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Antibody Responses in Patients with Rubella Infection Determined by Passive Hemagglutination, Hemagglutination Inhibition, Complement Fixation, and Solid-Phase Radioimmunoassay Tests

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Antibody responses in serial serum specimens collected from 31 patients with an acute rubella infection were determined by passive hemagglutination (PHA), hemagglutination inhibition (HI), complement fixation (CF), radioimmunoassay (RIA) immunoglobulin G (IgG), and RIA immunoglobulin M (IgM) tests to evaluate the effectiveness of these tests in diagnosing a recent infection. The HI, RIA IgG, and RIA IgM antibodies appeared almost simultaneously and reached the maximum level about 1 week after the onset of rash. Compared to these, the CF antibodies developed only slightly later, whereas the development of the PHA antibodies was much more delayed. The RIA IgM response was shown to be transient, lasting approximately 1.5 to 2.5 months postinfection. The results of this study indicate that demonstration of specific IgM antibodies is the best method for diagnosing a recent infection, one within 2 months after the onset of the illness. If an IgM test is not available, a combination of the HI and PHA tests is recommended.

The serological diagnosis of rubella virus infections is generally based on hemagglutination inhibition (HI) and complement fixation (CF) tests. These conventional tests have, however, some serious limitations, and a constant need for better laboratory tests has been recognized. During the last few years, several methods based on a separate determination of immunoglobulin G (IgG) and immunoglobulin M (IgM) class antibodies have been proposed for use in rubella diagnosis (4, 7, 17). These include the sensitive solid-phase radioimmunoassay (RIA) method recently developed in our laboratory (8, 11). In addition, a rubella antibody test based on the passive hemagglutination (PHA) reaction has recently been developed (Rubacell, Abbott Laboratories).

In this study, the antibody responses in serial serum specimens collected from 31 young adult patients with a postnatal rubella infection have been determined by PHA, HI, CF, RIA IgG, and RIA IgM tests, to evaluate the effectiveness of these tests in diagnosing a recent rubella infection.

MATERIALS AND METHODS

Serum specimens. A total of 144 serial specimens from 31 patients with acute rubella infection were tested. The patients were young, male army trainees with a mean age of 20 years, who contracted the infection during rubella epidemics occurring in an army base in southwest Finland. The patients were followed 29 to 200 days postinfection, and from each patient three to six serial serum specimens were collected. The sera were stored at −20°C until tested.

PHA test. PHA antibodies were detected by a commercially available test, Rubacell (Abbott Laboratories, North Chicago, Ill.). In this test, human erythrocytes, stabilized with formaldehyde-pyruvic aldehyde and sensitized with a soluble rubella virus antigen, agglutinates in the presence of a specific antibody (14). The tests were done according to the instructions given by the manufacturer.

HI test. HI tests were performed by microtechnique according to the modified test used at the Center for Disease Control, Atlanta, Ga. (16). Rubella hemagglutinin was prepared in BHK-21/13S cells maintained in a medium containing bovine serum albumin and no serum (6).

CF test. For CF tests, rubella antigen was prepared using the alkaline extraction procedure described by Halonen et al. (5). A standard microtechnique (1) was used.

RIA test. The details of the methods used have been described previously (8, 11). 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.

RESULTS

General patterns of the antibody responses as determined by the different tests are shown in

Fig. 1, and in Table 1, the individual titer values of four representative patients are given.

The delayed appearance of PHA antibodies is illustrated in Fig. 1a. The earliest positive specimen detected (titer 13.5) was taken 15 days postinfection, and the latest negative specimen was detected 21 days postinfection. The titers then increased constantly, but at a decreasing rate, up to the end of the follow up. A significant

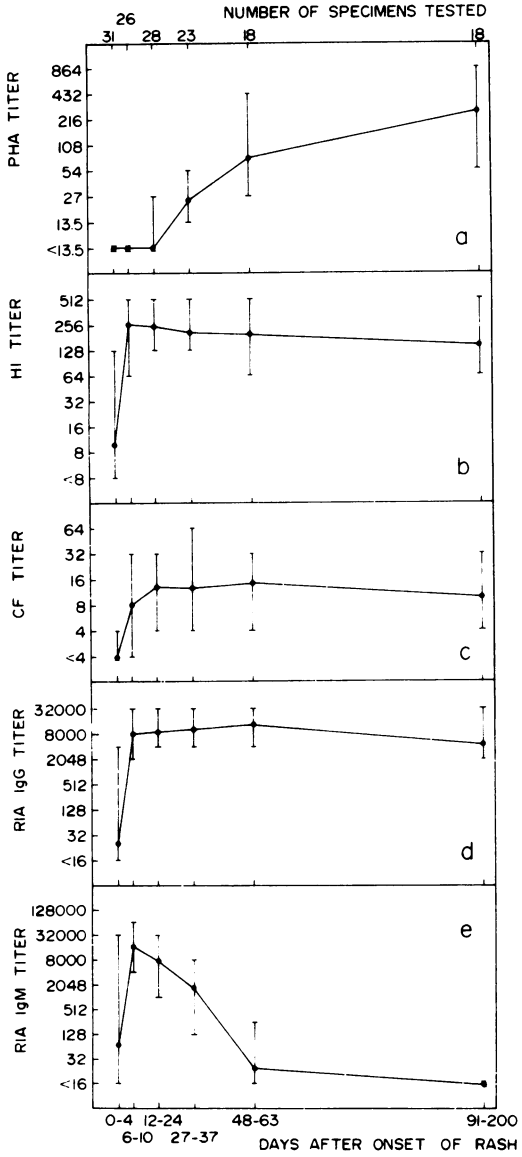

FIG. 1. *Development of PHA, HI, CF, RIA IgG, and RIA IgM antibodies in serial serum specimens collected from 31 young male patients with acute rubella infection. Geometric mean (solid line) and range of titers (bars) are shown.*

TABLE 1. *Rubella PHA, HI, CF, RIA IgG, and RIA IgM antibody titers in a series of serum specimens taken from four with rubella*

Patient	Days after onset of rash	Titer				
		PHA	HI	CF	RIA IgG	RIA IgM
I.M.	1	<13.5	<8	<4	<16	<16
	8	<13.5	256	4	16,000	64,000
	15	<13.5	256	16	16,000	32,000
	28	13.5	256	16	16,000	8,000
	58	27	256	16	8,000	<16
	170	432	128	16	4,096	<16
K.V.	2	<13.5	128	4	<16	16,000
	8	<13.5	512	16	16,000	64,000
	15	<13.5	256	16	16,000	32,000
	33	13.5	256	16	16,000	8,000
	58	54	256	16	16,000	256
	171	432	128	16	4,096	<16
M.H.	2	<13.5	<8	<4	<16	<16
	9	<13.5	256	16	16,000	16,000
	30	13.5	512	64	32,000	1,024
	59	108	128	32	32,000	<16
	184	432	128	16	8,000	<16
N.H.	2	<13.5	16	<4	<16	1,024
	8	<13.5	512	<4	4,096	32,000
	16	13.5	512	8	8,000	32,000
	29	54	256	8	8,000	8,000
	162	216	64	8	2,048	<16

(fourfold or greater) rise in the antibody titer was demonstrated in all patients before the end of week 9 postinfection. A steady or decreasing antibody level was not demonstrated in any patient during the 200-day study period.

The HI antibodies developed rapidly (Fig. 1b) and were detectable in all specimens taken on day 4 or later after the onset of rash. The maximum titers were usually reached within 1 week, after which a slow but constant decrease in titers was noticed. A significant rise in HI titer was demonstrated in all but two patients. The first serum specimens of these two patients were taken 3 and 4 days postinfection, respectively.

The appearance of CF antibodies (Fig. 1c) was somewhat delayed compared to that of the HI antibodies, but more rapid than the development of the PHA antibodies. CF antibodies were detected in all specimens taken on day 9 or later postinfection. A significant rise in CF titer was demonstrated in all but one patient, who also failed to show a significant increase in HI titer. After week 1 postinfection, when the maximal HI antibody response was already reached, a significant rise in CF antibody titer was demonstrated in 7 out of 26 patients from whom a specimen taken 6 to 10 days after the onset of rash was obtained. After reaching the

maximum level, the CF antibodies remained quite stable up to the end of the study period.

RIA IgG antibodies developed rapidly (Fig. 1d), almost parallel to the HI antibodies, and were detected in all specimens taken on day 3 or later postinfection. A relatively stable antibody level was reached at about 1 week postinfection. Titer increases were, however, noted up to month 2 postinfection, with slow but constant decrease after that time.

RIA IgM antibodies were detectable in all specimens taken between 4 and 37 days after the onset of rash. They increased parallel to the HI and RIA IgG antibodies up to about day 10 postinfection (Fig. 1e), after which time the titers decreased rapidly. The first negative convalescent specimen was taken at day 48 postinfection, and the latest positive specimen was taken at day 59 postinfection.

DISCUSSION

The rubella HI antibodies develop rapidly during the first days after the onset of rash (10), and the appearance of the CF antibodies, measured with crude CF antigen, is only slightly slower (2, 5). Therefore, a limitation of these two tests in the diagnosis of a recent infection is that a significant rise in the antibody titers can be demonstrated only if the first serum specimen is taken within a few days after the onset of rash. In this study, only 7 out of 26 cases of an acute rubella infection would have been diagnosed with these tests if the first serum specimen were taken on day 6 postinfection or later. The same limitation also applies to the RIA IgG antibodies, although they develop, on an average, somewhat more slowly than the HI antibodies. This can, for the most part, be explained by the fact that a great portion of the early HI antibodies are of IgM class (12, see also data for patients K.V. and N.H. in Table 1).

The PHA antibodies appeared slowly, about 2 to 3 weeks later than the other antibodies studied. Therefore, a significant rise in PHA antibody titer between the first and subsequent serum specimens was demonstrable even if the first specimen was not taken until 1 to 2 months postinfection. Moreover, since the PHA antibodies are persistent, comparable to the HI antibodies in measuring rubella immunity (14), a demonstration of HI antibodies in the absence of PHA antibodies is pathognomonic to the first 2 to 3 weeks after rubella infection, and would thus enable the diagnosis of a recent infection to be made from a single serum specimen.

Delayed rubella antibody responses resembling those of the PHA antibodies have been demonstrated previously by the CF test by using a soluble hemagglutinin-free antigen (18), the gel-precipitation tests (9, 13, 15), the platelet aggregation test (18), and immunoelectro-osmophoresis (3). None of these tests was, however, practical enough to be widely used in rubella diagnosis, whereas the Rubacell test can be easily adopted by any virus laboratory in which HI tests are performed.

The IgM antibody response after uncomplicated rubella infection is transient, and the demonstration of specific IgM antibodies provides a suitable tool for the diagnosis of a recent infection (7, 10a, 17). Thus, the use of an IgM test, together with an IgG antibody test mainly for immune status determinations, obviously caters best for the present needs in rubella serology. However, the separate demonstration of IgM and IgG antibodies by RIA, or by any other reliable method, requires special equipment and expertise, which limits the general use of these methods in the near future.

The results obtained here indicate that, although the Rubacell test is now marketed only for immune status determinations, it could be valuable also in the diagnosis of a recent infection. In fact, for laboratories that cannot adopt any specialized IgM rubella antibody test at the moment, a combination of the PHA and HI tests would be the easiest way to improve considerably the efficacy of rubella diagnosis, particularly in cases where the first serum specimen is taken late in the illness.

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