JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1986, p. 391–394
0095-1137/86/090391-04$02.00/0

Vol. 24, No. 3

Specific Immunoglobulin M Enzyme-Linked Immunosorbent Assay for Confirming the Diagnosis of Measles

ALAN W. LIEVENS AND PHILIP A. BRUNELL*

Department of Pediatrics, Division of Infectious Diseases, The University of Texas Health Science Center, San Antonio, Texas 78284

Received 3 February 1986/Accepted 23 May 1986

An enzyme-linked immunosorbent assay (ELISA) was evaluated for the detection of measles virus-specific immunoglobulin M (IgM) (MIgM). The ELISA was standardized by deriving a seronegative range of values from sera which should not contain MIgM (24 cord sera, 59 sera from immune health care workers, and 47 sera from infants before the administration of measles vaccine). These values were separable from those obtained from individuals convalescing from measles. Twenty sera containing rheumatoid factor were MIgM seronegative. Of 30 acute-phase sera from suspected measles cases, 26 contained MIgM; those that were seronegative were obtained on day 0, 0, 2, or 9. All 25 convalescent-phase samples contained MIgM. Of the 25 paired samples, 22 were IgG positive at the first sampling; 3 of the 22 did not show a rise in IgG titer. The MIgM ELISA can be used for confirming suspected measles cases, often requiring only a single serum specimen.

The widespread use of measles vaccine has resulted in a marked reduction in the number of cases of measles in the United States (2). Because of this, many health care workers have seen few or no cases of measles. Imported cases and outbreaks in schools are still reported. For this reason, it seemed desirable to develop and evaluate a method for the serological confirmation of clinically diagnosed cases of measles.

It was found that measles virus (MV)-specific immunoglobulin M (IgM) (MIgM) was detectable early after the onset of disease and that it was present transiently. Thus, the determination of MIgM appeared to offer some advantages for the laboratory confirmation of measles. In most cases, diagnosis could be confirmed by testing a single serum sample for MIgM. In this paper we describe the technique, note its limitations, and compare it with the enzyme-linked immunosorbent assay (ELISA) for MV-specific IgG (MIgG).

MATERIALS AND METHODS

Antigen preparation. Passage 21 of the Edmonston strain of MV (kindly supplied by Paul Albrecht) was grown in Vero cells as previously described (5). After infection of the monolayer with MV, the medium was replaced with Eagle minimum essential medium without calf serum. When extensive cytopathic effects were observed, the medium was removed, and the monolayer was washed twice with phosphate-buffered saline (PBS) (pH 7.4). The cells from a 150-cm^2 surface were scraped into 2.5 ml of PBS by using a rubber policeman and disrupted by sonication for 30 s (Branson sonifier; Heat Systems-Ultrasonics, Inc., Plainview, N.Y.). This material was then centrifuged at 600 × *g* for 10 min (model CRU-5000 centrifuge; International Equipment Co., Needham Heights, Mass.). The supernatant fluid obtained after centrifugation was used as the viral antigen. Similarly treated but uninfected cells were used as controls.

Antiserum preparation. Antiserum against MV was produced by immunization of Hartley strain guinea pigs with MV. A 75-cm^2 monolayer of transformed guinea pig cells (1) was infected with MV and then maintained in Eagle minimum essential medium containing 2% fetal calf serum. After washing the monolayer with PBS and scraping the cells into 1 ml of sterile PBS, we followed the procedure for the preparation of antigen described above. Uninfected transformed guinea pig cells were similarly treated. Equal volumes of infected or uninfected material were mixed with Freund complete adjuvant (GIBCO Laboratories, Grand Island, N.Y.) and inoculated into a footpad of separate, appropriately designated guinea pigs. Boosters were given intramuscularly 29 and 76 days after the initial inoculation. Serum samples obtained from the guinea pigs at intervals following immunization were diluted 1:100 in PBS containing 0.05% Tween 20 (PBS-T) and then tested for the presence of guinea pig MIgG. The ELISA used previously in this laboratory for testing human sera (5) was used, except for the substitution of goat anti-guinea pig serum (Antibodies, Inc., Davis, Calif.) conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) for an anti-human globulin conjugate. Alkaline phosphatase labeling of this serum was performed by the method of Voller et al. (14). When tested with this conjugate, none of the sera obtained from the animals before the initial inoculation or from the control animals reacted with MV antigen. Each of the animals injected with MV antigen had detectable antibody after the initial inoculation and increased reactivity with each subsequent booster.

MIgM ELISA. U-shaped polyvinyl chloride microtiter plates (Linbro/Titertek; Flow Laboratories, Inc., McLean, Va.) were coated with 0.1 ml of caprine anti-human μ antibody (Tago, Inc., Burlingame, Calif.) (6). By testing serial dilutions, a concentration of 1:5,000 in carbonate buffer (pH 9.6) was found to be optimal and was used in all subsequent experiments (14). Plates were stored at 4°C for a minimum of 24 h before use. Washing after removal of the coating fluid was repeated four times with PBS-T; all subsequent washings were done in this manner. The plate was then "postcoated" with 0.1 ml of 1% bovine serum albumin (Sigma Chemical Co.) in PBS-T (6) containing 0.2% sodium azide (Sigma Chemical Co.). This and all subsequent incubations were performed at 37°C in a moist chamber for 1 h. Incubation of the human sera at 4°C overnight (13) yielded

* Corresponding author.

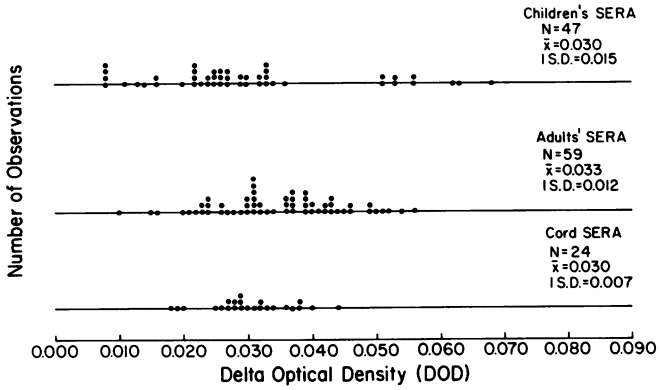

FIG. 1. MIgM DOD values obtained by testing 24 IgG-seropositive cord sera, 59 IgG-seropositive adult sera, and 47 sera drawn from IgG-seronegative children before the administration of measles-mumps-rubella vaccine. N, Number; $\bar{x}$, mean; S.D., standard deviation.

essentially the same results as 1 h of incubation at 37°C. The latter was chosen for all subsequent experiments, as the assay could be performed in a single day.

Serial dilutions of all reagents were tested in various combinations to achieve the clearest separation of MIgM seropositive and seronegative. All reagents were added in 0.1-ml volumes. Decanting and washing were performed between each of the successive steps. A 1:100 dilution of each serum sample was prepared in 1% heat-inactivated fetal calf serum–10% globulin-poor normal goat serum in PBS-T. The preparation of the latter by precipitation with Na_2SO_4 is described elsewhere (9). A 1:150 dilution of MV antigen or control (5) in PBS-T was then added to each of two "viral" or "control" wells, respectively. Subsequently, a 1:1,000 dilution of guinea pig serum collected after the second booster and diluted in PBS-T was added. Goat anti-guinea pig serum conjugated (14) to alkaline phosphatase (1:1,000 in PBS-T) was then added. Finally, the substrate, disodium *p*-nitrophenyl phosphate (Sigma Chemical Co.) in 10% diethanolamine (14) (Sigma Chemical Co.) (yielding 1 mg/ml), was added. After incubation for 45 min at room temperature, the enzymatic reaction was stopped by the addition of 3 N NaOH, and the color reaction was read spectrophotometrically with an enzyme immunoassay Biotek Reader (model EL 307 I; Biotek Instruments, Inc., Burlington, Vt.) at 405 nm. The result was expressed as the delta optical density (DOD), i.e., the mean of the optical density of two control wells subtracted from the mean of the optical density of two viral wells.

In both the MIgM and MIgG assays, aliquots of a known negative serum sample and low- and high-positive serum samples were included in each run. Variations of more than two standard deviations from the mean value of each serum sample were indicative of an unacceptable assay.

MIgG assay. The MIgG assay was performed as described previously (5, 15). The seronegative range was defined as DOD values of <0.056.

Serum specimens. Paired serum specimens from 25 individuals suspected of having measles and for which the date of onset and collection were available were tested in both the MIgG (5, 15) and MIgM ELISA. In addition, five unpaired acute-phase serum samples, 20 serum samples known to contain rheumatoid factor (RF), and acute- and convalescent-phase sera from five patients with rubella also were tested.

Three groups of sera which would not be expected to contain MIgM were also tested. These consisted of 24 cord sera, 59 sera from immune health care workers or from dental students, none of whom had known recent contact with measles or recent immunization with measles vaccine, and 47 sera drawn before the administration of measles-mumps-rubella vaccine from children between the ages of 14 and 35 months and without a history of measles (5, 15).

Treatment of serum samples. Serum samples were tested for the presence of MIgM before and after treatment with dithiothreitol (DTT) (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) by the modified method of McLaughlin (4) (0.005 ml of serum was added to 0.005 ml of DTT). They also were tested before and after treatment with staphylococcal protein A (SPA) (10).

RESULTS

Seronegative range for MIgM. The seronegative range of values was established by testing three groups of sera which would be expected to lack MIgM, namely, cord sera, sera from adults who had had measles many years previously, and sera drawn from seronegative children before the administration of measles-mumps-rubella vaccine. Sera from the first two groups were all positive for the presence of MIgG in the ELISA (15). Sera from all three groups were comparable in their lack of reactivity in the MIgM ELISA (Fig. 1).

The DOD values (means ± standard deviations) for the three groups in the MIgM ELISA were, respectively, 0.030 ± 0.007, 0.033 ± 0.012, and 0.030 ± 0.015. A DOD value (0.075) three standard deviations above the mean for sera from susceptible children was used to determine seronegative values, as most sera to be evaluated for MIgM were obtained from children.

Measles antibody following natural infection. Of 30 acute-phase sera, 26 had demonstrable MIgM. Samples drawn from patients 0, 0, 2, or 9 days after the onset of illness were seronegative (cases 1, 4, 6, and 13; Table 1). All of the convalescent-phase sera, including sera from patients whose acute-phase sera were MIgM negative, were shown to contain MIgM; one specimen was obtained as late as 41 days after onset. All of these individuals, then, had detectable MIgM in one or both serum specimens.

Only a single serum specimen was available from five additional individuals, all of whom had DOD values for both MIgG and MIgM above the seronegative range (Table 1).

All but three of the acute-phase sera were seropositive when tested for MIgG; indeed, many values exceeded those found in convalescent-phase sera. The three seronegative patients were the only ones who had a demonstrable seroconversion. Rises in antibody were observed in 22 of 25 individuals from whom paired sera were obtained; all convalescent-phase sera were MIgG positive (Table 1).

Specificity of the MIgM ELISA. To evaluate specificity, we treated sera with DTT and SPA. Treatment of sera with DTT during convalescence from measles caused a reduction in DOD values to seronegative values when tested for MIgM; MIgG DOD values were unaffected by DTT. In contrast, adult and cord sera showed no appreciable difference in the MIgM ELISA after treatment with DTT. After treatment with SPA, testing for MIgM revealed only a slight drop in DOD values in convalescent-phase sera, but there was a sizable drop in MIgG DOD values in convalescent-phase sera, in a serum sample from a normal adult, and in a cord serum sample (Table 2).

TABLE 1. ELISA DOD values for individuals clinically suspected of having measles

Case	ELISA DOD value for indicated sera on indicated day:					
	Acute phase			Convalescent phase		
	MIgG	MIgM	Days after onset	MIgG	MIgM	Days after onset
1	0.065	0.056[a]	0	1.406	0.388	5
2	0.674	0.167	3	0.929	0.359	13
3	0.016[b]	0.081	1	0.347	0.645	12
4	0.182	0.052[a]	0	1.798	0.127	14
5	0.352	0.079	0	1.411	0.160	13
6	0.767	0.057[a]	9	1.539	0.150	20
7	0.079	0.353	10	0.362	0.368	16
8	0.146	0.114	3	0.779	0.339	12
9	0.202	0.121	3	0.896	0.368	11
10	0.691	0.157	5	0.924	0.189	16
11	0.114	0.407	3	0.699	0.515	18
12	0.547	0.517	2	1.006	0.464	16
13	0.090	0.056[a]	2	0.775	0.233	16
14	0.044[b]	0.279	3	0.327	0.395	13
15	0.094	0.277	8	0.774	0.082	41
16	0.137	0.192	6	0.607	0.169	21
17	0.574	0.337	2	0.679	0.484	16
18	0.083	0.324	3	0.371	0.152	26
19	1.044	0.276	6	0.981	0.137	20
20	0.670	0.412	4	0.595	0.383	17
21	0.995	0.113	5	0.934	0.075	19
22	0.547	0.222	2	0.982	0.509	17
23	0.652	0.090	2	0.786	0.189	16
24	0.044[b]	0.268	0	0.264	0.358	14
25	0.087	0.217	1	0.376	0.388	15
26	0.197	0.513	11			
27	0.713	0.188	5			
28	0.108	0.287	5			
29	0.656	0.365	4			
30	0.467	0.486	4			

[a] Seronegative for MIgM (see text).
[b] Seronegative for MIgG (see text). The seronegative range was determined with 47 sera drawn from children before the administration of measles-mumps-rubella vaccine (mean, 0.008; standard deviation, 0.016) as previously described (1, 8).

The 20 sera containing RF were found to have MIgG (range of DOD values, 0.335 to 1.325); in the MIgM ELISA, all had DOD values below 0.075 (range, 0.034 to 0.057). The five pairs of sera obtained from patients with rubella all were in the MIgM-seronegative range.

DISCUSSION

The assay described is sensitive and specific and ideally suited for serological confirmation of recent measles infection. MIgM was detectable shortly after the onset of clinical measles and was present for more than 1 month following the onset of disease. No MIgM was found in the sera of normal MIgG-seropositive adults who had had measles many years previously.

TABLE 2. ELISA DOD values for MIgM and MIgG in several serum samples after treatment with DTT or SPA

Serum sample	ELISA	ELISA DOD value for indicated sera:		
		Untreated	DTT treated	SPA treated
Measles convalescent-phase serum	IgM	0.667	−0.008	0.570
	IgG	0.534	0.591	0.072
Measles convalescent-phase serum	IgM	0.181	0.014	0.135
	IgG	0.529	0.600	0.096
Cord serum	IgM	0.023	0.026	0.056
	IgG	0.351	Not Done	−0.038
Normal adult serum	IgM	0.052	0.030	0.032
	IgG	0.441	0.470	0.006

Sera having high titers of IgM directed against IgG, i.e., RF, have been shown in other IgM assays to produce false-positive results (3, 11, 12); additional manipulations have been necessary to remove RF. RF is commonly produced following viral infections (8, 11), including measles (11, 12). Thus, it is essential that this source of false-positive reactions be eliminated. By use of the antibody class capture assay, nonspecificity caused by RF was either markedly reduced (12) or not found (7). In our MIgM assay, none of 20 RF-positive sera were found to give false-positive results. The specificity of the system was further confirmed by finding that sera containing MIgM lost activity after treatment with DTT. Sera from patients with rubella, a disease sometimes confused with measles, lacked MIgM.

A single serum specimen submitted early in the course of an illness may provide an etiologic diagnosis in cases of suspected measles or in an uncertain febrile exanthema. At present, it is usually necessary to demonstrate a significant rise in total IgG to confirm a clinical diagnosis. For this reason, a second serum specimen must be obtained; if this is not available, the IgG assay of a single serum specimen may be difficult to interpret. Many of our patients already had higher levels of IgG in their initial serum specimens than in many convalescent-phase specimens. If a second serum

specimen is available, reporting of results must await the testing of that specimen. In several serum samples, moreover, a rise in IgG titer could not be shown. In contrast to this, MIgM was detected in 21 of 25 initial serum samples (84%) drawn up to 10 days after onset. In four other individuals who were initially seronegative, a subsequent serum sample was necessary; all of these were found to be positive for MIgM. In cases in which the initial serum sample is negative, therefore, a second serum sample drawn 1 to 3 weeks after the onset of illness should be tested. If this is also negative for MIgM, the diagnosis of measles is unlikely.

The MIgM ELISA has the advantage of not requiring the separation of IgM from the other serum antibodies or techniques to remove IgM or IgG. It can also be completed in one working day and requires only a small volume, 0.005 ml, of serum.

ACKNOWLEDGMENTS

We gratefully acknowledge the laboratory assistance of Z. M. Shehab, C. Grose, C. V. Sumaya, and E. K. Cobb. Serum samples were kindly supplied to us by V. Flannery, Houston City Health Department, Houston, Tex.; K. L. Herrmann, Centers for Disease Control, Atlanta, Ga.; L. M. Sehulster, Texas Department of Health, Austin; R. M. Pope, The University of Texas Health Science Center, San Antonio; R. Gonzales, Medical Center Hospital, San Antonio, Tex.; and B. Forghani, California Department of Health.

LITERATURE CITED

1. **Grose, C.** 1981. Immunization of inbred guinea pigs with varicella-zoster virus grown in a syngeneic transformed embryo cell line. J. Clin. Microbiol. **14**:229–231.
2. **Hinman, A. R., D. Brandling-Bennett, R. H. Bernier, C. D. Kirby, and D. L. Eddins.** 1980. Current features of measles in the United States: feasibility of measles elimination. Epidemiol. Rev. **2**:153–170.
3. **Kleiman, M. B., C. K. L. Blackburn, S. E. Zimmerman, M. L. V. French, and L. J. Wheat.** 1983. Rapid diagnosis of measles using enzyme-linked immunosorbent assay for measles immunoglobulin M. Diagn. Microbiol. Infect. Dis. **1**:205–213.
4. **McLaughlin, K. S.** 1982. Immunohematology; special test procedures, p. 404–410. *In* J. D. Bauer (ed.), Clinical laboratory methods, 9th ed. C. V. Mosby Co., St. Louis.
5. **Murphy, M. D., P. A. Brunell, A. W. Lievens, and Z. M. Shehab.** 1984. Effect of early immunization on antibody response to reimmunization with measles vaccine as demonstrated by enzyme-linked immunosorbent assay (ELISA). Pediatrics **74**:90–93.
6. **Naot, Y., G. Desmonts, and J. S. Remington.** 1981. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital *Toxoplasma* infection. J. Pediatr. **98**:32–36.
7. **Pedersen, I. R., A. Antonsdottir, T. Evald, and C. H. Mordhorst.** 1982. Detection of measles IgM antibodies by enzyme-linked immunosorbent assay (ELISA). Acta Pathol. Microbiol. Immunol. Scand. Sect. B **90**:153–160.
8. **Salonen, E. M., A. Vaheri, J. Suni, and O. Wager.** 1980. Rheumatoid factor in acute viral infections: interference with determination of IgM, IgG, and IgA antibodies in an enzyme immunoassay. J. Infect. Dis. **142**:250–255.
9. **Shehab, Z., and P. A. Brunell.** 1983. Enzyme-linked immunosorbent assay for susceptibility to varicella. J. Infect. Dis. **148**:472–476.
10. **Sumaya, C. V., Y. Ench, and R. M. Pope.** 1982. Improved test for IgM antibody to Epstein-Barr virus using an absorption step with *Staphylococcus aureus*. J. Infect. Dis. **146**:518–523.
11. **Tuokko, H.** 1984. Comparison of nonspecific reactivity in indirect and reverse immunoassays for measles and mumps immunoglobulin M antibodies. J. Clin. Microbiol. **20**:972–976.
12. **Tuokko, H., and A. Salmi.** 1983. Detection of IgM antibodies to measles virus by enzyme-immunoassay. Med. Microbiol. Immunol. **171**:187–198.
13. **van Griethuysen, A. J. A., R. de Graaf, J. A. M. van Druten, F. W. A. Heessen, J. T. M. van der Logt, and A. M. van Loon.** 1984. Use of the enzyme-linked immunosorbent assay for the early diagnosis of *Mycoplasma pneumoniae* infection. Eur. J. Clin. Microbiol. **3**:116–121.
14. **Voller, A., D. Bidwell, and A. Bartlett.** 1980. Enzyme-linked immunosorbent assay, p. 359–371. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology, 2nd ed. American Society for Microbiology, Washington, D.C.
15. **Weigle, K. A., M. D. Murphy, and P. A. Brunell.** 1984. Enzyme-linked immunosorbent assay for evaluation of immunity to measles virus. J. Clin. Microbiol. **19**:376–379.