JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1984, p. 376–379
0095-1137/84/030376-04$02.00/0

Vol. 19, No. 3

Enzyme-Linked Immunosorbent Assay for Evaluation of Immunity to Measles Virus

KRISTEN A. WEIGLE, M. DIANNE MURPHY, AND PHILIP A. BRUNELL*

Department of Pediatrics, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Received 26 August 1983/Accepted 8 December 1983

An enzyme-linked immunosorbent assay for the determination of immunity to measles virus was developed and standardized; it was compared to the hemagglutination inhibition and plaque reduction neutralization methods for sensitivity and specificity. The conditions of the enzyme-linked immunosorbent assay were adjusted such that groups of susceptible and immune individuals were clearly separable on the basis of the reactivity of a single (1:100) dilution of their sera to viral and control antigens. The range of values corresponding to susceptibility and immunity was defined by using the distribution of values observed from testing sera obtained from susceptible and immune control groups. The enzyme-linked immunosorbent assay was then applied in a study of measles vaccinees and found to be more sensitive than the hemagglutination inhibition method and equal in sensitivity to the plaque reduction neutralization method. The three methods were equal in specificity. Thus, the measles virus enzyme-linked immunosorbent assay is a rapid, reproducible, sensitive, and specific method for screening for the presence of measles antibody.

Although many tests are available for detecting measles antibody, an accurate method for determining immune status is sorely needed. The hemagglutination inhibition test (HI) is the most commonly used method of measuring measles antibody. This test is a less than ideal method for evaluating immunity against measles virus in that it is cumbersome to perform and subject to considerable variation (12). It is also less sensitive than the plaque reduction neutralization test (PRN) (1). A test for immune status that is easily performed, specific, and sensitive would be particularly useful in supporting the measles eradication program (6) to resolve the controversies regarding measles vaccine failure (3a, 5) or in supporting proposed changes in recommendations (M. D. Murphy, P. A. Brunell, A. W. Lievens, and E. Cobb, in press).

In this study, we used serum banks from groups of individuals with known immune status to standardize the enzyme-linked immunosorbent assay (ELISA) for determination of susceptibility. The measles virus ELISA was evaluated for its ability to separate immune individuals from susceptible individuals and was compared with the HI and PRN methods. When applied to a group of vaccinees, ELISA was as specific as, yet more sensitive than, HI.

MATERIALS AND METHODS

Human sera. Blood was obtained from 19 adults who were born before 1954 and who worked in a pediatric outpatient department. Sera from these adults served as the immune controls as the adults were born well before the introduction of measles vaccine and had undoubtedly been exposed to measles virus both during childhood and as a result of their occupations. For susceptible controls, blood samples were taken from 49 children between the ages of 14 and 35 months who had no history of exposure to natural or vaccine measles virus. These two groups of sera were used to standardize ELISA. Subsequently, blood was drawn from 301 children who had been immunized with measles, mumps, and rubella vaccines (MMR; Merck, Sharp, and Dohme Inc., West Point, Pa.) at 15 months of age or older, from 2 to 19 months previously. With these sera, we evaluated the utility of ELISA in screening for immune status. Parents, guardians, or subjects gave their written consent for participation.

Cells and virus. Vero cells (obtained from Paul Albrecht) were grown at 37°C in Eagle minimal essential medium supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine (MEM) containing 10% fetal bovine serum (FBS) (10% MEM). A 75-cm^2 monolayer of Vero cells was infected with the Edmonston strain of measles virus (obtained from Paul Albrecht). In 2 days when approximately one-half of the monolayer showed a cytopathic effect, the cells were scraped into 10 ml of 10% MEM, and 2.5-ml volumes were sonicated for 30 s. This virus stock was plaque titrated, diluted, and stored at −70°C in small volumes.

Antigen preparations. A 75-cm^2 monolayer of Vero cells grown in MEM with 5% FBS was rinsed in MEM lacking FBS and infected with ca. 1.2×10^4 PFU of the crude measles lysate. The cells were maintained in MEM lacking FBS. Three days later when a cytopathogenic effect was observed in 75% of the monolayer cells, the medium (M_1) was collected for antigen production and replaced with fresh MEM. Four days after infection when the entire monolayer showed a cytopathogenic effect, the cells of both the virus-infected flask and a mock-infected control flask were scraped into the medium, and then the medium was cleared of cells by sedimentation at $500 \times g$ for 20 min. Equal dilutions of this medium (M_2) and M_1 were compared in ELISA with known positive anti-measles virus sera; M_2 was found to bind a greater amount of antibody. Therefore, M_2 culture fluids were selected as the source of viral and control antigens. M_2 culture fluids were then centrifuged at $78,000 \times g$ for 2 h. The viral and control antigen pellets each were resuspended in 2 ml of 0.01 M phosphate buffer (pH 7.4) in saline (PBS). A subsequent sonication and low-speed centrifugation further clarified the antigens. Then the control antigen was adjusted to a protein concentration equal to that

* Corresponding author.

of the viral antigen (9). The optimum dilution of the viral antigen was determined by checkerboard titrations with the enzyme conjugate. Antigens were stored frozen at −70°C.

ELISA. ELISA was performed in polyvinyl microtiter plates (Costar, Cambridge, Mass.) with the following modifications of the method of Voller and Bidwell (11). Duplicate wells were coated with 0.1 ml of measles or control antigen in carbonate buffer for at least 18 h at 4°C. The fluid was removed, and wells were rinsed three times with PBS containing 0.05% Tween 20 (PBS-T). Serum diluted 1:100 in PBS-T containing 1% fetal calf serum and 10% globulin-depleted goat serum to decrease nonspecific reactivity (Z. Shehab, manuscript in preparation) was added to two wells containing control antigen and two wells containing measles antigen. After incubation at 37°C for 1 h and three PBS-T washes, 0.1 ml of a 1:1,500 dilution of goat anti-human immunoglobulin G (Antibodies, Inc., Davis, Cal.) conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) (kindly prepared by Ziad Shehab) was added to each well. After a 1-h incubation at 37°C, the plates were washed three times in PBS-T. Then 0.1 ml of the substrate (1 mg of *p*-nitrophenol phosphate per ml of diethanolamine buffer) was added to each well, and the plates were incubated at room temperature for 45 min. The enzyme reaction was stopped with 0.025 ml of 3 N NaOH. The optical density (OD) was measured at 405 nm with a Biotek EIA reader. The background, nonspecific reactivity of the two control wells was averaged and subtracted from the mean OD of the two wells containing measles antigen. This difference is referred to as the ΔOD.

HI antibody determination. Sera was heat inactivated at 56°C for 30 min and then absorbed with vervet erythrocytes (obtained from Eugene Buynak). Twofold dilutions, from 1:5 to 1:160, were tested by the method of Norrby and Gollmar (10) adapted to microtiter plates. Four hemagglutinin units of a measles antigen (obtained from Kenneth Herrmann) were used in each well; an antigen back titration was performed with each assay. A 0.4% suspension of erythrocytes (supplied by E. Buynak) drawn from one African green monkey less than 1 week previously was added to each well, and hemagglutination was read after 15 min. A serum control for each serum tested, three cell controls, and a standard high titer, low titer, and negative serum were included in each assay. Sera which did not inhibit hemagglutination when diluted 1:5 are subsequently referred to as negative for HI antibody.

PRN antibody determination. Selected sera were tested at a 1:8 dilution for neutralizing antibody as described by Albrecht et al. (1). Plastic 24-well culture plates (Linbro, Hamden, Conn.) were seeded with 150,000 Vero cells (passage, 160 to 190) per well and grown for 3 days in 10% MEM. Sera diluted in PBS were heat inactivated at 56°C for 30 min, then mixed with equal volumes of 4×10^2 to 8×10^2 PFU of measles virus, and incubated at 37°C for 1 h. Medium was removed from the culture wells, and 0.1 ml of the virus-serum mixture was adsorbed onto the monolayers for 1 h at 37°C. This mixture was then replaced with MEM containing 5% FBS and 1% (wt/vol) agarose (5% MEM-A; Sea Kem, FMC Corp., Rockland, Maine). Each dilution of sera was tested in triplicate. Four days later, 0.5 ml of 5% MEM-A containing 1:20,000 (wt/vol) of neutral red was layered onto each well. In 48 h, plaques became visible and were counted through the agarose. Reduction of 50% of the plaques by sera was calculated by using Karber's formula. Sera with titers of less than 1:8 are referred to subsequently as negative for PRN antibody.

RESULTS

Standardization of measles virus ELISA. The optimal concentrations of antigen, serum, and enzyme conjugate were determined by cross titrations, using the sera from known susceptible children to evaluate specificity and from immune adult controls to evaluate sensitivity. Dilutions were chosen which maximized the ΔOD values of the immune serum controls without increasing the ΔOD values of the susceptible controls. These standardization titrations thereby ensured that the susceptible population was clearly separable from the immune population on the basis of the ΔOD value of a single dilution of serum. The length of incubation of the enzymatic reaction (45 min) was chosen to maximize the difference between reactivity to viral antigen and that to control antigen (Fig. 1). The 45-min time period was also at the plateau phase of the enzyme reaction, thus minimizing the effect of minor variations in reaction time on the ΔOD value.

The mean ΔOD value for the 49 susceptible children was 0.016 ± 0.016. All ΔOD values that were three standard deviations above this mean, that is above 0.062 limits, were considered seropositive (Fig. 2). The serum samples from all 19 immune controls had ΔOD values above the 0.062 level.

Screening for immune status. Sera from 301 children previously vaccinated were tested with the standardized measles virus ELISA. Each assay included a low seropositive, a high seropositive, and a seronegative serum. This single dilution method permitted the testing of 24 different

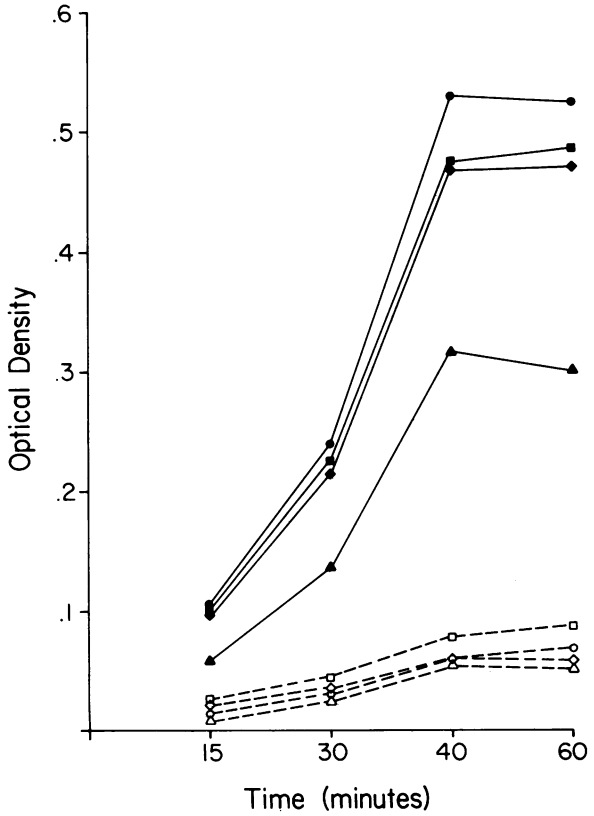

FIG. 1. Effect of enzyme reaction time on reactivity to viral and control antigens. Sera from four immune adults were reacted with measles antigen (●) and control antigen (○) in a measles virus ELISA. The OD measurements were made at the indicated times after the addition of enzyme substrate.

sera per microtiter plate. ΔOD values for all but 5 of these children were in the seropositive range. These five sera were in the seronegative range on repeated testing and were also lacking in HI and neutralizing antibody.

Comparison of ELISA to HI. Because HI has been the commonly used standard method for determining immune status, all sera tested by ELISA were also evaluated for HI antibody. Both ELISA and HI methods had specificities of 100%, in that the 49 susceptible children were found to be seronegative by both assays. However, 2 of the 19 immune adults and 11 of 296 vaccinees who were seropositive by ELISA had measles virus HI titers of less than 1:5. The sera of the 2 adults and 11 children which were negative by HI and positive by ELISA were also positive by PRN.

In general, those sera with greater levels of HI antibody levels had higher ΔOD values (Table 1). However, one could not predict the HI titer or the basis of the ΔOD value of a given serum. An HI titer is a discontinuous measurement and therefore is an approximation of antibody measurement. Each dilution interval represents a range of antibody measurements, perhaps explaining the range of ΔOD values for each dilution tested by HI.

Comparison of ELISA and HI to PRN. Because of the discrepancies between ELISA and HI we compared these two methods with PRN, a test previously found to be more sensitive than HI (1). To assure specificity of PRN, 15 susceptible children for whom sufficient volumes of sera were available were tested for neutralizing antibody. None of these sera reduced the number of measles virus PFU by more than 50% at a 1:8 dilution. Sera from 11 adults and 22 vaccinees, including the 2 adults and 16 vaccinees who were HI antibody negative, were tested by PRN. The results of ELISA and PRN were concordant for each serum, whereas HI was less sensitive than PRN (Table 2).

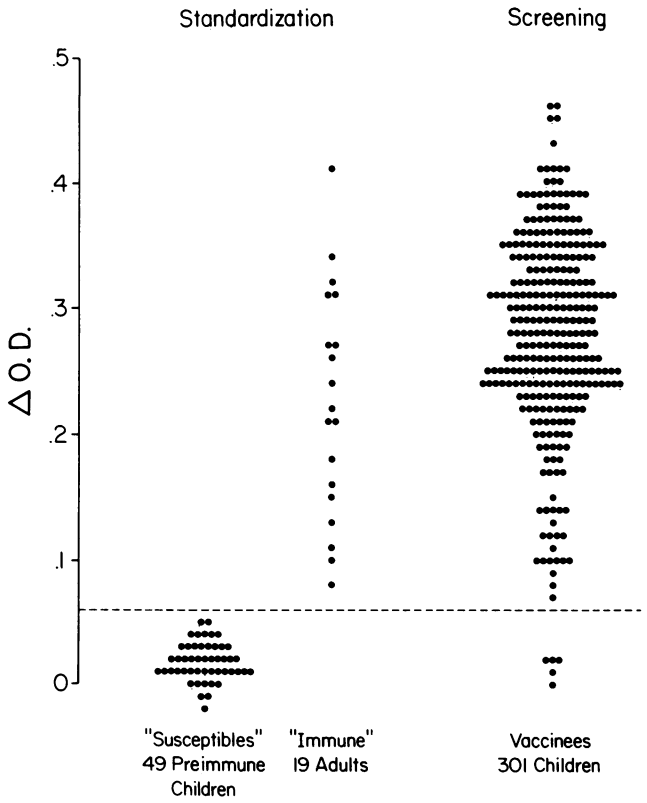

FIG. 2. Distribution of ΔOD values for control and test populations. ΔOD is the difference in reactivity (measured as OD) to viral and control antigens. The dotted line at 0.062 (ΔOD) separates the immune group from the susceptible group.

TABLE 1. Comparison between quantity of measles antibody as determined by ELISA and HI

No. of subjects	Measles antibody determined by	
	HI (titers)	ELISA[a]
5	<1:5[b]	0.02 (0.01)
11	<1:5[c]	0.14 (0.06)
61	1:5	0.23 (0.06)
102	1:10	0.29 (0.07)
89	1:20	0.31 (0.06)
28	1:40	0.34 (0.07)
5	1:80	0.40 (0.03)

[a] Values are expressed as mean Δ OD (SD).
[b] PRN-determined titers, <1:8.
[c] PRN-determined titers, ≥1:8.

Reproducibility of measles virus ELISA. Interassay variability was examined by testing three sera 11 times over a 4-month period. Two measles antibody-positive samples were always above the seronegative level, and the standard deviations were small (18 and 22%) relative to the ΔOD values. The third serum sample collected from a measles virus-susceptible child was consistently within the seronegative range.

DISCUSSION

We have standardized and evaluated a measles virus ELISA for the purpose of determining measles virus immune status. Serum banks of known immune status facilitated standardization of the assay to ensure maximal sensitivity without sacrificing specificity. Because the concentration of all of the reactants could be readily adjusted, standardization of ELISA to give a very sensitive and specific, but easily interpretable, test is much more feasible than it is with HI. This standardization clearly defined the relationship between reaction intensity and the presence of antibody, an essential feature for a test of immune status. The assay is reproducible and bears a close relationship to antibody titer as measured by HI. This measles virus ELISA, which used a single serum dilution, is an extremely rapid and simple method requiring a very small volume of serum and easily stored reagents. It is therefore ideal for screening large numbers of samples to assess vaccine efficacy or for epidemiological investigations.

The measles virus ELISA was more sensitive than the measles virus HI in the testing of relatively low-titered serum. In prior studies, ELISA was not observed to be more accurate than HI for the diagnosis of clinical measles (2, 4, 7, 8). After a primary measles virus infection, measles antibody rises to a high level easily detected by HI. It is only in the

TABLE 2. Measles antibody determined by ELISA and HI compared with that determined by PRN

Method	Measles antibody (no.) determined			
	Adults (n = 11)		Vaccinees (n = 22)	
	+	−	+	−
PRN	11	0	17	5
ELISA	11	0	17	5
HI	9	2	6	16

testing for low levels of antibody that the lack of sensitivity of HI is apparent. A recent study of a commercially available measles virus ELISA (3) found a small number of discrepancies in HI and ELISA methods and indicated that the measles virus ELISA may be more sensitive than HI. By the use of standard groups of sera and PRN, we determined that the differences between the results of ELISA and HI were due to the greater sensitivity of ELISA. In fact, the measles virus ELISA appears equivalent to the very time-consuming, but very sensitive, PRN.

The measles virus ELISA described in this communication should facilitate studies of large numbers of sera to determine measles virus immune status. It is particularly useful for the detection of low levels of antibody found long after natural infection or immunization.

ACKNOWLEDGMENTS

This work was supported in part by contract no. 200-80-0546 from the Centers for Disease Control, Atlanta, Ga.

LITERATURE CITED

1. **Albrecht, P., K. Herrmann, and G. R. Burns.** 1981. Role of virus strain in conventional and enhanced measles plaque neutralization test. J. Virol. Methods **3**:251–260.
2. **Blomberg, J., I. Nilsson, and M. Andersson.** 1983. Viral antibody screening system that uses a standardized single dilution immunoglobulin G enzyme immunoassay with multiple antigens. J. Clin. Microbiol. **17**:1081–1091.
3. **Boteler, W. L., P. M. Luipersbeck, D. A. Fuccillo, and A. J. O'Beirne.** 1983. Enzyme-linked immunosorbent assay for detection of measles antibody. J. Clin. Microbiol. **17**:814–818.

3a. **Brunell, P. A., K. Weigle, M. D. Murphy, Z. Shehab, and E. Cobb.** 1983. Antibody response following measles-mumps-rubella vaccine under conditions of customary use. J. Am. Med. Assoc. **250**:1409–1412.

4. **Forghani, B., and N. J. Schmidt.** 1979. Antigen requirements, sensitivity, and specificity of enzyme immunoassays for measles and rubella viral antibodies. J. Clin. Microbiol. **9**:657–664.
5. **Hayden, G. F.** 1979. Measles vaccine failure. Clin. Pediatr. **18**:155–167.
6. **Hinman, A. R., D. L. Eddins, C. D. Kirby, W. A. Orenstein, R. H. Bernier, P. M. Turner, and A. B. Bloch.** 1982. Progress in measles elimination. J. Am. Med. Assoc. **247**:1592–1595.
7. **Kahane, S., V. Goldstein, and I. Sarov.** 1979. Detection of IgG antibodies specific for measles virus by enzyme-linked immunosorbent assay (ELISA). Intervirology **12**:39–46.
8. **Kleiman, M. B., C. K. L. Blackburn, S. E. Zimmerman, and M. L. V. French.** 1981. Comparison of enzyme-linked immunosorbent assay for acute measles with hemagglutination inhibition, complement fixation, and fluorescent-antibody methods. J. Clin. Microbiol. **14**:147–152.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
10. **Norrby, E., and Y. Gollmar.** 1972. Appearance and persistence of antibodies against different virus components after regular measles infection. Infect. Immun. **6**:240–247.
11. **Voller, A., and D. E. Bidwell.** 1976. Enzyme-immunoassays for antibodies in measles, cytomegalovirus infections and after rubella vaccination. Br. J. Exp. Pathol. **57**:243–247.
12. **Yeager, A. S., J. H. Davis, L. A. Ross, and B. Harvey.** 1977. Measles immunization: successes and failures. J. Am. Med. Assoc. **237**:347–351.