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Evaluation of Monoclonal Antibody-Based Capture Enzyme Immunoassays for Detection of Specific Antibodies to Measles Virus

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Monoclonal antibodies to the hemagglutinin protein, fusion protein, phosphoprotein, matrix protein, and nucleoprotein of measles virus were evaluated as detector antibodies in capture enzyme immunoassays (EIAs) for the detection of specific serum immunoglobulin G (IgG), IgA, and IgM antibodies to measles virus. A pool of monoclonal antibodies to hemagglutinin protein and nucleoprotein proved optimal and was further evaluated. Specific IgM was detected in 97% of adolescents with clinical measles, 97% of infants 3 weeks postvaccination, and <1% of normal serum specimens. Specific IgA antibodies were found in 97% of adolescents with clinical measles, 97% of infants 3 weeks postvaccination, and <1% of normal serum specimens. Specific IgA antibodies were found in 97% of clinical measles cases and vaccinees, in 26% of healthy persons, and in 36% of infants 8 months postvaccination; consequently, IgA antibodies were not a useful indicator of recent measles infection. A significant increase in IgG antibodies between paired specimens was detected in 92% of clinical cases and all vaccinees. Only 59% of infant specimens had persistent IgG antibodies as detected by capture EIA at 8 months postvaccination, whereas all specimens had antibodies as detected by hemagglutination inhibition and plaque neutralization. An alternative indirect EIA, in which antigen was directly absorbed to the solid phase, was more sensitive than the capture design, detecting IgG antibodies in all infants postvaccination. When standardized with a microneutralization assay for the detection of persistent antibodies, the indirect IgG EIA gave predictive values for positive and negative tests exceeding 90%. Our capture IgM and indirect IgG EIAs provide a practical combination of serologic tests for the determination of acute measles virus infection and past exposure to measles virus or vaccine, respectively.

Since 1983, the annual incidence of measles in the United States has gradually increased, with a dramatic rise in the number of new cases reported in 1989 and 1990 (7). This increased incidence has created a renewed interest in measles diagnostics. Methods for detecting measles virus-specific antibodies have included complement fixation (CF) (17), hemagglutination inhibition (HI) (13), hemolysis inhibition (21), plaque neutralization (PNt) (1, 22), immunofluorescence (8), radioimmunoassay (4), and, more recently, indirect enzyme immunoassays (EIAs) for immunoglobulin G (IgG) (6, 9, 15, 16a, 29, 30) and IgM (23, 28) antibodies. Indirect EIAs—in which viral antigen is coated directly onto the solid phase—are particularly popular because of their sensitivity and simplicity of design (9), but they have also been criticized for their susceptibility to nonspecific reactions (11). To address these problems, reverse or capture EIAs—in which the solid phase is first coated with an anti-human immunoglobulin class-specific antibody and then with patient serum—have been developed to detect specific IgM antibodies (10, 16, 18). These assays are noted for their enhanced specificity and resistance to interference from rheumatoid factor (RF) (27).

In this study, we evaluated capture EIAs for measles virus-specific IgG, IgA, and IgM antibodies by using detector monoclonal antibodies (MAbs) to measles virus proteins, as well as an indirect EIA for measles virus-specific IgG. We further assessed the utility of these assays in the diagnosis of acute measles virus infection and the determination of host immune status.

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MATERIALS AND METHODS

Serum specimens. Human serum specimens used in this study included the following: (i) acute- and convalescent-phase specimens collected from 59 previously vaccinated adolescents with clinical measles, (ii) specimens from seven unvaccinated infants with clinical measles, (iii) prevaccination and postvaccination specimens from 32 children ≥15 months of age receiving primary vaccination with MMR_{II} (Merck Sharp & Dohme, West Point, Pa.), (iv) a single specimen from a person with subacute sclerosing panencephalitis (SSPE), (v) single and paired specimens from 120 normal adult blood donors, healthy children <4 years of age, and persons with various other virus infections, (vi) 123 specimens with negative or low positive EIA values from 5- to 6-year-old children vaccinated at the appropriate age (approximately 18 months of age), and (vii) 26 specimens positive for RF by latex agglutination.

Antigen. Antigen for capture EIAs was prepared from cell culture-adapted Edmonston strain measles virus (American Type Culture Collection, Rockville, Md.). Virus was absorbed for 1 h at 37°C onto monolayers of E-6 Vero cells followed by the addition of Eagle's minimal essential medium (MEM) supplemented with fetal calf serum (2%), glutamine (2 mM), penicillin (200 U/ml), streptomycin (200 μg/ml), and amphotericin B (10 μg/ml). Cultures were then incubated until the monolayers developed 3–4+ cytopathic effect. Cultures were frozen and thawed three times, and the cellular debris was pelleted by low-speed centrifugation (500 × *g*) for 15 min. Supernatants were collected and stored at −70°C until use. Uninfected cells were similarly processed for negative control antigen.

Antigen for indirect EIAs was prepared by a modification

TABLE 1. MAbs to measles virus-specific proteins

Antibody	Protein[a]	Fusion inhibition	Neutralization
83VIIKK2	N	−	−
81I168	N	−	−
81I366	H	−	+
79XVV17	H	−	+
84IM7	F	+	+/−
83VIIY2	P	−	−
84IIB7	M	−	−

[a] Protein specificity was determined by immunoprecipitation of radiolabeled or biotinylated proteins.

of a method previously described (14). Virus was cultured as described above, except that cells were dislodged into the medium with a scraper and pelleted by low-speed centrifugation. The pelleted cells were then washed once with Eagles's MEM without fetal calf serum, pelleted, and resuspended in 1/20 of the original culture volume of 0.1 M glycine buffer (pH 9.5). The cell suspension was then placed on ice and sonicated with three 10-s bursts at a 50% probe intensity setting by using a Microson Ultrasonic Cell Disrupter (Heat Systems-Ultrasonics, Inc, Farmingdale, N.Y.). Supernatants were collected and stored as described above.

MAbs. Detector MAbs with specificities for the fusion (F) protein, hemagglutinin (H) protein, nucleoprotein (N protein), phosphoprotein (P protein), and matrix (M) protein of measles virus were kindly provided by Dale McFarlin, National Institutes of Health, Bethesda, Md. (5) (Table 1). Biotinylation of MAbs was conducted as previously described (3).

Capture EIAs. Goat anti-human IgG, IgA, and IgM antibodies (Organon Teknika Corp., West Chester, Pa.) diluted 1:1,000 in 0.01 M phosphate-buffered saline (PBS), pH 7.2, were separately added to Immulon II microtiter plates (Dynatech Laboratories, Alexandria, Va.) and incubated for 1 h at 37°C. All reagent volumes were 75 μl per well, and PBS with 0.5% gelatin and 0.15% Tween 20 (PBS/G/T) was used as the diluent for all subsequent steps. Plates were then washed three times with PBS containing 0.05% Tween 20, and a 1:100 dilution of each serum specimen was added to four consecutive wells and incubated for 1 h at 37°C. Plates were washed three times, and measles virus-positive antigen and negative control cells diluted 1:10 in a 1:4,000 final dilution of biotinylated anti-measles virus MAbs were added to duplicate wells for each specimen and incubated for 3 h at 37°C. Plates were then washed three times, and a 1:3,000 dilution of streptavidin-peroxidase (Amersham International, Amersham, United Kingdom) was added and incubated for 20 min at 37°C. After five washes, a solution containing 0.1 mg of 3,3',5,5'-tetramethylbenzidine per ml (TMB) (Sigma Chemical Co., St. Louis, Mo.) and 1.6 μl of 3% H_2O_2 per ml in 0.1 M citrate-acetate buffer, pH 5.5, was added and incubated for 15 min at room temperature. Color development was stopped by the addition of 2 M H_2PO_4, and the absorbance was read at 450 nm with a Vmax Microplate Reader (Molecular Devices Corporation, Palo Alto, Calif.). Results were expressed as P−N values, defined as the average differences in measured absorbance values between measles virus-positive antigen and negative control cells in duplicate wells of test sera. To determine the cutoff values for a positive test, 32 serum specimens from prevaccinated infants with no detectable HI or PNt antibodies were tested. The cutoffs chosen for the IgG, IgA, and IgM capture EIAs were the average values determined for these specimens plus 3 standard deviations, i.e., 0.11, 0.053, and 0.085, respectively.

Indirect EIAs. An indirect measles EIA was performed by a modification of the method described by Boteler et al. (6). Briefly, measles virus-positive antigen and negative control cells were each diluted 1:40 in deionized H_2O and dried onto microtiter plates overnight. Following complete drying, the plates were washed three times and a 1:200 dilution of the serum specimens in PBS/G/T with 10% normal control cells was added to duplicate positive and negative antigen wells and incubated for 1 h at 37°C. Plates were then washed three times, and a 1:40,000 dilution of peroxidase-labeled goat anti-human IgG antibody (Kirkegaard & Perry, Gaithersburg, Md.) was added and incubated for 1 h at 37°C. Plates were washed five times and the assay was completed as described above.

In both capture and indirect EIAs, a significant increase in the level of measles virus-specific antibodies was defined as a ≥50% increase in P−N values between acute- and convalescent-phase specimens.

Microneutralization EIA. A microneutralization EIA was conducted by a modification of a method previously described (2). Starting with a dilution of 1:5, serum specimens were serially diluted (50 μl per well) in flat-bottomed, 96-well tissue culture plates (Costar, Cambridge, Mass.), and 50 μl of 100 50% tissue culture infective doses of Edmonston virus was added to each well and incubated for 2 h at 4°C. Uninfected E-6 Vero cells were harvested by trypsinization and diluted to 7.5×10^4 cells per ml in Eagle's MEM with 1% fetal calf serum, and a 100-μl suspension was added to each well. Plates were incubated for 3 days at 37°C. After incubation, the contents of all wells were aspirated, washed three times, and fixed by incubation for 15 min at 4°C with 75 μl of 80% (vol/vol) acetone in PBS per well. Plates were aspirated and precoated with 150 μl of PBS/G/T per well for 30 min at 37°C. Plates were then washed three times, and biotinylated MAb 83VIIKK2 diluted 1:300 in PBS/G/T (75 μl per well) was added to all wells and incubated at 37°C for 1 h. The assay was completed with the addition of streptavidin-peroxidase and TMB as described above. Each dilution of antibody was run in quadruplicate, and each assay included control, wells with diluent instead of sera, wells with uninfected cells, and a back titration of virus to monitor assay performance. End point titers were determined as previously described (2).

Other measles antibody assays. HI and CF assays were performed previously at the New Jersey Public Health Department, Trenton, N.J., by using standard methods. The PNt assay was performed by Paul Albrecht, Food and Drug Administration, Bethesda, Md.

Determination of total IgM and IgG antibodies. Selected serum specimens were fractionated by rate zonal centrifugation in continuous 10 to 40% sucrose gradients at 100,000 × *g* for 16 h at 4°C (Beckman Instruments Inc., Palo Alto, Calif.), and the first 12 fractions (approximately 0.4 ml each) from each gradient were collected and assayed for total and measles virus-specific IgM and IgG antibodies. Total antibodies were measured by coating each fraction directly onto microtiter plates—or plates previously coated with anti-human IgM or IgG antibodies—and incubating the plates for 1 h at 37°C. After a washing, peroxidase-conjugated goat anti-human IgM or IgG antibodies (Kirkegaard & Perry) were added and the assay was completed as described above. Measles virus-specific antibodies in each fraction were measured by capture EIA.

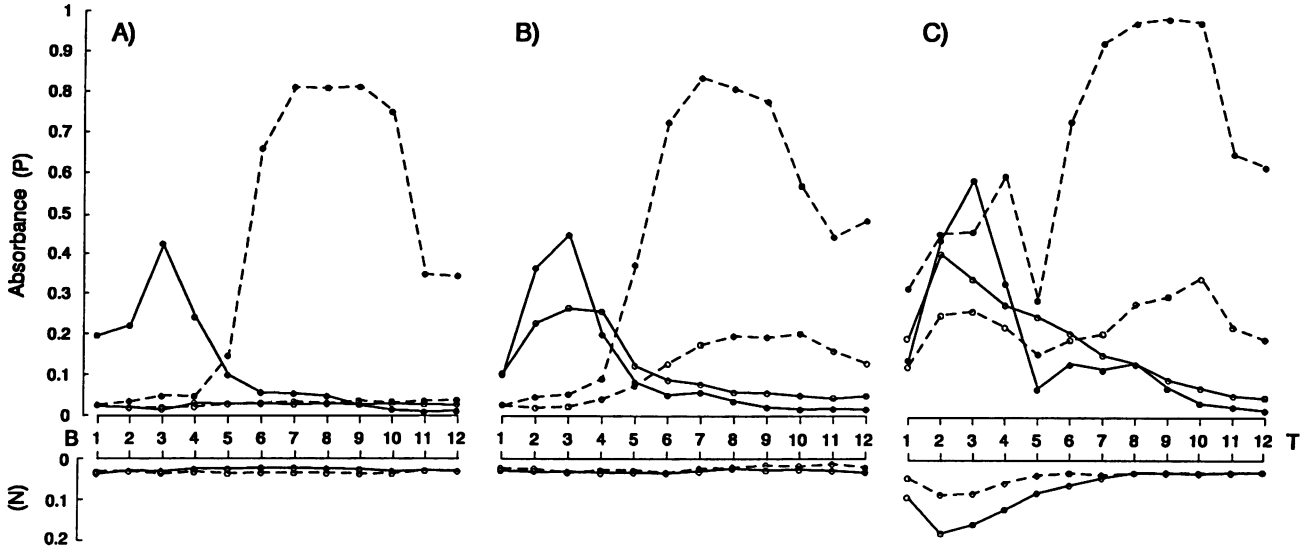

FIG. 1. Sucrose gradient fractions (bottom [B] to top [T]) of measles virus antibody-negative (A), measles virus IgG- and IgM-positive (B), and measles virus IgG- and RF-positive (C) serum specimens. Total serum IgM (-●-) and IgG (-●-) antibodies and measles virus-specific IgM (-○-) and IgG (-○-) antibodies are represented. Absorbance values are against positive (P) and negative (N) measles virus antigen as measured by EIA.

Determination of RF. RF was assayed by an EIA previously described (19). Results were standardized with a serially diluted RF reference serum obtained from the World Health Organization International Laboratory of Biological Standards, Copenhagen, Denmark.

RESULTS

Evaluation of MAbs. To determine the relative reactivity of the detector MAbs in capture EIAs, each MAb was tested against a panel of acute- and convalescent-phase serum specimens obtained from 10 persons with recent measles infection and 1 person with SSPE. Assays based on MAbs to P protein and M protein failed to detect measles virus-specific antibodies in all serum specimens tested. F protein MAb assays detected a very weak rise in IgG, IgA, and IgM antibodies in persons with acute measles but failed to detect specific IgG in the SSPE specimen. H protein MAb assays were strongly reactive with serum specimens from patients with acute measles but detected relatively low levels of IgG in the SSPE specimen. N protein MAb assays were moderately reactive with sera from acute measles cases and detected very high levels of IgG in the SSPE specimen. Assays using MAbs 79XVV17 (H protein) and 81I168 (N protein) gave the best signal-to-noise ratios for their respective proteins and were pooled for subsequent studies.

Capture EIA specificity. To evaluate the specificity of the capture EIAs, single and paired serum specimens from 120 healthy adult serum donors, healthy children, and persons with other virus infections were tested for measles virus-specific IgG, IgA, and IgM antibodies. Of the 120 persons tested, specific IgG was detected in 77 (64%), IgA in 31 (26%), and IgM in 1 (<1%). No significant increases in antibody levels between paired specimens were detected.

When 24 serum specimens positive for RF by latex agglutination were tested, 3 were positive by IgM capture EIA. These three specimens had the highest combined RF and measles virus-specific IgG values. To determine whether IgM RF mediated false-positive results, these specimens were fractionated on sucrose gradients and the successive fractions were assayed for total and measles virus-specific IgG and IgM antibodies. The distributions of IgG and IgM antibodies in the gradient fractions of representative measles virus antibody-negative, measles virus IgG and IgM antibody-positive, and measles virus IgG- and RF-positive specimens are illustrated in Fig. 1. Total IgG and measles virus-specific IgG antibodies were absent in the IgM fractions of the measles virus antibody-negative and measles virus antibody-positive specimens but were detected in the IgM fractions of the RF-positive serum specimens; background signal was also elevated in the IgM fractions of the RF-positive specimens.

Capture EIA sensitivity: confirmation of measles virus infection. Of 59 previously vaccinated adolescents with clinical measles, 46 (78%) and 41 (69%) had detectable increases in antibody levels as determined by HI and CF, respectively, whereas 54 (92%) had detectable increases in specific IgG antibody levels by capture EIA (Fig. 2). Fifty-seven (97%) adolescents also had detectable IgM and IgA antibodies in either or both serum specimens over the course of the study. IgM antibodies were detected in a high proportion of cases at the onset of rash; 51 of 55 (92%) were IgM positive within a week after the onset of symptoms, with the peak of the antibody response at about 2 to 3 weeks after onset. IgA and IgG responses peaked at about 1 to 2 weeks after onset. Of seven infants with clinical measles and no history of vaccination or evidence of past measles infection (specific IgG and IgA antibodies were not detected in their acute-phase specimens), all developed IgG and IgA responses and all had detectable IgM antibodies (data not shown).

Capture EIA sensitivity: detection of measles virus-specific antibodies postvaccination. Of 32 infants tested prior to vaccination, none had antibodies as detected by HI (titer of <8), PNt (titer of <4), or capture EIA (Fig. 3). At 3 weeks postvaccination, all developed antibodies by HI and PNt and specific IgG antibodies by capture EIA, and 31 (97%) developed both IgA and IgM antibodies. At 8 months postvaccination, all infants had antibodies detectable by HI and PNt whereas only 19 (59%), 10 (31%), and 4 (13%) had IgG, IgA,

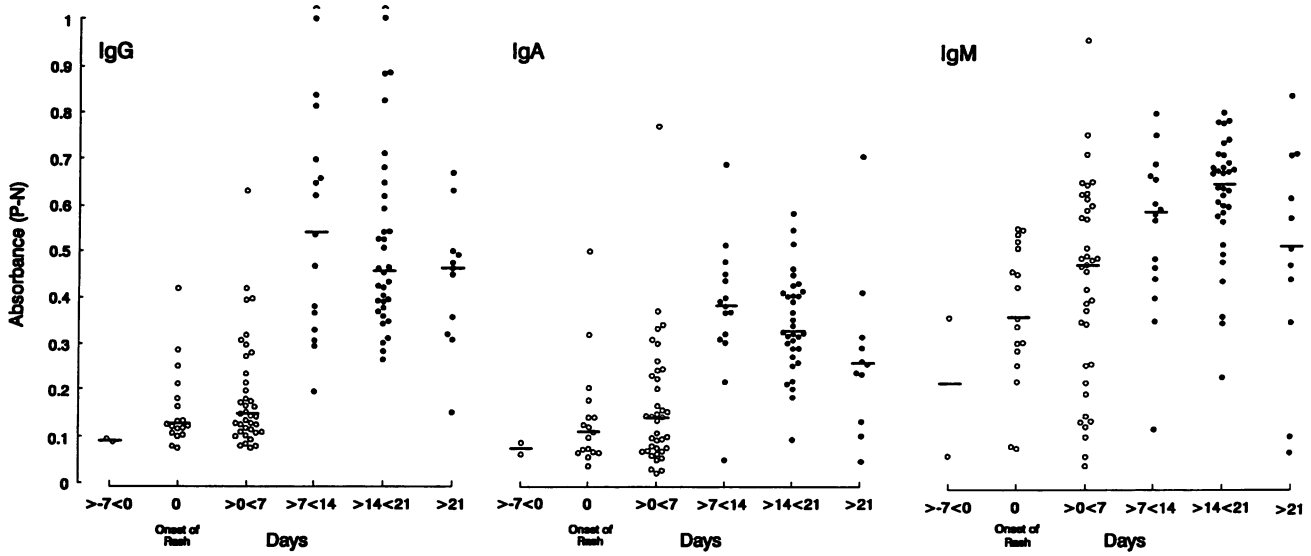

FIG. 2. Measles virus-specific IgG, IgA, and IgM antibody responses in 59 previously vaccinated adolescents with clinical measles. Paired acute-phase (○) and convalescent-phase (●) serum specimens were collected for each case. Each bar represents the median value for that data group.

and IgM antibodies, respectively, detectable by capture EIA.

Comparison of capture and indirect IgG EIAs. The low sensitivity of the IgG capture EIA with serum specimens collected 8 months postvaccination prompted evaluation of an indirect assay format. The indirect EIA was more sensitive than the capture EIA, detecting specific IgG antibodies in all infants 8 months postvaccination. When the kinetics of the different assays were compared by using the vaccinee sera, average PNt, HI, and capture EIA values declined between 3 weeks and 8 months postvaccination, whereas indirect EIA values continued to rise.

Comparison of indirect IgG and microneutralization EIAs. To standardize our indirect IgG EIA with a microneutralization EIA, 123 serum specimens with lower absorbance (P−N) values—75% of values from the lower 25th percentile and all values from the lower 50th percentile of approximately 900 specimens submitted for immune screening—were tested for measles virus neutralizing antibodies. By using the microneutralization EIA as a standard, the relative sensitivities, specificities, and predictive values for positive and negative tests for the indirect EIA were determined by selecting cutoff values based on 1 to 4 standard deviations above the mean value of previously tested measles virus

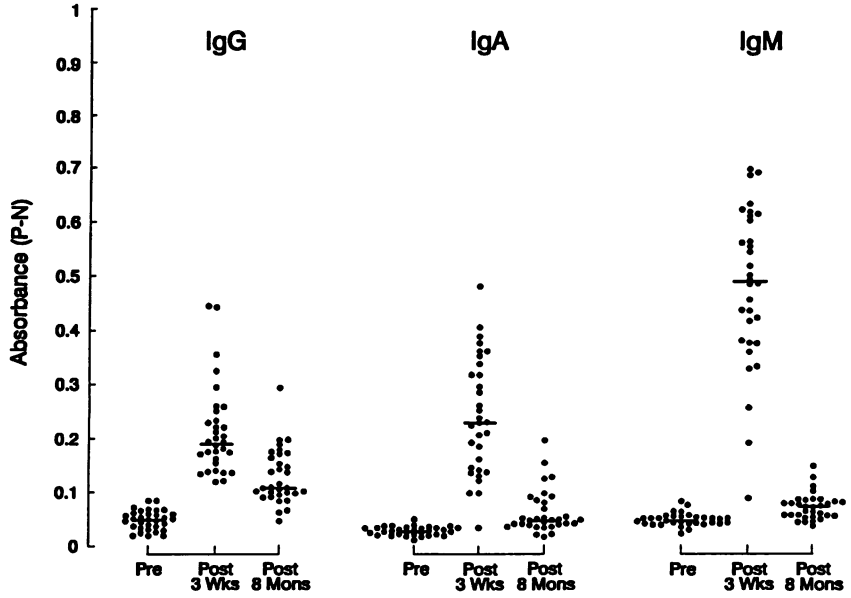

FIG. 3. Measles virus-specific IgG, IgA, and IgM antibody responses in 32 infants receiving primary vaccination. Serum specimens were collected prior to vaccination and at 3 weeks and 8 months postvaccination. Each bar represents the median value for that data group.

TABLE 2. Comparison of indirect IgG and microneutralization EIAs[a]

SD[b]	Cutoff	Result	Indirect EIA				Microneutralization EIA	
			Sensitivity (%)	Specificity (%)	Predictive value for positive test (%)	Predictive value for negative test (%)	No. of positive serum specimens	No. of negative serum specimens
1	0.033	Positive	100	79	75	100	47	16
		Negative					0	60
2	0.048	Positive	94	91	86	96	44	7
		Negative					3	69
3	0.064	Positive	87	97	95	93	41	2
		Negative					6	74
4	0.080	Positive	68	99	97	83	32	1
		Negative					15	75

[a] One hundred and twenty-three serum specimens from 5- to 6-year-old children vaccinated at the appropriate age were tested by both indirect IgG and microneutralization EIAs. Cutoff values for the indirect EIA were selected on the basis of the mean value of known negative serum specimens (0.017) plus 1 to 4 standard deviations (1 standard deviation equals 0.016). Sensitivity, specificity, and predictive values for positive and negative tests are calculated for each cutoff value.

[b] SD, standard deviation.

antibody-negative serum specimens (Table 2). A cutoff at 3 standard deviations gave the best overall correlation between EIA values and the presence of neutralizing antibodies and was selected for routine use. At this cutoff, 41 of 43 specimens (95%) positive by indirect EIA had detectable neutralizing antibodies; 2 specimens negative by indirect EIA were positive when the assay was repeated. Of 80 specimens with values below this cutoff, 74 (93%) had no detectable neutralizing antibodies; all false-negative specimens were between 1 and 3 standard deviations above the mean value.

DISCUSSION

Detection of specific IgM antibodies by capture EIA proved to be a rapid, sensitive, and specific method for confirming cases of clinical measles. The use of detector MAbs offered the additional advantage of providing a continuous supply of standard high-quality reagents. An indirect EIA for IgM antibodies was not pursued because in our experience (data not shown) and that of others (27) this method is less sensitive and specific than the capture design for IgM detection. Although the time course of the IgM response to natural measles virus infection suggested that the optimal time for specimen collection was approximately 2 to 3 weeks after the onset of symptoms, more than 90% of cases were IgM positive at or within a few days of the onset of rash. The ability of our assay to detect specific IgM antibodies in clinical measles cases with a history of prior vaccination—in contrast to those which found IgM to be reduced or absent in cases of postvaccination measles (20, 26)—may reflect the increased sensitivity of capture design.

Although RF appears to be less problematic for capture EIAs, the presence of IgG antibodies in the IgM gradient fractions of RF serum specimens suggests that complexes of RF and measles virus-specific IgG can bind to the capture phase and yield false-positive results. In this study, only those specimens with high levels of both RF and measles virus-specific IgG antibodies, which would be expected to constitute a very small percentage of specimens encountered in a normal population, gave false-positive results. These results could usually be recognized by the presence of high background signal in the negative control wells. Methods to reduce RF interference have been described (11).

Relatively little is known about the utility of specific serum IgA detection in the diagnosis of measles virus infection. In this study, 97% of adolescents with clinical measles had specific IgA antibodies in their acute- or convalescent-phase serum specimens. Like Friedman et al. (12), we found that the peak IgA response to measles occurred at about 1 to 2 weeks after the onset of symptoms and then gradually declined. An IgA response developed in 97% of infants receiving primary vaccination, with 37% positive at 8 months postvaccination. This contrasts with the findings of Pedersen et al. (24), who reported a lower IgA seroconversion rate of 59%, with 14% positive at 1 year. The lower rates observed by those authors probably reflect the lower sensitivity of their assay. Our ability to diagnose recent measles virus infection was not improved by the detection of specific IgA antibodies, and the epidemiologic value of this assay was undermined by the high percentage of specific IgA antibodies found in healthy persons. We could not confirm the diagnostic utility of polymeric IgA observed by Ponzi et al. (25) because our capture EIA did not distinguish between monomeric and polymeric forms of IgA. It is unlikely, however, that the detection of measles virus-specific IgA antibodies would improve upon IgM detection in cases of recent measles virus infection.

Although our IgG capture EIA was more sensitive than CF or HI for detecting significant increases in the levels of measles-specific antibodies in clinical measles cases with documented vaccine history, it was less sensitive than the indirect EIA in detecting low levels of persistent IgG antibodies and thus was less sensitive for assessing a person's immune status. This lower sensitivity may reflect (i) competition between total and measles virus-specific IgG antibodies for available sites on the capture phase; (ii) the nonspecific binding of envelope proteins in the capture EIA, resulting in higher cutoff values for a positive test; and (iii) the detection of specific antibodies to a subset of the measles virus proteins by capture EIA—a consequence of our choice of MAbs—compared with the indirect EIA which, theoretically, detects antibodies to all measles virus antigens. Evidence for the latter was found in the continued rise in antibody levels in vaccinated children as measured by indirect EIA at the same time that antibody levels as measured by HI, PNt, and capture EIA were falling.

Because neutralizing antibodies are considered the best predictor of host immune status, we standardized our indirect EIA to a microneutralization assay, selecting a cutoff that would provide acceptable sensitivity with minimum

false-positive results. For this comparison, most serum specimens were selected from the lower 25th percentile of EIA values from a population of 5- to 6-year-old children vaccinated at the appropriate age. These specimens included those interpreted as negative or low positive by indirect EIA and should have highlighted any differences between the two assays. For these specimens, predictive values for positive and negative tests exceeded 90%. Invariably, specimens from the upper 75th percentile had detectable neutralizing antibodies. Like the results of Cremer et al. (9), our results demonstrate the high concordance between the indirect EIA for measles virus IgG antibodies and neutralizing antibodies even among specimens with low levels of antibodies.

This study, therefore, suggests that (i) a capture IgM EIA should provide sensitive and specific diagnosis of acute measles infection and (ii) an indirect IgG EIA should be a reasonably good predictor of prior measles virus infection and a suitable substitute for the neutralization assay for determining host immune status. The extent to which the indirect IgG EIA predicts protection from disease awaits further study.

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