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Use of Monoclonal Antibodies to Human Immunoglobulin M in "Capture" Assays for Measles and Rubella Immunoglobulin M

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Monoclonal antibodies to human immunoglobulin M (IgM) were used in a four-phase enzyme immunofluorescence "capture" assay for determination of IgM antibodies to measles and rubella viruses. Little or no background reactivity was seen in the test system, and interfering effects of rheumatoid factor were avoided by preabsorption of test sera with aggregated human IgG. Virus-specific IgM antibody was demonstrable in 23 of 24 patients with serological evidence of measles virus infections and in 36 of 36 patients with serological evidence of postnatal rubella infection. A few of the rubella patients did not show IgM antibody until 5 days after onset of illness. The enzyme immunofluorescence assay was able to demonstrate rubella IgM antibody in congenitally infected newborns, whereas indirect immunofluorescence results for virus-specific IgM were negative. Viral IgM antibody was not detected in persons with past infections with the test viruses, in young children without evidence of past infection, or in patients infected with heterotypic viruses, rickettsiae, chlamydiae, or mycoplasmas.

Demonstration of virus-specific immunoglobulin M (IgM) antibodies in acute-phase serum specimens, in cerebrospinal fluids, or in sera from newborns with congenital disease can often permit a specific diagnosis of viral infection more rapidly than can be achieved by conventional serological procedures or by virus isolation. However, the development and application of viral IgM antibody assays has been hampered by lack of anti-human IgM reagents of adequate specificity, potency, and consistency. We have recently described the production of monoclonal antibodies to human IgM, together with preliminary data on their suitability for assay of viral IgM antibodies (7). In the present report we describe results obtained by using the monoclonal antibodies as "capture" antibodies in a solid phase for assay of IgM immunoglobulins to measles and rubella viruses by enzyme immunofluorescence assays (EIFA).

MATERIALS AND METHODS

Immune reagents. The production and characterization of a clone of monoclonal antibody to human IgM have been described in detail (7). Antiserum to measles virus was produced by intraperitoneal inoculation of hamsters with suckling hamster brain infected with the neurotropic HNT Philadelphia strain 26 (2) of measles virus. Rubella virus antiserum was produced by immunization of rabbits with virus propagated in RK-13 rabbit kidney cells grown and maintained on rabbit serum (17); this antiserum was absorbed with BHK-21 hamster kidney cells at a ratio of 1 volume of packed cells to 10 volumes of undiluted serum to make it adequately specific for use in the EIFA tests. Goat anti-hamster serum obtained from Antibodies, Inc., Davis, Calif., was labeled with alkaline phosphatase as described previously (9). Alkaline phosphatase-labeled goat anti-rabbit serum was purchased from Miles Laboratories, Inc., Elkhart, Ind. The optimal working dilution of each immune reagent for use in the capture IgM assay was determined by preliminary block titrations.

Antigens. Antigen for measles virus was prepared from cultures of human fetal diploid lung (HFDL) cells infected with the LEC virus strain (1, 8). When the cultures showed a 3- to 4-plus viral cytopathic effect, cells were dislodged into the fluids by shaking with glass beads, and the cells were sedimented by centrifugation at 700 × *g* for 30 min. The supernatant fluid was removed and saved, and the cells were frozen and thawed three times in a small volume of the supernatant fluid. After clarification of the cell lysate by centrifugation at 6,000 × *g* for 20 min, the lysate was added back to the infected culture fluids. Uninfected control antigen was prepared in the same manner. By preliminary block titration, the optimal working dilution of the measles antigen was determined to be 1:10.

Rubella antigen was prepared in the same manner from BHK-21 baby hamster kidney cells infected with the RV virus strain (8). However, the antigen consisting of pooled infected cell culture fluid and the supernatant fluid from the disrupted cells was then concen-

trated eightfold by dialysis against polyethylene glycol 6000. Control antigen was prepared in the same manner from uninfected BHK-21 cell cultures. The optimal working dilution of the rubella antigen was found to be 1:2, corresponding to a fourfold concentrate of the original antigen.

Sera. The following sera were examined: paired sera from 27 suspected measles virus infections, paired sera from 36 patients with postnatal rubella infections diagnosed by significant increases in hemagglutination inhibition or complement-fixing antibody (or both) or by significant enzyme immunoassay ratios between convalescent- and acute-phase sera, and sera from six infants with suspected congenital rubella. Negative control sera included those from 12 persons with past measles or rubella infections, sera from 10 children without rubella or measles viral antibodies, and paired sera from 27 patients with serologically confirmed infections with heterologous viral, rickettsial, mycoplasmal, or chlamydial agents.

Absorption of test sera with aggregated human IgG. Based upon results described below which indicated that rheumatoid factor (RF) could give false-positive or falsely high viral IgM antibody titers, sera were routinely absorbed with aggregated human IgG to remove RF before they were examined for viral IgM antibodies. A 50% slurry of human IgG insolubilized with 2.5% glutaraldehyde was prepared as described elsewhere (5). For each serum to be examined, a 0.6-ml volume of the slurry was centrifuged at 700 × *g* for 5 min, the supernatant fluid was removed, and to the sediment was added 0.4 ml of a 1:8 dilution of test serum. The mixture was incubated at 37°C for 30 min with constant shaking, and after centrifugation at 700 × *g* for 5 min, the supernatant fluid (absorbed serum) was removed for testing.

Capture EIFA for determination of viral IgM antibodies. Cups in microtiter plates (Immulon II round bottom; Dynatech Laboratories, Inc., Alexandria, Va.) were sensitized by the addition of monoclonal antibodies in human IgM (mouse ascitic fluid). These were diluted 1:1,000 in 0.05 M bicarbonate buffer (pH 9.5) and added in a volume of 0.2 ml per cup. After overnight incubation at room temperature, the contents of the wells were aspirated, and wells were washed twice in 0.01 M phosphate-buffered saline, pH 7.3 (PBS), containing 0.05% Tween 20, with a washer-aspirator (Dynatech). To saturate protein adsorption sites, 5% bovine serum albumin in PBS was added in a volume of 0.35 ml per well, and plates were incubated for at least 4 h at room temperature. The fluids were then aspirated, the wells were washed once with PBS–Tween 20 buffer, and the plates were used immediately or stored at −70°C.

Test sera, viral antigens, and viral antisera were diluted in PBS with 1% bovine serum albumin and conjugates were diluted in PBS with 3% bovine serum albumin. Two sets of serum dilutions, starting at 1:16, were prepared in the plates in volumes of 0.1 ml by using microdiluters. After incubation for 1 h at 37°C, plates were washed three times with PBS–Tween 20 buffer. To one set of serum dilutions was added 0.1 ml of the optimal dilution of viral antigen, and to the other was added 0.1 ml of the same dilution of control antigen. After overnight incubation at room temperature, the plates were washed three times, and optimal dilutions of hamster antiserum to measles virus (1:1,000) or rabbit antiserum to rubella virus (1:500) were added in 0.1-ml volumes. Plates were incubated for 2 h at 37°C and washed three times with PBS–Tween 20 buffer, and 0.1 ml of a 1:1,000 dilution of alkaline phosphatase-conjugated goat anti-hamster serum was added to the measles test system, or 0.1 ml of a 1:1,000 dilution of conjugated goat anti-rabbit serum was added to the rubella test system. After incubation for 1 h at room temperature, the plates were washed three times, and 0.1 ml of the fluorogenic substrate 4-methyl umbelliferyl phosphate was added at a concentration of 0.025 mg/ml in 10% diethanolamine buffer (pH 9.8) with 10^{-3} M $MgCl_2$. Tests were incubated at room temperature, and after 12 to 15 min the enzymatic action on the substrate was stopped by the addition of 0.05 ml of 1 M K_2HPO_4–KOH (pH 10.4). Readings were made visually by using a light box with UV illumination (6). Antibody endpoints were the highest serum dilutions showing clear-cut blue fluorescence with the viral antigen, and results were considered to be specific only when all corresponding wells containing uninfected control antigen showed no fluorescence. Sera known to be positive and negative for virus-specific IgM antibody were included as controls in each run.

Reference serological tests. Hemagglutination inhibition (3, 12) and complement fixation (11) tests for measles or rubella viral antibodies were performed by standard microtiter procedures. The enzyme immunoassay for rubella IgG antibody was the Rubazyme system (Abbott Laboratories, North Chicago, Ill.), and positive results were based upon the demonstration of a ratio of ≥1.65 between optical density readings of convalescent- and acute-phase sera tested in duplicate at 1:20 dilutions. RF was assayed by using a latex agglutination kit (RA; Hyland Laboratories, Inc., Costa Mesa, Calif.).

RESULTS

Effect of RF on viral IgM antibody assays. In one set of experiments to examine the effect of RF on viral IgM antibody assays performed by the capture technic, the positive control provided with a latex agglutination kit (Hyland Laboratories) was mixed with various test sera. A 1:50 dilution of the RF serum was mixed with an equal volume of a 1:8 dilution of serum positive or negative for measles IgM antibody. Serial dilutions of these mixtures were made and assayed in parallel with samples of the sera without added RF. Representative results are shown

TABLE 1. Effect of RF on assays for measles IgM antibody

Serum	Titer[a] in measles IgM antibody assay	
	Without RF	With RF
Low titer of measles IgM	128	512
High titer of measles IgM	1,024	4,096
Negative for measles IgM	<16	512
RF control serum	256	

[a] Reciprocal of antibody titer.

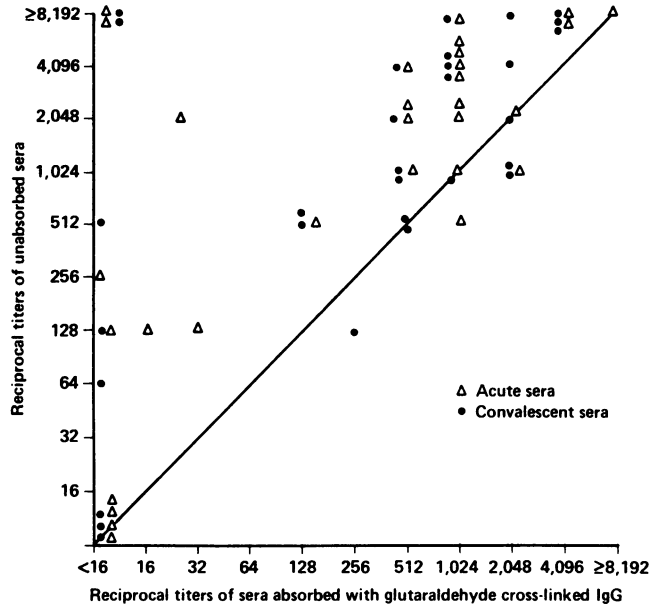

FIG. 1. Effect of absorption with aggregated human IgG on titers of IgM antibody to measles virus.

in Table 1. The presence of RF increased titers of sera positive for measles IgM antibody and caused false-positive reactions with sera that were negative for measles IgM antibody, and the RF serum itself was falsely positive in the assay for measles IgM antibody.

Additional evaluation of the effect of RF in the test system was done by testing a set of sera for measles IgM in parallel with and without preliminary absorption with aggregated human IgG. These included acute- and convalescent-phase sera from persons with suspected current measles virus infections and a few sera from persons with past measles infections. The results shown in Fig. 1 indicated that unabsorbed sera could give false-positive or falsely high viral IgM antibody titers in the test system. Based upon these results, sera were routinely absorbed with cross-linked human IgG before they were examined for viral IgM antibody, and the results described below were obtained with absorbed sera.

Assays for measles IgM antibody. The ability of the capture assay with monoclonal anti-human IgM to detect measles virus-specific IgM antibody was evaluated by testing paired sera from 27 persons with suspected measles infections. Twenty-four of the patients showed diagnostically significant rises in CF antibody to measles virus, and 23 of these also gave positive reactions for measles IgM antibody. Development and decay of viral IgM antibody showed great individual variation in the different patients, with some having higher titers in their acute-phase sera, others having higher titers in their convalescent-phase sera, and a few having the same titers in both sera. The distribution of measles IgM antibody titers for the 24 patients is shown in Fig. 2A. One patient showed IgM antibody only in the convalescent-phase serum; the acute-phase serum was collected at 3 days after onset. Two patients showed IgM antibody only in acute-phase sera, and their convalescent-phase sera were collected at 42 and 53 days after onset.

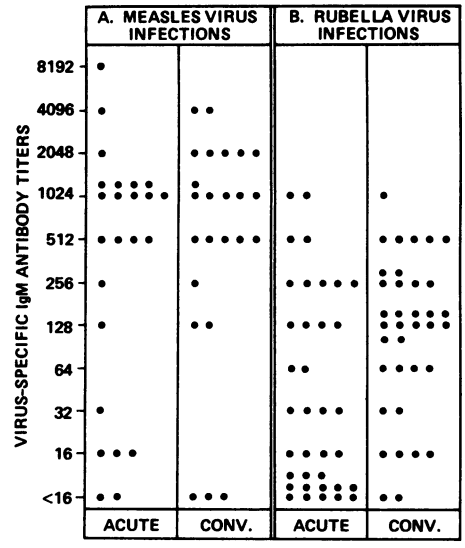

FIG. 2. Distribution of homologous IgM antibody titers in measles and rubella virus infections.

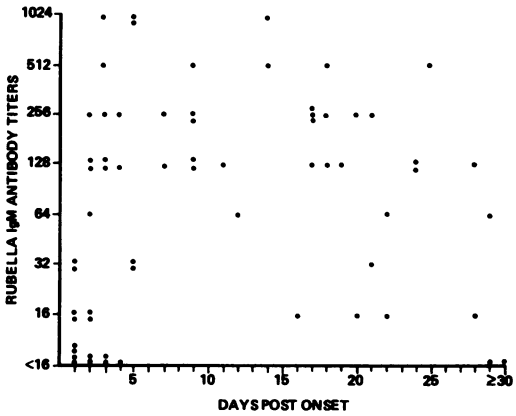

FIG. 3. Titers of IgM antibody to rubella virus in relation to time after onset of illness.

Three of the 27 patients with suspected measles virus infections had stationary titers of CF antibody, and they were negative for measles IgM antibody, suggesting that their current infections were not measles. In addition, 12 sera from persons with past measles infections and who had measles IgG antibody were negative for measles IgM antibody, as were sera from 10 children who lacked measles IgG antibody and apparently had never been infected.

Assays for rubella IgM antibody. Paired sera from 36 patients with postnatal rubella infections (diagnosed by hemagglutination inhibition or complement fixation [or both] or the Rubazyme test) were examined for rubella-specific IgM antibody, and virus-specific IgM antibody was detected in all 36 patients. Figure 2B shows that rubella IgM antibody titers tended to be lower than those for measles virus. Again, development and decay of viral IgM showed great individual variation. Figure 3 shows the relationship of rubella IgM antibody titers to time after onset of illness for those patients on whom dates of specimen collection were available. There was wide variation in antibody titers at the different time intervals, but it is noteworthy that a number of sera were negative for IgM antibody during the first 4 days after onset. However, all of the 5-day specimens were positive for rubella IgM antibody. Some of the patients showed elevated IgM antibody titers as early as 2 to 5 days after onset. Two patients had become negative for rubella IgM antibody by 29 or 30 days after onset of illness.

Five of the patients failed to show a significant increase in rubella hemagglutination inhibition antibody titers, despite appropriate spacing of their acute- and convalescent-phase sera, and rubella infections were diagnosed on the basis of complement fixation or Rubazyme results. These five patients also showed positive results for rubella IgM antibody in the EIFA test.

Ability of the capture system to detect rubella IgM antibody in congenital infections was examined by testing sera from six infants with suspected congenital rubella. Virus was isolated from nasopharyngeal or urine specimens of five of the infants. As shown in Table 2, the five infants with positive virus isolation results showed rubella-specific IgM antibody in the capture EIFA system, but not by indirect immunofluorescence staining (10). The one infant with negative virus isolation results also had negative results for rubella IgM antibody.

Specificity of the capture EIFA for rubella IgM antibody was further evaluated by testing paired sera from 27 patients with heterotypic viral, rickettsial, mycoplasmal, or chlamydial infections. These included four patients with measles, three each with herpes simplex virus, varicella-zoster, or *Mycoplasma pneumoniae* infections, two each with influenza A virus, adenovirus, Q-fever, rickettsial pox, psittacosis, or parainfluenza virus infections, one with respiratory syncytial virus infection, and one with mumps. Sera from all of these patients were negative for rubella IgM antibody.

DISCUSSION

These studies have demonstrated the reliability of an EIFA capture system for assay of viral IgM antibodies which is based upon the use of monoclonal antibodies to human IgM on a solid phase to bind IgM from the test serum. The availability of anti-mu chain reagents of this source can be expected to overcome some of the problems that have been encountered in the past with reagents of low specificity and sensitivity. Some reports on the use of standard anti-mu chain reagents in capture assay systems for viral IgM antibodies have noted high background absorbance, which was overcome by using antibody-negative human sera or normal animal sera in diluents for the test reagents (13, 18). However, with our monoclonal reagent we encountered

TABLE 2. Detection of virus-specific IgM antibody in congenital rubella infections by the capture system

Patient	Age (days)	Virus isolated	Rubella IgM antibody titer[a]	
			Capture EIFA	Indirect immunofluorescence
1	1	+	64	<8
2	1	+	256	<8
3	13	+	64	<8
4	19	+	128	<8
5	21	+	128	<8
6	21	0	<16	<8

[a] Reciprocal of antibody titer.

little or no background reactivity, and only low concentrations of bovine serum albumin were required in the diluents.

Our assay system differed from other capture assays that have been described recently for determination of rubella and measles IgM antibody (4, 13–16, 18) in that it was a four- rather than a three-phase system. This provides greater versatility for assay of IgM antibodies to a variety of viruses, since the only labeled immunoglobulins required are those directed against the species in which the viral antibodies are produced, and these labeled immunoglobulins are available from commercial sources. Workers using three-phase systems with labeled viral antibodies have often encountered problems with nonspecific reactivity of animal viral antisera, and some have resorted to the use of human sera as a source of viral antibodies for labeling (13, 18). Possible reactivity of these human IgG antibodies with RF has been countered through the use of $F(ab')_2$ fragments for labeling (13) or by using aggregated human IgG in the diluent to bind and saturate RF (14, 18).

Although purified rubella viral antigens are generally required in indirect enzyme immunoassays for viral IgG where antigen is used in the solid phase (8), we found that unpurified antigens were satisfactory for use in the capture EIFA for assay of measles and rubella IgM antibodies; this increases the practicality of the test systems. Visual reading of results is another feature of the tests described herein which may make them more widely applicable than enzyme immunoassays based upon spectrophotometric or fluorometric readings.

There have been somewhat conflicting reports as to the effect that RF can have on the sensitivity and specificity of viral IgM antibody assays performed by the capture technic. However, it is generally recognized that RF of the IgM class has the potential to reduce sensitivity of the assays by competing with virus-specific IgM for binding sites on the solid phase. RF bound to the solid phase may also produce false-positive results for viral IgM antibodies. This can result from RF complexing with virus-specific IgG antibodies to which viral antigen attaches and subsequently binds the detector viral antibodies. False-positive reactions can also result from IgM RF in the solid phase, binding directly to the labeled IgG reagent. Our results clearly showed that RF could produce false-positive results for measles IgM antibody. Many of the sera that showed high levels of false-positive reactivity gave only a weak positive reaction for RF by latex agglutination, confirming that this is not a satisfactory method for evaluating the specificity of results obtained in viral IgM antibody assays. Absorption of test sera with aggregated human IgG was adopted for routine pretreatment of test sera, and this virtually eliminated false-positive reactions. However, it is also important to test each serum against uninfected control antigen together with viral antigen, so that nonspecific reactivity of the test sera can be recognized and false-positive readings can be avoided.

A major advantage of the capture EIFA for rubella IgM antibody was its ability to detect virus-specific IgM in congenital infections. In viral IgM antibody assays such as indirect immunofluorescence which use viral antigen in the solid phase, high concentrations of viral IgG antibodies in newborns' sera compete with IgM for antigen binding sites, resulting in tests of low sensitivity. However, the sensitivity of the capture assay depends upon the proportion of IgM in the test serum which is virus specific, and since this is high in congenitally infected newborns, the capture system is particularly suitable for examination of sera from patients with suspected congenital viral infections.

We found that a number of patients with postnatal rubella infections were negative for rubella IgM antibody during the first 4 days after onset of illness. Similar results were reported by Kurtz and Malic (14), and thus it would appear that in attempting to diagnose postnatal rubella infections by demonstration of virus-specific IgM in a single early serum, tests should be done on sera collected at least 5 days after onset of illness.

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