Detection and Titration of Measles Virus Antibody by Hemagglutination Inhibition and by Dot Immunobinding

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Received 31 May 1990/Accepted 25 September 1990

Measles continues to be a major disease of both human and nonhuman primates. The dot immunobinding assay, a modified enzyme immunoassay, permits the detection of measles virus antibody in the nonlaboratory setting with either serum or whole blood collected on filter paper.

Measles is a significant disease of both human and nonhuman primates. Since the introduction of human vaccine programs, the incidence of measles in humans has sharply decreased. Outbreaks continue to occur, however, and the determination of the presence or absence of antibody continues to be the method of choice for detection of immunity. Measles is also an important disease in nonhuman primates, particularly among young animals. Species variation in susceptibility to measles virus infection is recognized. Subclinical infection is the most common manifestation, but fatalities do occur, particularly in New World species. Monkeys in the wild apparently are free of measles, contracting infection only after contact with humans (21).

A number of diagnostic tests for detecting antibody response to measles virus are available. Of these, the hemagglutination inhibition (HI) test has been most extensively used (19). The specificity and sensitivity of the HI test are equal to those of the serum neutralization test; moreover, the HI test is more sensitive than the complement fixation test, but it is reportedly less sensitive than the enzyme immunoassay (3, 15). The HI test has the disadvantage that erythrocytes are required for the test and for the treatment of test sera to remove nonspecific agglutinins. The dot immunobinding assay (DIA), a modified enzyme immunoassay, has been shown to equal enzyme immunoassays in the detection of antibodies to a wide variety of viral antigens, and it is easier to perform (2, 10, 25). The use of filter paper to apply serum or blood directly to the nitrocellulose sheet permits the direct testing of whole blood, thus eliminating the need for venipuncture and the separation of serum from the cells (11, 17). Another advantage of the DIA over the HI test is that the DIA can be used in the field or office setting, as no laboratory equipment is needed.

In this report, the HI test and the DIA are compared for their abilities to detect measles virus antibody in human sera and whole blood and in nonhuman primate sera and whole blood. Preliminary studies comparing the DIA with the HI test indicated a good correlation (11). Other investigators have demonstrated the use of filter paper for the collection and testing of measles virus antibody (4, 6, 22, 27). It was also of interest to ascertain how the DIA compares with the HI test for detecting and titrating measles virus antibody.

Serum samples from humans or from nonhuman primates (Macaca mulatta) were obtained after venipuncture in the usual manner. Whole blood was collected on precut filter paper after finger puncture and either was used directly in the DIA or, after being dried, was stored at 4°C until it was used (17). A variety of filter papers have been used in the DIA; for this study, Whatman filter paper (grade 4CHR, catalog no. 3004633) in 1-in. (ca. 2.5-cm)-wide rolls was cut into 3- to 5-mm strips.

Measles virus antigen was used in the HI test and the DIA as previously described (11). The DIA procedure has been reported in detail (11–14). Sera were tested either undiluted or at various dilutions by dipping precut filter paper strips into the serum or serum dilution and draining excess fluid off by carefully passing the strip over the lip of the tube. After being dried, blood-saturated strips were applied directly to the nitrocellulose sheet, care being taken that the entire strip was in contact with the nitrocellulose sheet, as evidenced by the strip becoming moist. If titrations were done on the whole-blood strips, two strips were placed in 0.5 ml of phosphate-buffered saline and allowed to stand overnight at 4°C, and twofold dilutions of this eluate were made after an initial dilution of 1:10. All results reported here are based on initial dilutions.

Fifty-two human specimens were tested by both the HI test and the DIA either as whole blood (with or without elution) or as serum. Thirty-two of these specimens were triturated for antibody endpoint. Sixteen of the 32 serum samples had titers of less than 1:8 (negative) by the two procedures; 8 showed a one-tube difference, 1 indicated a two-tube difference, and the remaining 7 had the same titer by both the HI test and the DIA (Table 1). Of the 20 blood specimens tested as whole blood and tested after elution, 18 were in complete agreement, with the numbers of positives and negatives the same for both the whole blood and the eluate. For the remaining two samples, the eluates were positive at a 1:10 (negative at 1:20) dilution and equivocal results were obtained with the corresponding whole blood. A total of 325 rhesus monkey (M. mulatta) serum samples were individually tested at different times and at different dilutions by both the DIA and the HI test. A total of 258 serum samples were positive by both the HI test and the DIA (79.0%), and 275 were positive by the DIA (85.0%). Another 78 serum samples were then simultaneously tested at the same dilution (1:10), and 52 (67.0%) samples were positive by both procedures. The remaining 26 serum samples (33.0%) were negative (<1:10) by both the DIA and the HI test.

In a vaccine study, 60 animals whose serum samples had titers of <1:10 all seroconverted (fourfold) after vaccination, as determined by both procedures. It is of interest that only a one-tube difference in serum titers (with one exception) was observed between the HI test and the DIA results. In

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Table 2, 25 representative serum samples are listed, showing the pre- and postvaccination titers obtained by the DIA and the HI test.

The development of a viral diagnostic test that is rapid, sensitive, specific, and cost-effective has merit, particularly if it requires little laboratory equipment and needs no highly specialized, trained personnel and therefore can be done in the office setting or in the field. An added advantage, particularly when individuals are being screened for the presence or absence of antibody, is that whole blood can be applied directly to filter paper in the DIA. A number of well-documented reports indicate the suitability of using filter paper to collect whole blood for detecting antibody, including measles virus antibody (1, 4, 5, 7–9, 16–20, 22–29.) As shown herein, there is excellent agreement between the DIA and the HI test for detecting measles virus antibody.

Excellent reproducibility was also shown when pre- and postvaccination sera were compared by the DIA and the HI test. Preliminary data comparing human patient sera following measles virus infection indicate the appropriateness of the DIA for detecting an antibody increase when acute-phase and convalescent-phase sera are tested. These results suggest that the DIA may serve for serodiagnosis of measles virus infection as well as for evaluation of vaccination programs and epidemiological surveillance. This comparison of the DIA with such standard procedures as the HI test and the immunofluorescence assay (data not shown) for detecting measles virus antibody, as well as its applicability to other test antigens, suggests wider employment of the DIA in the laboratory setting.

The technical assistance of Melisande Meyers and Frances Pfuger is gratefully acknowledged.

Portions of this study were supported by Small Business Innovation Research Program grants 1R43 AI29317-01 and 1R43 AI128604-01.

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