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Persistence of Immunoglobulin G and Immunoglobulin M Antibodies After Postnatal Rubella Infection Determined by Solid-Phase Radioimmunoassay

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The appearance and persistence of immunoglobulin M (IgM) and IgG antibodies in postnatal rubella infections were studied by employing a solid-phase radioimmunoassay test. Altogether, 222 serial serum specimens from 51 patients with acute rubella infection were tested. Both IgG and IgM antibodies developed rapidly and appeared in all patients within 4 days after the onset of rash. In some patients, the IgM antibodies clearly preceded the IgG antibodies; however, the reverse situation was also noticed in a few cases. The IgG antibodies showed only minor changes after 8 to 10 days from the onset of rash. The IgM titers also reached a maximum level at approximately 8 to 10 days after the onset of rash, after which time a rapid decrease was normally seen. The mean half-life of IgM antibodies after 15 days from the onset of rash was 4.5 days, giving for IgM antibodies persistence times from 43 to approximately 80 days. Two patients with a prolonged IgM antibody response were detected. One of these patients had bilateral arthritis of the knee as a complication, whereas in the other patient no complication caused by rubella virus was detected. The IgM antibody response and its value in diagnosis are discussed.

Demonstration of a recent viral infection by the determination of specific immunoglobulin M (IgM) antibodies is dependent upon knowing the "normal" persistence of these antibodies after an uncomplicated infection and the incidence of prolonged or persistent IgM antibody response in the normal population. In rubella infections, IgM antibodies have been reported to persist from 20 to 40 days (4, 7, 30) up to over 1 year (1). This variation is considerable, but can for the most part be explained by differences in sensitivity between the techniques used. This demonstrates that for clinical diagnostic use, the results of any IgM test can be reliable only if knowledge of the general behavior of IgM antibodies, as studied by the method in question, has been obtained by testing a large number of serial serum specimens from patients with an acute rubella infection.

The present investigation was initiated to study the immune response of postnatal rubella infections, with special aattention being paid to the persistence of IgM antibodies. The method employed was the sensitive and reliable solid-phase radioimmunoassay (RIA) method recently developed in our laboratory (19, 20).

MATERIALS AND METHODS

Serum specimens. A total of 422 serum specimens were tested. Included were 222 serial specimens from 51 patients with acute rubella infections. The patients were male army trainees with a mean age of 19.9 years (range, 17 to 24 years), who contracted the infection during rubella epidemics occurring in three army bases in southwest Finland. The patients were followed for 29 to 200 days postinfection, and three to six serial serum specimens were collected from each patient.

In addition, 200 sera, positive in the rubella hemagglutination inhibition (HI) test, were collected from 50 males and 50 females belonging to the age group of 15 to 25 years and from 50 males and 50 females belonging to the age group of 40 to 50 years. The mean ages of these groups were 19.5 and 44.2 years, respectively, and the geometric mean HI titers were 79 and 56, respectively.

Sera with high rheumatoid factor activity were excluded from this study. The specimens were tested immediately or stored at −20°C until used.

RIA procedure. The methods used in this study have been described in detail elsewhere (19, 20). Briefly, purified rubella virus antigen was adsorbed onto polystyrene balls, and serum antibodies binding to the antigen were detected by ^{125}I-labeled anti-human gamma and anti-human mu immunoglobulins. The criteria for positive specimens, as well as the estimation of end-point titers, were the same as previously described (20).

HI tests. HI tests were carried out with a modified microtechnique (28).

RESULTS

IgG antibody response. The appearance

and persistence of IgG antibodies in 51 cases of acute rubella infection are shown in Fig. 1. The antibodies developed quite rapidly and were present in all specimens taken between 4 and 200 days after the onset of rash. The maximum titers were generally reached in about 1 week, although in some patients slight increases in titers were still noticed up to month 2 after the onset of rash. After that time, the individual titers decreased, having, at the end of the follow-up, values of one-half or one-quarter of the detected peak titers.

IgM antibody response. Figure 2 shows the IgM antibody reponse in 49 cases of acute rubella infection. The IgM antibodies developed on an average somewhat sooner than did IgG antibodies; however, the difference in time was very small. Among the first serum specimens that were taken 0 to 3 days after the onset of rash and in which rubella antibodies were demonstrable, IgM antibodies without IgG antibodies were found in 10 cases, and IgG antibodies without IgM antibodies were found in three cases. In an additional 10 cases the IgM antibody titer was markedly higher than the IgG titer, whereas in the rest approximately equal titers were found. The IgM antibodies also reached maximum titers about 1 week after the onset of rash. During week 2, the titers decreased slightly or, in some cases, remained stable. After that time, the titers decreased constantly, with a half-life of 4 to 5 days. Since this half-life value was rather constant in all 49 patients, the persistence of IgM antibodies depended upon the peak rubella IgM titer reached in each individual. The observed persistence of IgM antibodies, excluding two patients who will be described below, varied from 43 to 59 days. However, since some of the sera still had titers of 128 to 256 at 57 to 58 days after the onset of rash, the persistence of IgM antibodies in these patients appeared to be approximately 80 days.

In two patients, the behavior of IgM antibodies followed a different pattern, with a slow decline and prolonged persistence of these antibodies. The IgM antibody responses of these patients are shown in Fig. 3 together with normal IgM antibody responses of five other patients (also included in Fig. 2) for comparison. One of the patients with a prolonged IgM antibody

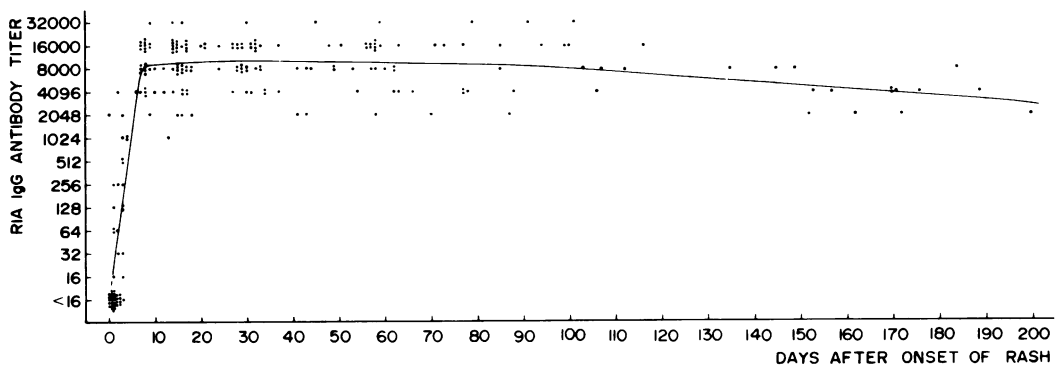

FIG. 1. *Rubella RIA IgG titers obtained on 222 serial serum specimens from 51 patients with acute rubella infection. The solid line represents the geometric mean titer.*

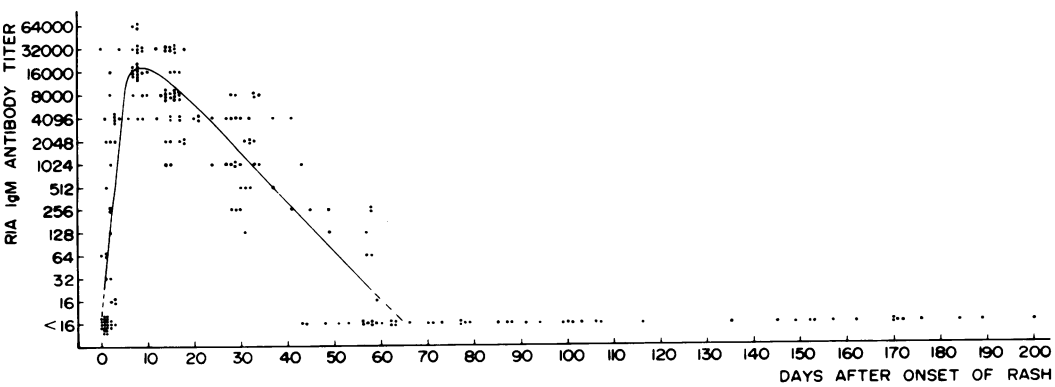

FIG. 2. *Rubella RIA IgM titers obtained on 213 serial serum specimens from 49 patients with acute rubella infection. The solid line represents the geometric mean titer.*

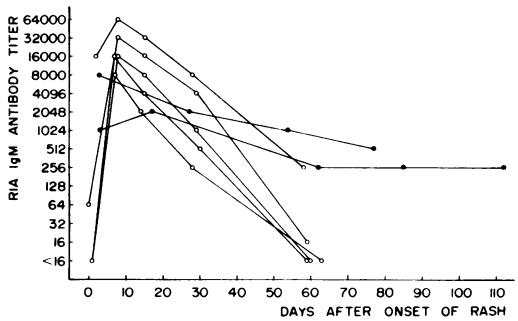

FIG. 3. *Rubella RIA IgM titers of two patients with a prolonged IgM antibody response* (●) *and of five patients with a normal IgM antibody response* (○).

response had bilateral arthritis of the knee as a complication. The other patient had no apparent complication caused by rubella virus, but a bacteriologically verified streptococcal tonsillitis occurred at the same time as the rubella infection.

A study of the possible persistence of rubella IgM antibodies in a normal population was also undertaken. A total of 200 rubella HI-positive sera collected from persons belonging to the age groups of 15 to 25 and 40 to 50 years were tested by the RIA test. All of these serum specimens were negative for IgM antibodies against rubella virus.

DISCUSSION

The first methods used to study the IgM antibody response in rubella infections, a comparison of HI titers before and after treatment with 2-mercaptoethanol (2) and immunofluorescence (4, 7), are rather insensitive. With these methods, rubella IgM antibodies could be detected for only a short time postinfection and not consistently. More detailed knowledge about the pattern of IgM antibody response was then obtained with more sensitive and reliable techniques, such as serum fractionation by sucrose density centrifugation followed by HI testing (6, 10, 29, 30) or radioimmunodiffusion (22), serum fractionation by column chromatography on Sephadex G-200 (15, 24) or on agarose (5) followed by the HI test, and improved immunofluorescence methods (8, 17, 18). Specific IgM antibodies were found to appear during the first 5 days after the onset of rash, sometimes, but not always preceding the IgG antibodies (4, 8, 17, 22). The maximum titers were generally observed 7 to 14 days after the onset of rash, after which time the titers decreased (5, 6, 17). In uncomplicated infections, the persistence of IgM antibodies was found to be for 1 to 3 months (5, 6, 10, 22, 30).

The results obtained in this study are in agreement with earlier publications. IgM antibody titers increased rapidly up to 8 to 10 days after the onset of rash, during which time rubella virus can be recovered from the body (14). About the time when free antigen disappears, the antibody production is known to complete the switchover from IgM formation to IgG formation, after which the IgM antibodies follow a half-life of approximately 5 days (3). In this study the observed mean decrease rate of the IgM titer was linear, beginning from about day 15 after the onset of rash. The calculated half-life for the rubella IgM antibodies was 4.5 days.

By using sucrose density fractionation followed by an HI test with overnight incubation of sera with the antigen, Al-Nakib et al. (1) reported the persistence of IgM antibodies for 1 year in four out of nine cases of uncomplicated postnatal rubella. It has been shown, however, that immunodiffusion, the method that they used to confirm the purity of the IgM fractions, is not sensitive enough when overnight incubation is used for the HI test (26). Small amounts of contaminants, undetectable by immunodiffusion, can give rise to IgM results that are false positives as verified by the resistance of those titers to 2-mercaptoethanol (26). Caul et al. (6), using a technique similar to that used by Al-Nakib et al. followed by 2-mercaptoethanol treatment of the IgM fractions, could not find IgM responses exceeding 117 days.

A prolonged IgM response after uncomplicated rubella infections has also been reported by Pattison et al. (23), who detected IgM antibodies in three patients as late as 7 and 11 months postinfection. Prolonged IgM responses have been reported by several authors in rubella infections complicated by carpal tunnel syndrome, arthritis, or thrombocytopenic purpura (16, 25), as well as in women who have contracted the infection in early pregnancy and have given birth to congenitally infected children (4, 9, 27). The longest reported persistence time has been over 4 years (27).

In this study two patients with a prolonged IgM response were detected. Unfortunately, the patients were lost from follow-up 77 and 112 days after the onset of rash, so the actual persistence times remained unclear. Nevertheless, the unusually low decrease of the IgM titers (Fig. 3) clearly shows that these patients had prolonged responses that might have lasted at least 200 days if the decrease rates had remained constant. Although one patient without a doubt had a complication caused by rubella virus, the other obviously had not. Rubella arthritis was also diagnosed in two patients with a normal IgM response, and it is evident that the rubella

arthritis incidence, 6% among adult males (13), is much higher than the incidence of a prolonged rubella IgM response.

The reason for a prolonged IgM response in complicated infections has been suggested to be a continuous antigenic stimulus caused by a chronic infection in the exposed tissue, e.g., in the fetus (4). However, in the case reported by Stallman et al. (27), the mother had IgM antibodies years after the delivery without any clinical or laboratory evidence of a chronic or continuing rubella infection. Similar unexplained prolonged IgM antibody responses have also been reported in connection with two other togaviruses, after Japanese encephalitis virus infections (11) and after vaccination with live yellow fever vaccine (21). As with rubella virus, a chronic infection by these two viruses was not indicated.

The results of this study verify that the demonstration of IgM antibody by RIA is a suitable method for the diagnosis of a recent infection (19, 20). The scatter of the titers and the variation in the persistence times were reasonably small, which makes the interpretation of IgM RIA results quite easy. If more heterogeneous patient groups, such as children or pregnant women, had been included in the study, this might have increased the observed variation. Prolonged IgM responses do occur, but they are rare. In this study, 2 out of 51 cases, or 1 out of 50 cases if complicated infections are excluded, were found to have prolonged persistence of rubella IgM antibody. The present study clearly supports the view (12) that a low incidence of prolonged rubella IgM production should not be allowed to interfere with the diagnosis of an acute rubella infection. This is particularly true when only one specimen is available and the determination of the presence or absence of rubella IgM antibodies is the only diagnostic test available.

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