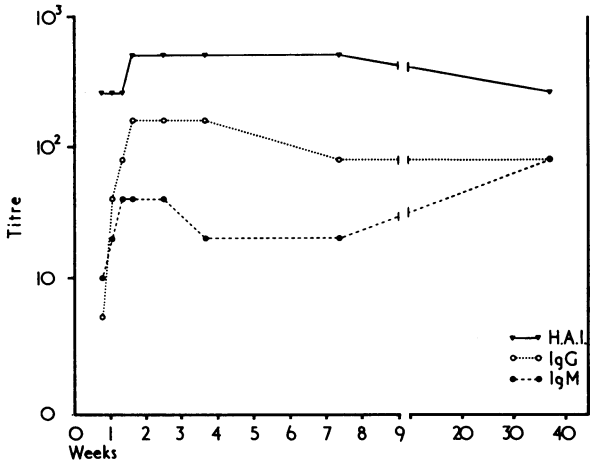

FIG. 5.—Rubella H.A.I., rubella-specific IgG, and rubella-specific IgM antibody response in a boy aged 14 years with severe thrombocytopenic purpura following rubella.

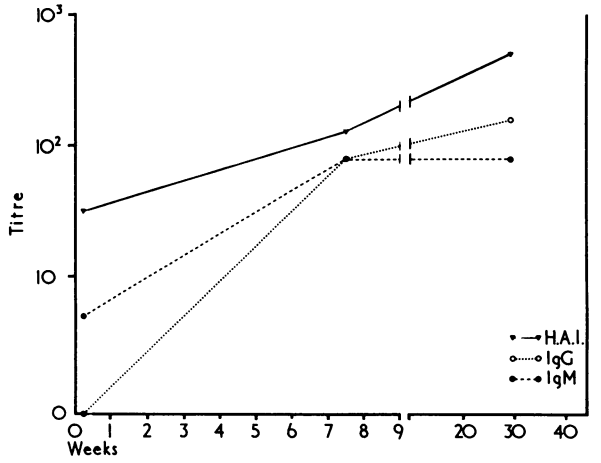

FIG. 6.—Rubella H.A.I., rubella-specific IgG, and rubella-specific IgM antibody response in a woman aged 54 who had carpal-tunnel compression following rubella infection.

Discussion

At least 100 cases of thrombocytopenic purpura following rubella have been reported (Pitten, 1929; Ackroyd, 1949; Steen and Torp, 1956; Tadžer, 1958; Cohen *et al.*, 1960; Ferguson, 1960; Sladden, 1963; S. J. Wallace, 1963; Plotkin, 1964; D. C. Wallace, 1964; Adkins and Fernbach, 1965; Bayer *et al.*, 1965; Lokietz and Reynolds, 1965; Svenningsen, 1965; Morse *et al.*, 1966; Sander, 1966; Staub, 1968; Volpato *et al.*, 1969), though laboratory confirmation of diagnosis is available in only four instances (Plotkin, 1964; Volpato *et al.*, 1969). The finding by Myllylä *et al.* (1969) of higher levels of platelet aggregating antibody in seven patients with this abnormality compared with a control group of patients with uncomplicated rubella suggests that this may wholly or partly result from an immunological mechanism. Most of the information regarding the carpal-tunnel syndrome complicating rubella is from clinical notes and the pathogenesis is not discussed (Bailey, 1962; Courtenay, 1962; Heathfield, 1962; Brodribb, 1963; Chambers and Bywaters, 1963). The work of Lee *et al.* (1960), though inconclusive, suggests that in rubella arthritis there is an alteration of the euglobulin fraction of serum in some cases.

By showing a prolonged rubella-specific IgM response in three cases of rubella with complications our results suggest that persistence of infection or alteration of the immunological response may be factors in pathogenesis. The virus infection may have continued or, if absent, may at some stage have extrinsically stimulated the antibody-forming cells to produce this particular response. When infection occurs early in pregnancy the virus has the predilection for differentiating immunocompetent cells in the embryo; our findings may be evidence that a similar differentiation may occur in some cases in the mature individual with resulting clinical complications.

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Measurement of Recovery from Outpatient General Anaesthesia with a Simple Ocular Test*

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Summary: Measurements of extraocular muscle balance with a Maddox wing can be a useful clinical test of the rate of recovery from general anaesthesia. In 65 dental outpatients recovery was found to be most rapid in those patients given only nitrous oxide, oxygen, and halothane, whereas the previous administration of methohexitone, propanidid, or thiopentone for induction was associated with slower recovery. Recovery rates after methohexitone and propanidid were similar and rapid enough to confirm their choice for intravenous induction of anaesthesia in outpatients, but delayed recovery after thiopentone showed that this agent is best avoided in these circumstances.

Introduction

When new plans for the deployment of medical resources in the community come into effect probably more patients will receive general anaesthesia for diagnostic and minor surgical procedures as outpatients, and in this area one of the new "best-buy" hospitals is being built which will include a special department for day surgery (Harrington and Goodman, 1969).

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