Use of Enzyme Immunoassays and the Latex Agglutination Test to Measure the Temporal Appearance of Immunoglobulin G and M Antibodies After Natural Infection or Immunization with Rubella Virus

JAMES M. MEEGAN,* BRIGITTE K. EVANS, AND DOROTHY M. HORSTMANN

Departments of Epidemiology & Public Health and Pediatrics, Yale University School of Medicine, New Haven, Connecticut 06510

Received 18 April 1983/Accepted 16 June 1983

The time course of appearance of antibodies after infection with rubella virus was determined with an immunoglobulin G (IgG) detection enzyme-linked immunosorbent assay, a latex agglutination test, and an IgM detection enzyme-linked immunosorbent assay. In six naturally infected rubella patients and 26 vaccinees, antibodies measured by either the IgG enzyme-linked immunosorbent assay or the latex agglutination test generally appeared in parallel with those detected by the hemagglutination inhibition test. By 28 days after inoculation of live virus vaccine and by 2 days postonset of clinical rubella symptoms caused by natural infection, antibodies were found by the two tests for all individuals. A commercially available enzyme-linked immunosorbent assay kit was used to detect rubella-specific IgM. After natural infection, IgM appeared earlier than IgG, and although IgM titers decreased rapidly postinfection, in four of five patients antibodies were still detectable 40 to 43 days after the onset of clinical symptoms. After vaccine-induced infection, rubella-specific IgM was lower in titer than after natural infection and was detected in only three of seven vaccinees 70 days post-immunization.

The hemagglutination inhibition (HAI) test is the most widely used method for both the serological diagnosis of rubella infection and the determination of immune status. However, the test is time-consuming, labor intensive, and difficult to standardize between laboratories. Several other antibody assays have recently been developed and tested, including radioimmunoassay (14), passive hemagglutination (3), single radial hemolysis in gel (5, 6), indirect immunofluorescence (5, 7), enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) and IgM (8, 11, 21, 22, 26), and latex agglutination (LA) (13, 24). These tests are rapid, simple, and usually require no pretreatment of sera; some now are available commercially in kit form. Numerous reports (4, 5, 7–10, 15, 19, 20, 25) revealed that in general they correlate well with the HAI as assays for immune status. Because several of the newer methods are gaining widespread acceptance, we extended the comparison with HAI by investigating the time of appearance of antibodies to rubella virus after vaccine-induced and natural infection by using LA and ELISA for detection of rubella-specific IgG and IgM.

A series of sera collected from 26 susceptible school children and young adults who had been immunized with RA27/3 vaccine were available for the present study. We also included a second group of sera which had been collected previously from six military recruits who experienced natural rubella infection. All preexposure sera were negative for antibodies to rubella virus by HAI and by a semi-microneutralization test (18).

The procedure followed for the LA test was that recommended in the Rubascan LA kit (Hynson, Westcott and Dunning, Baltimore, Md.). Serum and latex beads coated with rubella antigen were mixed on a Teflon-coated card and incubated at room temperature on a rotating shaker, and agglutination was read after 8 min. The kit provides positive and negative control sera. To determine endpoint titrations, we made twofold dilutions of sera with the dilution buffer contained in the kit. The antibody titer was recorded as the reciprocal of the dilution which, when mixed with a drop of latex beads, produced agglutination.

The protocol for detection of IgG by ELISA was based on the test developed by Voller and co-workers (22, 23), which was modified as previously detailed (13). In this system, gradient-purified rubella virus and control antigen
from uninfected Vero cells were coated to 96-well polystyrene plates. Goat anti-human IgG (gamma chain specific) conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) was used to detect bound IgG. For each serum dilution a specific activity was calculated by subtracting the average optical density in wells coated with negative control antigen from that in wells coated with rubella antigen. The dilution was considered positive only if it yielded a specific activity which was 2.5 standard deviations greater than the mean specific activity of a series of reference negative sera. In comparative tests, antibody titers determined by this ELISA method were generally two- to four-fold higher than those obtained with two commercially available ELISA kits. We showed previously that titers in our ELISA correlated closely with those we obtained in HAI, LA, and semi-microneutralization assays (13).

The IgM ELISA was performed with a Rubelisa M kit (Whittaker M.A. Bioproducts, Walkersville, Md.), following the procedure recommended with the product. Sera were first treated to remove interfering IgG by adsorption with a modified protein A preparation; they were then added to wells of a 96-well plate coated with either rubella or control antigen. After incubation for 2 h at 20 to 25°C, the plates were washed and alkaline phosphatase-conjugated goat anti-human IgM (mu chain specific) was added, followed by incubation for 2 h at 20 to 25°C. After a final wash, substrate was added, and the plate was held at 20 to 25°C for 45 min. The reaction then was stopped with 2 N NaOH, and the absorbance was read at 405 nm. Two positive sera, one of high and one of low IgM titer, and an antibody-negative serum were included with each test as calibrators. As a further control, a reference IgM-negative serum containing rheumatoid factor was included to ensure that interfering IgG had been removed. For each serum, the absorbance value in the well coated with control antigen was subtracted from the value in the rubella-coated well. Following the guidelines in the kit, the absorbance values of the three reference sera were used to construct a calibration curve from which an IgM value (roughly comparable to the absorbance value) could be determined. IgM values of 0.20 to 0.27 were interpreted as equivocal; higher or lower values were considered IgM positive or negative, respectively. According to unpublished information enclosed with the product, this test has the same sensitivity, specificity, and accuracy as HAI tests of sera fractionated to separate IgM on sucrose gradients.

For the HAI test, the dextran sulfate-calcium chloride system was employed to remove non-specific inhibitors before the sera were tested.

Incubation was for 1 h, and human type O erythrocytes were used by the method of Liebhaber (12).

At 14 days after inoculation of the live, attenuated rubella virus vaccine, 2 of the 26 subjects developed antibodies detected in the LA IgG and ELISA but not in the HAI test (Fig. 1A). At 28 days post-immunization all sera had antibodies detectable by each of the three tests, and by day 42 to 70 titers had reached maximum values.

The six young adult patients who were followed during natural rubella infection all had typical clinical courses: a prodrome of malaise, fever, headache, and sore throat lasting 1 to 4 days, followed by the appearance of a maculopapular eruption on the face. Antibodies were detected first by the IgG ELISA test, with a low titer demonstrable in the serum of one patient 1 day after appearance of the rash (Fig. 2A). All patients had antibodies detectable by the three tests on day 2 after the rash appeared. The development of high titers measured by the LA method appeared to be delayed compared with those measured by HAI and ELISA.

Sufficient quantities of sera were available to allow samples from seven vaccinees and five naturally infected patients to be tested for rubella-specific IgM. In vaccinees, IgM detected by ELISA was present in one serum on day 14 and
FIG. 2. Time course of appearance of antibodies to rubella virus after natural infection. (A) Geometric mean antibody titers and range for sera from six patients tested by HAI, IgG detection ELISA, and LA. (B) Mean IgM ELISA values for sera from five patients.

RA27/3 vaccine showed that complement-fixing antibodies had disappeared, but LA and ELISA titers correlated with persisting titers of HAI antibody (13).

The ELISA test for IgM showed that these antibodies appeared earlier than did IgG antibodies in patients experiencing natural rubella infection. IgM antibodies persisted in four of the five patients for longer than 40 to 43 days. Gradient centrifugation methods for measuring IgM antibodies show a comparable pattern of appearance and indicate that rubella-specific IgM usually persists for 2 to 4 months postinfection (16), although occasionally it is detectable for longer periods (1, 17).

After vaccine-induced infection, there was less IgM as measured by ELISA than after natural infection, and it was detected in only three of seven individuals 70 days postimmunization. Ogra et al. (16) reported that IgM antibodies disappeared within 2 months after immunization, whereas other reports indicated that these antibodies can persist for more than 6 months (1, 2). ELISA methods, particularly those which trap IgM on a solid phase and determine its specificity by the addition of rubella antigen and then of conjugated rubella antiserum (8), might prove more sensitive and reliable than the tests used in past studies. These differences in detecting the disappearance of IgM after immunization should be resolved, since in some instances the presence of IgM is used to determine whether women who were inadvertently vaccinated during pregnancy were susceptible to rubella. Frequently such women are not tested until a month or more postimmunization.

Finally, as new serological tests replace the HAI test, standardizing the various assays so that results from different laboratories are comparable, as well as establishing guidelines for equating results obtained by different assays, will become increasingly difficult. This emerging problem will require extensive evaluation and review.

We are grateful to Wilma B. Bias, Department of Medicine, Division of Medical Genetics, The Johns Hopkins University School of Medicine, for graciously supplying some pre- and post-immunization sera. We are also indebted to J. Emmons and R. Capper for performing HAI assays.

This study was supported in part by Public Health Service grant 731D-41-49836 from the National Institutes of Health and Department of Defense contract N000014-78C-0104.

LITERATURE CITED
NOTES

748


