Abnormal responses to rubella infection

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SYNOPSIS Two cases of rubella are described which caused initial problems in laboratory diagnosis due to abnormal features in the immune response. One patient presented with thrombocytopenic purpura and associated circulating immune complexes. The other patient, who was in early pregnancy, had an unusually prolonged rash and a delayed humoral immune response. The possible reasons for the difficulties in serological confirmation are discussed.

The usual features of clinical postnatal rubella are a rash, lymphadenopathy, suffusion of the eyes, sore throat, a rise in temperature rarely exceeding 38°C, and slight malaise. Arthralgia and arthritis occasionally occur, particularly in the adult. The rash rarely lasts for more than four days (Christie, 1974). A rare but well established complication of post-natal rubella is thrombocytopenic purpura (Ackroyd, 1949; Bayer *et al.*, 1965), and this has also been described after rubella vaccination (Bartos, 1972).

The patterns of immune response to rubella infection have been well documented (Vesikari, 1972). Haemagglutination-inhibiting (HI) antibodies normally rise rapidly after the onset of the rash and reach a maximum within a week. This early HI antibody is mainly IgM, and its presence is taken to indicate that infection has recently occurred.

With the realization that a clinical diagnosis alone is unreliable, the laboratory diagnosis of rubella has become of the utmost importance, particularly in the investigation of possible infection during pregnancy. Laboratory diagnosis is normally reliable if samples are obtained soon after infection. Because most laboratories rely mainly on serological investigations in rubella diagnosis, and occasionally recommend termination of pregnancy on the strength of these results, it seems important to report instances of abnormal responses which may give rise to confusion in diagnosis.

Patient 1

A 29-year-old Malaysian man appeared in the casualty department on 6 May 1974 with persistent epistaxis, gingival haemorrhage, and purpura. These

features had been present for three days and he was found to have macroscopic haematuria. Further questioning revealed that he had been quite well until seven days before admission when he developed a generalized rash. Associated with this were myalgia, pharyngitis, and conjunctival suffusion. At that time his general practitioner diagnosed acute rubella.

A platelet count on admission showed less than 10×10^9 platelets/litre, and a diagnosis of thrombocytopenic purpura, probably due to rubella, was made. The patient was started on corticosteroid drugs, and the purpura and haematuria gradually resolved in association with a gradual rise in platelet numbers (fig 1). The platelet count had returned to normal in three months and he has remained well since discharge from hospital.

Serum which had been treated with heparin and

Fig 1 Patient 1. Platelet counts after rubella infection. The times of admission to hospital and testing for rubella haemagglutination-inhibiting antibodies (HI) are indicated.

manganese chloride to remove non-specific inhibitors (Cooper *et al*, 1969) was tested for HI antibody (Stewart *et al*, 1967) on three occasions at intervals of 10, 16, and 69 days after the rubella-like illness. The first serum (10 days) was removed directly from the clotted blood and was tested before freezing. This gave an antibody titre of 1/32. However, when the second sample (16 days) arrived, this was tested in parallel with the first sample, and results of >1/4096 were obtained from each specimen. As a result of this discrepancy between the two results on the first serum, some of the original unfrozen serum was retrieved, and it was clearly demonstrable that by one cycle of freezing and thawing the antibody level was increased from 1/32 to >1/4096. This phenomenon did not occur with the second serum sample or with the late convalescent sample (69 days) which had an antibody titre of 1/128 using either unfrozen or frozen serum. Rubella-specific IgM was present in the first two sera and absent in the third, confirming the diagnosis of recent rubella leading to thrombocytopenic purpura.

Serum samples collected 9, 11, 33, and 89 days after the onset of rubella were stored at -70°C until studied for the presence of circulating immune complexes both by the anticomplementary technique and also the solubilization of C₁q by complexes (Johnson *et al*, 1975). Sera at 9 and 11 days were positive by both methods, and the anticomplementary complexes in the second of these were removable

by ultracentrifugation of the whole serum at 100 000 *g* for 30 min. From this same serum, the pseudoglobulin preparation which contained C₁q was adjusted to pH 5 and the acid euglobulin precipitate contained C₁q and immunoglobulin. The pellet was dissolved in 0.1 M phosphate buffer pH 7.2 and examined by negative staining on a carbon-coated grid in a Phillips 300 electron microscope. This revealed particles complexed mainly with IgM-like protein molecules (fig 2). No circulating complexes were detected in the sera collected at 33 and 89 days.

No evidence of a reduction in total haemolytic complement or C₃ was found in any of the samples, and immunoglobulin levels tested at 10 days after rubella were normal. There was virtually no transformation of peripheral blood lymphocytes to phytohaemagglutinin at 12 days after rubella. A number of authors have described this phenomenon in association with rubella infection (McMorrow *et al*, 1974). To investigate the possibility that rubella antibody levels may change after freezing, 30 randomly chosen sera were subjected to one cycle of freezing and thawing. The sera which had been frozen were tested in parallel with the unfrozen sera, and HI antibody levels were found to be identical in each group.

Patient 2

A 29-year-old woman Caucasian patient presented at

Fig 2 Patient 1. Negatively stained preparation of the acid euglobulin precipitate from serum taken 11 days after rubella. This shows particles which are presumably aggregates of rubella antigen, complexed with IgM-like protein molecules. ($\times 122\,000$)

12 weeks of pregnancy on 7 March 1975 with a rubella-like rash which had been present for 10 days. The rash was accompanied by a persistent sore throat, cervical lymphadenopathy, arthralgia, and an intermittent low-grade fever which had never exceeded 38.4°C. The rash finally disappeared 17 days after onset, although the lymphadenopathy persisted for a further two weeks. The arthralgia involved mainly the finger and wrist joints and was present for the first five days of the rash. The rash was seen on several occasions by two medical practitioners, and it was described as rubella-like during the entire 17-day period. The patient had received no drugs for several months before this illness.

A blood sample collected 10 days after the onset of the rash was tested for HI antibodies and a titre of 1/16 was obtained. Seven days later a further sample was tested and the result was 1/2048. The second sample was found to contain rubella-specific IgM (27% of the HI antibody). There was insufficient antibody in the first sample to check for specific IgM using sucrose density gradient or Sephadex column separation. Therefore a serological diagnosis would not have been made if a single sample had been studied within approximately two weeks of the onset of the rash.

As a result of the finding of rising titre and the presence of rubella specific IgM, the pregnancy was terminated at 13 weeks. Follow-up specimens taken 46 and 199 days after the onset of illness showed a return to a low level of antibody (1/32 and 1/16 respectively).

The first three samples (10, 17, and 46 days after onset) were examined for the presence of immune complexes; none was detected and the total haemolytic complement, C₁, C₄, and C₃ levels were all within the normal range.

Immunoglobulin estimations were performed on the third sample (46 days) and were within the normal range. Phytohaemagglutinin transformation of the peripheral lymphocytes was normal 199 days after the onset of the illness, as were the standard haematological values.

In view of the atypical clinical picture in this case the possibility of glandular fever was considered. No atypical mononuclear cells were seen on blood films, and the Paul Bunnell test was negative on two occasions.

Discussion

In both patients, despite a correct clinical diagnosis at presentation, initially a serological diagnosis of recent infection was not made. Although this was not significant in the management of either patient,

it could be of importance with other patients at similar phases of the illness.

In patient 1 it appears that the majority of the antibody was complexed to antigen, *vide* fig 2, and that this complex, despite treatment with heparin and manganese chloride, was dissociated only after freezing. Freezing and thawing is known to be a potent method for the denaturation of lipoproteins (Rosenberg, 1964), and the damage to the lipoprotein antigen may have resulted in the release of free antibody which was then detectable in the HI antibody test. Normally the sera would have been frozen and thawed before testing so that this situation would not arise. Occasions may arise, however, particularly when a result is required urgently, when the serum is used as soon as the blood has clotted and without a temporary storage in a frozen state. No difference was found between frozen and unfrozen sera in 30 random samples tested, but these all contained IgG resulting from past infections, and it seems likely that this phenomenon is related to the period immediately after illness. It is probable that patients with thrombocytopenia and detectable immune complex formation are most likely to show masking of antibody response in this way, but as some depression of platelet numbers frequently follows postnatal rubella (Vesikari, 1972), it may occasionally be present after uncomplicated rubella.

The second patient presented a different situation in that no complexes were detected and no effect of freezing was found. It appears that she was very slow in developing immunity manifest as IgM antibody. The clinical picture is also compatible with a delay in elimination of the virus. After recovery, no general defect in immunity was demonstrable, although not all aspects of the immune response were tested, and it is also possible that there is a specific defect to this particular virus. It has been suggested that the rash of rubella may be immune-complex in type (Heggie, 1971), but we have failed in this patient to find C₁ binding immune complexes, although some HI antibody was present in all three samples tested. The method for detecting anti-complementarity is, however, relatively insensitive for IgM complexes which might have been present early in the illness. It is possible, although it seems unlikely, that the first result of 1/16 was falsely low due to the formation of a more stable IgM complex than in patient 1.

Thus, although the causation of the rash in this patient is still uncertain, it was sufficiently typical of rubella to allow a clinical diagnosis to be made during a prolonged period when serological confirmation was not available.

Obviously the association of circulating immune complexes with a poor antibody response is open to

a causal interpretation. There is considerable evidence that immunity, particularly but not exclusively of the cellular type, may be inhibited by complexes (MacLennan, 1972). The reaction of platelets with immune complexes has been shown to be due to IgG complexes rather than IgM, with which platelets reacted poorly (Pariyananda and Mowbray, 1971). However, in the two patients discussed here, the apparently impaired response to the infection occurred in the patient without detectable complexes, and the thrombocytopenia occurred in a patient in whom the free antibody in the serum appeared to be exclusively IgM. These findings do not deny the assumption that IgG complexes may have been specifically immunosuppressive, or may have produced thrombocytopenia; they merely emphasize that very minor components of the overall antibody or complex that are not normally measured may be responsible for some of the phenomena.

We thank Dr Helen Dodsworth and Dr B. Kustow for their help in investigating these patients, and the clinicians at St. Mary's Hospital for allowing us to study them. We are grateful to Dr D. S. Dane, of the Middlesex Hospital, for supplying one serum and confirming the results on the second patient, and to Mr C. Kenny for technical assistance. Some of this work was supported by grants from the Medical Research Council.

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