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

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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 IgA 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 IgG1 (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.

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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 HA1 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 (Dako) 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 checkerboard 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 (Sterlin, 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 log$_2$ HAI antibody titres for all sera gave a straight line relationship.

Results

In all patients' sera, IgG$_1$ 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 log$_{10}$ specific absorbance and the log$_{2}$ 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 from two of the immune adults and the end point was within one doubling dilution of the HAI antibody end point, showing that in immune individuals IgG$_1$ is the antibody that binds to envelope polypeptides on the virion.

In the sequential sera taken after natural infection or vaccination, the earliest IgG antibody detectable, 10 days after the rash, was IgG$_1$. This maintained the only type of IgG antibody detected in all these patients; the levels rose during 6 months, but not always in parallel with the rise in HAI and SRH antibody titres. Sera from two of the patients also showed a small amount of rubella-specific IgG$_4$. These patients had high HAI antibody titres (1280 and 10 240) but in eight others with equally high HAI antibody titres, IgG$_4$ was not detected. At no time was rubella-specific IgG$_2$ or IgG$_3$ detected. The single and sequential serum samples from naturally infected individuals (groups 3 and 4) were also tested for rubella-specific IgA responses. IgA antibody was detected in serum from six of the 12 patients as early as 3 days and up to 28 days after the onset of the rash, but not in three of these patients from whom serum was available after 3–24 months. IgA$_2$ antibody was not detected.

Synovial fluids from the 21 patients with arthritis were also tested, and in two of them there was evidence for the synthesis of rubella antibody in the affected joint (Mims et al., 1985). In all these synovial fluids, IgG$_1$ was the only rubella-specific antibody detected. Of the congenitally infected group, both cord sera and four of the post-natal sera contained rubella-specific IgG$_1$ antibodies; the remaining four gave high positive results with control (virus negative) antigen, and were not tested further.

Discussion

The pattern of the IgG subclass response to most virus infections is not known. Indeed, the biological significance of these different antibodies is obscure. Our results, however, confirm the findings of Skvaril and Schilt (1984) that in immune individuals rubella specific antibodies are virtually confined to the IgG$_1$ subclass. They also show that even in the earliest stages of the antibody response, IgG$_1$ is the principle type of IgG antibody. In many of the sera studied, rubella HAI and SRH titres were high so that the other subclasses, if represented in proportion to their total content in serum, would have been detected. The only one detected, however, was IgG$_4$. Although rubella-specific IgG$_4$ was found in two sera, it was not present in eight others with equally high HAI titres ($\geq 1280$).
In the 21 synovial fluids tested, Ig\textsubscript{G1} 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 IgA\textsubscript{1} subclass. The IgA\textsubscript{2} 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 \textit{et al.}, 1975; Halonen \textit{et al.}, 1979).

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**REFERENCES**


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