Subclass distribution of IgG and IgA responses to rubella virus in man

ANN STOKES, C. A. MIMS and R. GRAHAME*

*Departments of Microbiology and *Rheumatology, Guy's Hospital Medical School, London SE1 9RT*

Summary. Monoclonal anti-subclass antibodies were used in a micro-ELISA method to determine rubella-specific IgG subclass antibodies in serum from 22 subjects who had acute rubella or had been vaccinated, from 10 infants with congenital rubella, and in serum and synovial fluid samples from 21 patients with chronic arthritis. In nearly all samples IgG₁ was the only type of IgG antibody detected. In acute infections it was present within 10 days of the onset of the rash. IgG₄ antibody was detected in sera from two immune individuals. Rubella-specific IgA₁ subclass antibody was detected by the same technique in sera from 6 of 12 subjects with acute rubella as early as 3 days but not later than 28 days after the appearance of the rash.

Introduction

Studies of IgG₁₋₄ subclass antibody responses in viral infections of man and in other conditions have been encouraged by the availability of monoclonal antibodies to these subclasses. IgG₁ antibodies comprise 60–70% of the total IgG in adults. Antibodies active against polysaccharide antigens—mainly IgG₂ (30% of total IgG)—rise slowly with age (Schur *et al.*, 1979) and IgG₂ deficiencies have been linked with susceptibility to infection by bacteria with polysaccharide capsules (Oxelius, 1984). Often, however, there is a co-existing deficiency in IgG₄ (Heiner, 1984), a subclass comprising 4% of the total IgG that does not fix complement but that has been associated with allergic conditions. There have been a few studies of the subclass distribution of antibodies to viruses, including that of Sundqvist *et al.* (1984) on herpes simplex and varicella-zoster and three studies on rubella. Skvaril and Schilt (1984) used monoclonal antibodies to show that antibodies in immune individuals were almost exclusively IgG₁. Both Doerr *et al.* (1984) and Beck (1981), however, using either polyclonal antibodies or density gradient separation for identification of IgG subclasses, had reported that rubella-specific IgG₃ (normally 6% of the total IgG) was present in both acute- and immune-phase sera. Rubella-specific IgA responses have been described (Al Nakib *et al.*, 1975; Halonen *et al.*, 1979) but their subclass distribution has not been reported. We therefore examined rubella antibodies in sera from immune subjects and from recently infected patients.

Materials and Methods

Sera

The following rubella antibody-positive sera were studied: (1) single samples from 21 adult immune patients with rheumatoid or seronegative arthritis (Mims *et al.*, 1985); (2) successive samples taken 7 weeks and later after rubella vaccination (RA27 or Cendehill) from 10 individuals with joint complications; (3) single samples from six naturally infected individuals taken 1–10 days after the appearance of the rash; (4) successive samples from six naturally infected individuals taken 5 days–2 years after infection; (5) cord-blood sera from two and non-cord sera from eight congenitally infected infants. Serum series 2–5 were very kindly provided by Dr Jennifer Best, St Thomas's Hospital, London. Synovial fluid samples from the 21 patients with arthritis were also tested.

Antibody titrations

Rubella specific IgG subclass antibodies. A micro-ELISA system was employed. Dynatech M12GA micro-ELISA plates coated with rubella HAI antigen diluted 100-fold (produced from infected BHK21 cells; Wellcome Laboratories, Beckenham, Kent) were allowed to react with test fluids diluted 100-fold, and then with monoclonal antibodies to human IgG subclasses 1, 2, 3 and 4 (Seward Laboratory, Bedford; clone nos. NL16, GOM1, ZG4 and RJ4) diluted 1 in 1600 (anti IgG₁), 400 (anti IgG₂ and anti IgG₄) and 200 (anti IgG₃). Finally, horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts) diluted 1 in 800, was added, followed by the substrate *o*-phenylene diamine (BDH). After incubation in the dark for 30 min the reaction was terminated by adding 4N H₂SO₄ and the OD read at 492 nm. The OD reading obtained with plates coated with negative antigen control (Wellcome) was then subtracted from the test reading to give the specific OD value. The optimal

dilutions of antigen, test fluids, monoclonal antibodies and conjugates were determined after preliminary check-board titrations. Throughout the procedure, PBS Tween (0.05%) was used as a diluent and washing agent. The ELISA method was also used to obtain an end point titration of antibodies to rubella by determining the final serum dilution that gave an OD reading at least 0.2 units higher than the control value.

Rubella-specific IgA subclass antibodies. The same method was used with monoclonal antibodies to human IgA₁ and IgA₂ (Seward Laboratory; clone nos NIF2 and 2E2) diluted 1 in 400 and 1600 respectively. In these tests the antigen-coated wells were treated with 1% bovine serum albumen (BSA) for 2 h at 37°C, and 1% BSA was added to the conjugate.

Haemagglutination inhibiting (HAI) and single radial haemolysis (SRH) antibody determinations. Rubella haemagglutinating antigen (HA) was obtained from Wellcome Laboratories and HAI antibody titrations were performed in V-well microtitration plates (Sterilin, Teddington, Middlesex). For SRH, "Rubascreen" SRH plates (Northumbria Biologicals, Cramlington, Northumberland) were used; the diameters of the haemolytic zones were recorded and the zone areas calculated. The areas plotted against log₂ HAI antibody titres for all sera gave a straight line relationship.

Results

In all patients' sera, IgG₁ antibody was the major subclass detected and it was generally present in proportion to the rubella HAI antibody titre. A linear relationship was shown between the log₁₀ specific absorbance and the log₂ of the HAI titre of sera from the 21 patients with chronic arthritis, with a Spearman's rank correlation coefficient of 0.77 (figure). ELISA titrations were performed with sera

In the 21 synovial fluids tested, IgG₁ was the only rubella-specific antibody detected, i.e., there was no evidence for the synthesis of other subclasses of antibody in the inflamed joints of patients with rheumatoid or seronegative arthritis.

The rubella-specific IgA tests on sequential sera suggested that the IgA response is detectable before the IgG response. Not unexpectedly, it is confined to the IgA₁ subclass. The IgA₂ subclass normally comprises <20% of serum IgA, but is present in

larger amounts in secretory IgA. In the small number of individuals tested, the IgA response appeared to be ephemeral. The sensitivity of the test, however, was not determined, and others have shown that the IgA response to rubella persists for longer periods (Al Nakib *et al.*, 1975; Halonen *et al.*, 1979).

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