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Persistence of Rubellavirus-Specific Immunoglobulin M and Immunoglobulin A Antibodies: Investigation of Successive Serum Samples with Lowered Immunoglobulin G Concentration

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The persistence of rubellavirus-specific immunoglobulin (Ig) M and IgA antibodies has been studied in seven patients with primary postnatal rubella infections. Successive blood samples obtained over a period of several years after the onset of disease have been investigated, employing the fluorescent antibody technique and the sucrose gradient centrifugation method. IgM antibodies were found to persist for 4 to 5 weeks after the onset of disease, with only moderate variation being observed with respect to the different patients and the method of investigation being studied. The persistence of IgA antibodies (as examined by the fluorescent antibody technique) varied from a few weeks to several years after the onset of the disease. The detection of IgA antibodies cannot be considered as conclusive for the diagnosis of recent rubella infections.

According to previous investigations, virus-specific serum immunoglobulin (Ig) M antibodies persist for a period of a few weeks up to several months after the onset of acute virus infections. The observed persistence of these antibodies varies to a certain extent according to the type of virus infection examined and the method of investigation employed.

Rubellavirus IgM antibodies are present 4 to 5 weeks after onset of the disease (in primary postnatal infections) according to a number of investigations (2, 3, 7, 10, 13, 16). Some investigators have nevertheless reported this type of antibodies to persist 8 to 10 weeks after the start of the disease (3, 8, 15).

After acute infections with influenza A, herpes simplex, varicella-zoster, or coxsackie B viruses, IgM antibodies have been reported to be present for varying periods up to 6 weeks (17, 18, 21, 22).

Epstein-Barr virus and cytomegalovirus IgM antibodies were found to be present in cases of Paul-Bunnell-positive and -negative cases of infectious mononucleosis, respectively, for periods lasting several months after the onset of disease (1, 4, 19, 20).

Some investigators (3, 4, 5) have reported that virus-specific IgA antibodies are present for 1 to 2 months after the onset of disease, whereas others (15, 19, 22) have found this type of antibodies to be present during periods varying from a few months up to a year or more after the acute infections examined.

In a recent publication (12) we described a method for the detection of rubellavirus IgM and IgA antibodies in serum samples with lowered IgG content, using the fluorescent antibody (IF) technique. In the present report, the persistence of IgA and IgM antibodies has been studied in seven patients with primary postnatal rubella infections. Sera sampled at regular intervals for several years after the onset of the disease have been investigated, using this IF technique (12).

MATERIALS AND METHODS

Patients examined. Patients were one man (20 years old) and six women (13 to 44 years old) with primary rubella infections diagnosed by the hemagglutination inhibition (HI) technique in 1968 and 1969. None of the women were pregnant at the time of onset of the disease.

Sera examined. A total of 62 serum samples from the seven patients mentioned above were used, obtained at regular intervals during 3 to 5 years after the onset of the disease. The sera were stored at -20 C until the time of this investigation.

Antibody determinations performed. HI test on whole sera and on serum fractions was done according to the technique described by Halonen et al. (11), using the Takatzy microtitration system. IF tests on

whole sera and on serum fractions were done as described earlier (12).

Fractionation of serum samples. By sucrose density gradient centrifugation, as described earlier (12), fractions of 0.3 ml were collected from the bottom of the tube. Fractionation was also done by treatment with diethylaminoethyl (DEAE)-Sephadex-50 (Pharmacia Chemicals, Sweden), as described earlier (12, 23).

Determination of IgA, IgG, and IgM concentrations in whole sera and in serum fractions. This was done by rocket immunoelectrophoresis in antibody-containing gels, as described earlier (12, 24).

Specificity of the rubellavirus IgA, IgM, and IgG antibody determinations. Figure 1 shows the immunoglobulin content of the first 10 fractions (0.3 ml), collected from the bottom of the tube, after ultracentrifugation of a serum sample on a sucrose gradient. It can be seen that the first four fractions contained IgM only. A serum sample was scored as IgM antibody positive by the HI technique only if the first three fractions showed a definite inhibition of hemagglutination. For the IF technique, only anti-human antiglobulin conjugates with the following qualities were employed: (i) the anti-IgM conjugate gave positive IF with fractions 1 to 3 (showing positive HI), which had been pooled and further concentrated, but negative IF with fractions 7 to 10 (showing positive HI), which had similarly been pooled and concentrated. (ii) and anti-IgA and the anti-IgG conjugates gave negative IF with fractions 1 to 3, but positive IF with fractions 7 to 10.

A serum sample with high IgG concentration may occasionally give traces of IgG in the first fractions collected after sucrose-density gradient centrifugation. It is therefore important that a method with high sensitivity for the detection of these traces of IgG is employed. Rocket immunoelectrophoresis usually has a higher sensitivity than passive immunodiffusion (Mancini) for the detection of immunoglobulins.

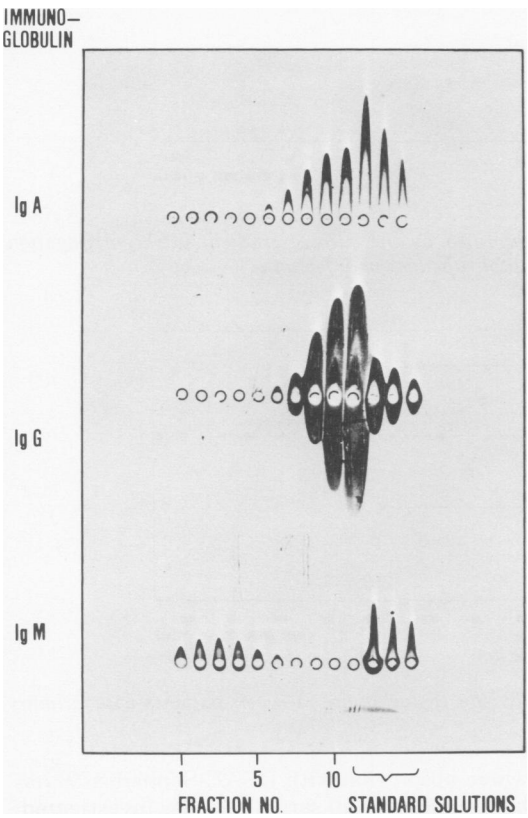

FIG. 1. *Representative findings of IgA, IgM, and IgG concentrations in the first 10 fractions (0.3 ml) harvested after 18 h of ultracentrifugation ($10^5 \times g$) of 0.25 ml of serum sample (diluted 1:2) on a 4.5-ml sucrose gradient (12.5 to 37.5%). The three holes to the right on the plates were filled with standard solutions of the respective immunoglobulins.*

RESULTS

Persistence of IgM antibodies. Figure 2 shows the period after the onset of disease where rubellavirus IgM antibodies could be detected by the IF-antibody technique in seven patients. An increase of HI titer was found from the first to the second blood sample in all seven patients. IgM antibodies could be found from day 1 to day 24 with only moderate variations between the seven patients investigated. All results shown in Fig. 2 have been obtained using serum samples which have been absorbed with DEAE-Sephadex-50, according to the methods described earlier (12, 23). This absorption lowers the ratio between the IgG and the IgM concentrations (in milligrams per 100 ml) from approximately 10:1 to approximately 2:1, as described earlier (12). The lowest dilution of the IgM containing serum fraction examined in the IF technique was calculated to correspond to approximately a 1:6 dilution of the original serum sample. Calculated in this way, the IgM antibody-positive serum fractions (after DEAE-Sephadex-50 absorption) had serum titers in the range of 6 to 48. Specific and reproducible results could not be obtained when the IF technique for IgM antibody determination was applied to whole, untreated sera.

Five of the seven patients listed in Fig. 2 also appear in Fig. 3, which shows the persistence of IgM antibodies as detected by sucrose-density gradient centrifugation and HI tests. Using this technique, IgM antibodies were detected from day 0 to day 36. A comparison of Fig. 2 and 3 reveals that the two methods have probably the same level of sensitivity.

Persistence of IgA antibodies. The variable persistence of IgA antibodies is shown in Fig. 4.

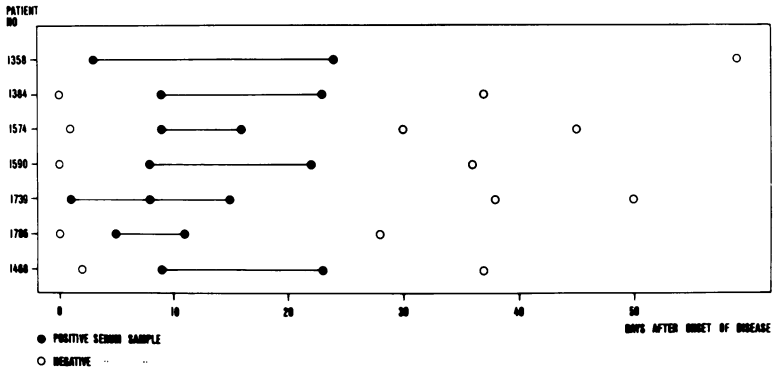

FIG. 2. *Transient presence of IgM antibodies as determined by the IF technique in seven patients with primary postnatal rubellavirus infections.*

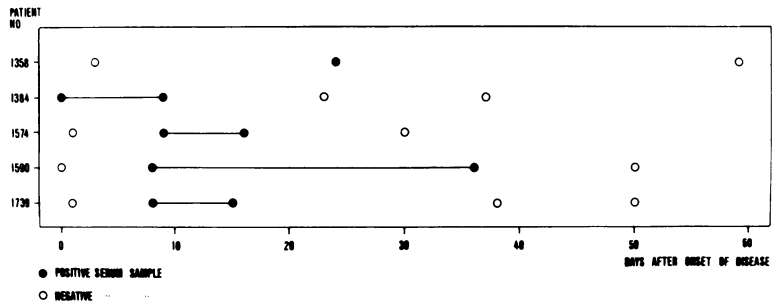

FIG. 3. *Transient presence of IgM antibodies as determined by the sucrose gradient ultracentrifugation method and HI tests in five patients with primary postnatal rubellavirus infections.*

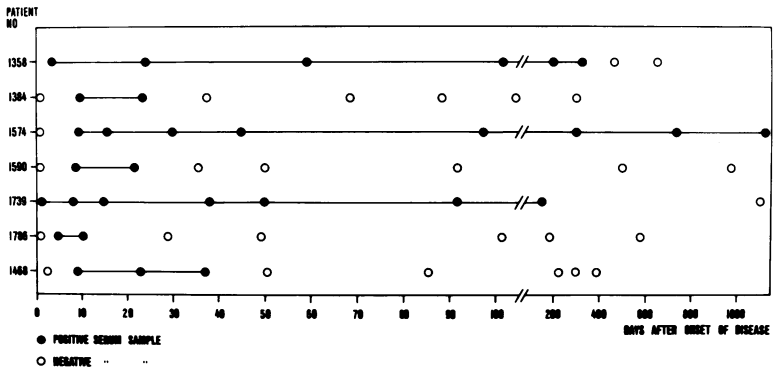

FIG. 4. *Persistence of IgA antibodies as determined by the IF technique in seven patients with primary postnatal rubellavirus infections.*

This type of antibody persisted for only a few weeks in four of the patients examined, but persisted for approximately 6 to 10 months, respectively, in two other patients. In patient 1574, these antibodies could be detected for at least 4 years. A blood sample obtained from this patient on day 1597 (not listed in the figure) was also found to be positive for IgA antibodies.

Also, Fig. 4 gives the results of experiments where absorption with DEAE-Sephadex-50 has been applied to all serum samples investigated. The IF technique usually gave better results with respect to IgA antibodies when absorbed sera instead of whole sera were employed. This was the case especially for serum samples obtained more than a few weeks after the onset of the disease. This is evident from Table 1, which shows the titers obtained with and without

TABLE 1. *Comparison of IgA antibody titers obtained by the IF technique when serum samples absorbed with DEAE-Sephadex-50 were examined in parallel with the corresponding unabsorbed sera*

Patient no.	Day after onset of disease	IgA titer	
		Untreated serum	Absorbed serum
1739	8	80–160	48–96
1739	15	<10	12–24
1786	11	<10	6–12
1786	28	<10	<6
1590	0	<10	<6
1590	8	20–40	12
1590	22	<10	24
1590	36	<10	<6
1574	1	<10	<6
1574	9	10–20	24–48
1574	30	10–20	12–24
1574	45	<10	12–24

absorption with DEAE-Sephadex-50, respectively. For patient 1739 at day 15, patient 1590 at day 22, and patient 1574 at day 45, definite IgA antibody titers could be demonstrated only when absorbed sera were examined by the IF technique.

DISCUSSION

In the present study, serum IgM antibodies could be detected (with the exception of one serum sample) during the first 4 weeks after onset of disease in patients 13 to 44 years old, irrespective of the sucrose-gradient centrifugation technique or the IF technique employed. This has been shown in a number of previous studies (2, 4, 7, 10, 13, 16). In a recent investigation (15), it was found that rubellavirus serum IgM antibodies, whether appearing after subcutaneous immunization with rubella vaccine or after natural infection, persisted for 8 to 10 weeks in children 2 to 8 years old after immunization or onset of disease, respectively. A more sensitive technique may have been used in this investigation (15), the results of which have yet to be confirmed. The persistence of IgM antibodies for several months after the onset of virus infections has nevertheless been reported for cases of Epstein-Barr virus infections and for cases of cytomegalovirus infections (1, 14, 19, 20).

Some authors (6, 9) have pointed out the existence of the possibility of obtaining nonspecific (secondary) IgM antibody staining when examining sera which contain both rubellavirus IgG antibodies and rheumatoid factor (anti-IgG IgM antibodies). In the present investigation the sensitivity of the IgM antibody determination was improved by removing the majority of the IgG content present in the serum samples concerned, employing absorption with DEAE-Sephadex-50. If some of the sera had contained a rheumatoid factor, the anti-IgM fluorescence would have been decreased by this absorption and not increased as was the case.

According to our experience, the IF antibody technique seems to be the most valuable method presently available for rubella IgA antibody determinations. We have not been able to confirm the results reported from a recent investigation (3) where IgA containing serum fractions were obtained free of IgG, using gel filtration on an agarose column. We have found that the IF technique employed for IgA antibody determination also displays a higher sensitivity when, instead of unabsorbed serum samples, DEAE-Sephadex-50-absorbed serum samples are investigated. Our findings indicate that the determination of rubellavirus IgA antibodies probably is blocked by IgG antibodies present in the same serum samples. This is in accordance with the observation of the blocking effect being more evident in serum samples taken during later phases than in serum samples taken during earlier phases of the acute infections examined.

A transient presence of IgA antibodies during acute rubella infections has been reported previously (3, 4). According to our findings this type of antibody may in some patients persist for several months (or even several years) after the onset of disease. The diagnosis of a recent rubella infection by determination of IgA antibodies in a single serum sample cannot be considered as conclusive evidence. The finding of a high IgA antibody titer in a single serum sample from a case suspected of being a rubellavirus infection nevertheless supports this supposition to a large degree.

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