JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1986, p. 731–735
0095-1137/86/110731-05$02.00/0

Vol. 24, No. 5

Accuracy of Immunoglobulin M Immunoassay for Diagnosis of Chlamydial Infections in Infants and Adults

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Received 16 April 1986/Accepted 30 July 1986

An improved solid-phase enzyme immunoassay (EIA) with *Chlamydia trachomatis* L2 434/Bu elementary bodies was developed for the measurement of immunoglobulin M (IgM) antibody to *C. trachomatis* in serum. Comparison of EIA and microimmunofluorescence IgM antibody titers of 156 serum samples revealed an EIA sensitivity and specificity of 100% for infants, but reduced sensitivity (85%) and specificity (76%) for sera from adults. Sera containing IgM class rheumatoid factor produced false-positive IgM results which could easily be eliminated by pretreatment of the sera with anti-human IgG. Analysis of sera from infants with chlamydial infections revealed that 17 of 17 infants with *C. trachomatis* pneumonia had high IgM antibody titers (geometric mean titer, 1:64,812), whereas two infants with conjunctivitis only lacked detectable IgM antibody. EIA detected IgM antibody to several serovar groups in serum, including serovars B, BDE, FG, and J. IgM antibody to *C. trachomatis* in serum was detected as early as 5 days after the infection that was acquired at delivery and persisted for 3 months. The availability of an EIA possessing good sensitivity and specificity for the detection of IgM antibody to *C. trachomatis* may permit more laboratories to diagnose perinatal chlamydial infections.

Chlamydia trachomatis infections in humans include infections with both the trachoma and lymphogranuloma biovars and are manifested as hyperendemic blinding trachoma, lymphogranuloma venereum, urethritis, proctitis, epididymitis, cervicitis, salpingitis, conjunctivitis, and pneumonia (11, 12, 14, 16, 22). Infections with *C. psittaci* are rare and usually limited to bird handlers. Existing serological methods for diagnosing trachoma biovar infections in humans are generally not satisfactory. Existing tests are plagued with either poor sensitivity or high antibody prevalences in certain populations. Available tests such as the microimmunofluorescence (MIF) test use antigens which are expensive and technically difficult to prepare, and commercial support has been slow in developing. The MIF test is useful but for the reasons listed above its use is restricted to reference centers or institutions with ongoing chlamydial research and is not generally available to routine diagnostic laboratories.

Recently, solid-phase immunoassays have appeared for the measurement of chlamydial antibody (2, 4–6, 8, 10, 13, 15, 20). These assays have been used to measure antibodies in patients with psittacosis and lymphogranuloma venereum (8), as well as oculogenital infections in both adults and infants (5, 10). Immunoassays for the measurement of chlamydial immunoglobulin M (IgM) antibody have been less numerous and have suffered from poor sensitivity and poor specificity (4, 5, 10, 13). We previously described a solid-phase enzyme immunoassay (EIA) for measuring IgG and IgM in serum to several serovars of *C. trachomatis*, but the IgM assay possessed poor sensitivity and specificity (10). In this report we describe an improved EIA with excellent sensitivity and specificity for measuring chlamydia IgM antibody in sera from infants.

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MATERIALS AND METHODS

Cells and media. Mycoplasma-free heteroploid mouse McCoy cells were grown in Eagle minimal essential medium supplemented with 10% fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.)–2 mM glutamine–50 μg of streptomycin per ml.

Sera. Sera were obtained from males and females attending a sexually transmitted disease clinic with a prevalence of *C. tractomatis* infection of approximately 26%, from women attending gynecological practices in Hamilton or San Francisco, and from infants with chlamydial pneumonia (1), pertussis, or viral respiratory infections in Hamilton, New York, or San Francisco.

Treatment of sera. Sera were treated to remove rheumatoid factor (RF) by reaction with sheep anti-human IgG antisera as described previously (24). Removal of RF was confirmed by either latex agglutination with the RF latex reagent (Behringwerke AG, Marburg, Federal Republic of Germany) or by nephelometry with World Health Organization calibration standards and an Auto ICS instrument (Beckman Instruments, Inc., Fullerton, Calif.). RF values of <140 IU were considered negative.

MIF test. The MIF test was performed with prototype antigens grown in yolk sac culture by the method of Wang et al. (21). Each serum sample was tested with serial twofold dilutions beginning at an initial dilution of 1:8. Serovar reactivity in positive serum samples was characterized by a predominant pattern of reactivity with prototype antigens.

EIA. *C. trachomatis* L2 434/Bu was grown in cycloheximide-treated (2μg/ml) McCoy cells to 95% infectivity, as assessed by iodine staining. Monolayers in 25-cm^2 Falcon flasks were inoculated with 5 ml of chlamydia (10^6 inclusion-forming units per ml) and then centrifuged at 2,800 rpm (2,400 × *g*) for 30 min at 35°C in a centrifuge (model PR-J; International Equipment Co., Div. Damon Corp., Needham

TABLE 1. Effect of the presence of RF on the determination of chlamydial IgM antibody by EIA

Serum sample	Treatment with RF[a]	RF (IU/ml)[b]	IgM titer[c]	
			MIF	EIA
1	−	5,856	<1:8	1:25,600
	+	68	<1:8	<1:200
2	−	7,638	<1:8	1:51,200
	+	92	<1:8	<1:200
3	−	6,478	1:1,024	1:51,200
	+	74	1:1,024	1:51,200

[a] RF and IgG were removed from the serum samples by treatment with anti-IgG antibody, as described in the text.
[b] RF levels in serum were determined by nephelometry, as described in the text, with RF standards.
[c] Chlamydial IgM titers were determined by MIF and EIA, as described in the text.

Heights, Mass.). Flasks were incubated for 2 h at 37°C, and then the inoculum was replaced with 5 ml of growth medium supplemented with 2 μg of cycloheximide per ml. Chlamydial elementary bodies (EBs) were harvested postinfection by disruption of cell monolayers with 5-mm glass beads. Cell debris was pelleted by centrifugation at 500 × *g* for 10 min, and chlamydiae were collected by centrifugation at 18,500 rpm (30,000 × *g*) for 20 min. EBs were suspended in phosphate-buffered saline (pH 7.4), layered over 2 ml of 30% (vol/vol) Renografin-76 (E. R. Squibb & Sons Ltd., Montreal, Canada), and centrifuged in an SW 50L rotor at 15,000 rpm (20,000 × *g*) for 40 min in an ultracentrifuge (model L3-50; Beckman). The final pellet (from six flasks) was suspended in 1 ml of phosphate-buffered saline. Preparations of chlamydial antigen were standardized by titration against a positive serum sample with a known IgM titer (1:51,200) and were used at the appropriate dilution. The EIA was performed as described previously (10) with the following modifications. Prior to testing, each serum sample was treated with antiserum to human IgG to remove IgG and RF as described previously (24). Microtiter plates were coated with either chlamydial antigen or McCoy cell lysate (control antigen) diluted in freshly prepared carbonate buffer overnight at 25°C. All reagent volumes were 0.05 ml, and affinity-purified horseradish peroxidase-labeled goat anti-human IgG antibody (Kirkegaard and Perry Laboratories) was used as the indicator reagent. Stop solution (3 N HCl) was added, and plates were read with an automated microenzyme-linked immunosorbent assay reader (model MR 580; Dynatech Laboratories, Inc., Alexandria, Va.) at a wavelength of 492 nm. Chlamydial IgM antibody titers were determined as the highest serum dilution with a signal-to-noise (S/N) ratio of >2.1, in which the S/N ratio is calculated by dividing the absorbance obtained with the chlamydial antigen by the absorbance obtained for the control antigen-coated well. Antibody titers of 1:1,600 and 1:800 were used as the positive cutoff values for adult and infant sera respectively. Microtiter plates could be coated with antigen, sealed, and stored for up to 1 month with no effect on antibody titers of positive sera.

Chlamydial isolation. Isolation of *C. trachomatis* was performed as described previously with cycloheximide-treated McCoy cells and iodine staining in either a microculture system (3) or in shell vials.

Pertussis. Pertussis was diagnosed by growth of *Bordetella pertussis* on oxoid CM119 medium or by fluorescent antibody staining of nasopharyngeal secretions with fluorescein-conjugated pertussis antibody.

RESULTS

Since the presence of IgM class RF has been shown to cause false-positive results in EIAs we tested serum samples containing high titers of RF for chlamydia IgM antibody. Two serum samples with levels of RF greater than 5,000 IU/ml had high IgM titers >1:25,600 by EIA but were negative when tested by MIF (Table 1). Removal of RF from both serum samples by treatment with anti-IgG antibody decreased the IgM titer to <1:200 and eliminated the false-positive result. Treatment of a serum sample containing chlamydial IgM antibody (serum sample 3) did not affect the IgM antibody titer (Table 1). Treatment of serum samples with anti-IgG antibody was therefore incorporated into the procedure to ensure that all positive results were true positives.

To ascertain whether the IgM titer of a serum sample could be determined by testing it at a single dilution, a standard curve was constructed from EIA results of 94 serum samples. Data for adults and infants were combined because graphs of a subset of adult and infant sera were similar. The absorbance of each serum sample at a dilution of 1:800 was plotted against the actual IgM titer determined by testing serial dilutions of serum with a S/N ratio cutoff of 2.1. Figure 1 shows the mean absorbance at 492 nm of each group of sera with various IgM titers. The absorbance increased linearly with IgM titer (correlation coefficient r = 0.96), thus establishing that the IgM titer of a serum sample could be determined by testing it at a single dilution.

To determine the sensitivity and specificity of the improved assay, we tested sera from 152 adults and 58 infants with two gold standards of comparison: (i) MIF IgM and (ii) diagnosis of chlamydial infection by isolation of the organism. Table 2 summarizes the data for adults showing EIA IgM sensitivities of 85.7 and 70.4% and specificities of 76.2 and 85.2%. The sensitivities and specificities of the EIA for infant sera were 100% whether compared with MIF or isolation of the organism (Table 3). The latter group of sera were from 56 infants (aged 12 days to 6 months) with chlamydial pneumonia, pertussis, or viral respiratory tract infections all proven by culture. All 17 infants with *C. trachomatis* recovered from the nasopharynx had chlamyd-

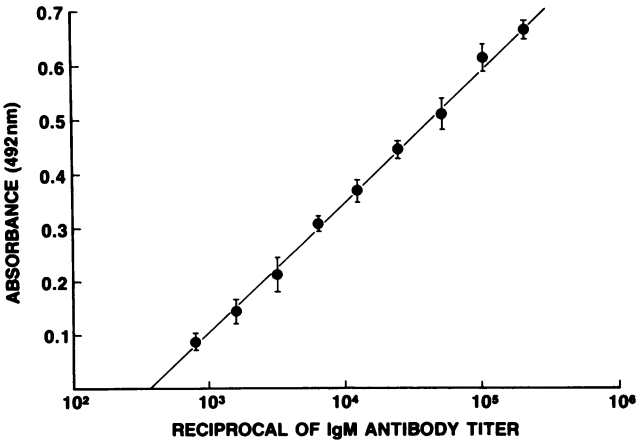

FIG. 1. Correlation of chlamydial IgM antibody titer with absorbance. The EIA IgM titers of 94 serum samples (determined by testing serial serum dilutions, as described int the text) were plotted against the absorbance obtained at a dilution of 1:800 for each serum sample. The data points represent the mean absorbance ± standard deviation of each group of sera with the indicated IgM titer.

ial IgM antibody in serum, whereas none of the others did. Two additional infants with *C. trachomatis* conjunctivitis only had no detectable IgM antibody to chlamydia in serum by either EIA or MIF. The IgM antibody titers for the 17 infants with *C. trachomatis* recovered from the nasopharynx ranged from 1:6,400 to 1:204,800, with a geometric mean titer of 1:64,812. The mean IgM antibody titer of the same serum sample by MIF was 1:1,081. EIA detected IgM antibody in infants infected with several different serovars of *C. trachomatis*, including B, BDE, FG, and J (data not shown).

IgM antibody titers of 19 serum samples collected at various times after birth from 17 infants born to culture-positive women and who developed pneumonia are shown in Fig. 2. All infants with chlamydial pneumonia were treated with antibiotics following culture. Two infants had IgM antibody detectable as early as 15 and 17 days of life, while sera collected from two other infants at 3 and 7 days of age had no detectable chlamydial IgM antibody. IgM antibody titers in serum rose quickly and approached maximal titers by the second week of life and persisted for up to 3 months (83, 97, and 105 days) in three infants.

DISCUSSION

We developed an improved EIA to measure IgM antibody to *C. trachomatis*. EIA possesses excellent sensitivity and specificity (100%) for measuring chlamydial IgM antibody in infants using the MIF test and isolation as gold standards. All infants studied with chlamydial pneumonia had high chlamydial IgM antibody titers which appeared 2 to 3 weeks after infection and persisted for up to 3 months.

The serological diagnosis of infections due to *C. trachomatis* has in the past relied heavily on the complement fixation and MIF tests. The lack of sensitivity of the complement fixation test and scarcity of reagents for the MIF test have necessitated a search for newer serological tests. EIAs for the measurement of IgG and IgM antibody to *C. psittaci* and *C. trachomatis* have been described recently (2, 4–6, 8, 10, 15). These assays showed a poor correlation of IgM results by EIA and MIF (5, 10). We improved the EIA for measuring chlamydial IgM antibody by using affinity-purified enzyme conjugates and control antigen-coated wells. The improved assay is rapid and easy to perform; it can determine IgM antibody titers by testing sera at a single dilution and can detect IgM antibody to the major serovars of *C. trachomatis* found in North America (7). Furthermore, the assay uses a single strain of *C. trachomatis*, LGV 434, which can be grown in tissue culture, and commercially available enzyme-labeled conjugates. This should allow many laboratories the opportunity to employ the test.

In a large study of infants with pneumonia, Schachter and colleagues (18) demonstrated that infants with systemic chlamydial infection had high IgM antibody titers. Nineteen infants with chlamydial pneumonia had high MIF IgM antibody titers, ranging from 1:64 to 1:4096 with a geometric mean titer of 1:256. Infants with chlamydial inclusion conjunctivitis or nasopharyngeal carriage of the organism without signs of pneumonia did not, however, develop high levels of IgM antibody. In the study by Schachter et al. (18) only 42 of 91 IgM antibody-positive infants with radiologically confirmed pneumonia yielded chlamydia, suggesting that IgM serology may be more sensitive than isolation of *C. trachomatis* for diagnosing chlamydial pneumonia. We tested sera from 17 infants with chlamydial pneumonia for IgM antibody by EIA and found that all 17 had high levels of IgM antibody. Puolakkainen et al. (13), using an EIA employing purified chlamydial major outer membrane protein (MOMP), reported similar results, showing that 15 of 16 infants with chlamydial pneumonia had high levels of serum IgM antibody in serum. Collectively, results of these studies indicate that chlamydial pneumonia is accurately diagnosed by the presence of high IgM antibody titers in serum.

Previously described immunoassays for the measurement

TABLE 2. Correlation of chlamydial IgM antibody with isolation of the organism from adults

Identification method	No. with EIA IgM antibody[a]: Positive	Negative	Sensitivity (%)	Specificity (%)
MIF				
Positive	12	2	12/14 (85.7)	
Negative	20	64		64/84 (76.2)
Culture[b]				
Positive	19	8	19/27 (70.4)	
Negative	4	23		23/27 (85.2)

[a] Chlamydial IgM antibody in sera from adults attending a sexually transmitted disease clinic or a gynecological practice was measured by EIA and MIF, as described in the text, with 1:1,600 and 1:8 as cutoffs of positivity for EIA and MIF, respectively.

[b] *C. trachomatis* was isolated from the urethra of 19 males and from the endocervix of 8 females.

TABLE 3. Correlation of chlamydial IgM antibody and isolation of chlamydia in infants

Identification method	No. with EIA IgM antibody[a]: Positive	Negative	Sensitivity (%)	Specificity (%)
MIF				
Positive	21	0	21/21 (100)	
Negative	0	37		37/37 (100)
Chlamydial pneumonia[b]	17	0	17/17 (100)	
Other infections[c]	0	39		39/39 (100)

[a] Chlamydial IgM antibody was determined by EIA and MIF, as described in the text, with 1:800 and 1:8 as cutoffs for positivity for EIA and MIF, respectively.

[b] Chlamydial pneumonia was defined as the isolation of *C. trachomatis* from the nasopharynx of an infant with clinical signs and symptoms characteristic of chlamydial pneumonia (1).

[c] Other infections included 34 cases of pertussis diagnosed by isolation of the organism from the nasopharynx, positive fluorescent antibody test, or positive serology and 5 viral infections diagnosed by isolation of adenovirus (1 case), respiratory syncytial virus (1 case), herpes simplex virus (1 case), and untyped hemadsorbing myxoviruses (2 cases).

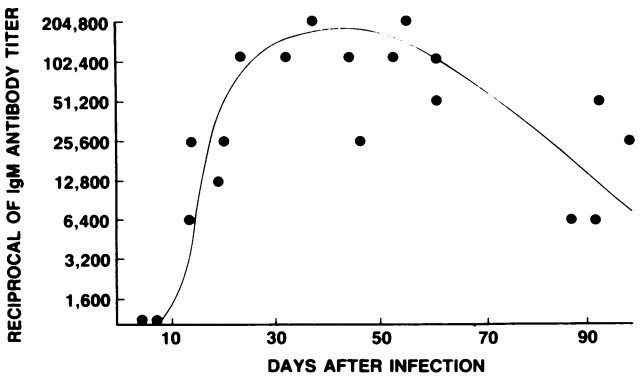

FIG. 2. IgM antibody titers in serum in infants at various times after infection with *C. trachomatis*.

of chlamydial antibodies have employed different solid-phase antigens. Purified intact (EBs), reticulate bodies (RBs), purified MOMP, or genus-specific antigens have been employed. Jones et al. (6) compared RBs and EBs in an EIA and concluded that both antigens gave similar results to the MIF test which employs yolk sac grown EBs. Mahony et al. (10) and Finn et al. (5) employed EBs grown in tissue culture and showed a good correlation between MIF and EIA for measuring chlamydial IgG antibody but a poorer correlation for measuring IgM antibody. Puolakkainen et al. (13) more recently compared an IgM EIA employing RBs, purified MOMP, or *Salmonella* RE mutant lipopolysaccharide (which cross-reacts serologically with chlamydia) and an immunofluorescent antibody test employing infected McCoy cells. Results for EIA employing MOMP were in good agreement with those obtained by the immunofluorescent antibody test, while the commercial RB-based EIA performed poorly for measuring IgM antibody. Collectively, these results suggest that several different antigens, including intact EBs, detergent-treated EBs, and MOMP, but not *Salmonella* RE lipopolysaccharide can be used in an EIA to measure chlamydial IgM antibody. The MOMP EIA titers reported by Puolakkainen et al. (13) were not as high as our titers. In their study, the mean titer for 6 infants with chlamydial pneumonia was 1:759, whereas, our mean titer for 17 infants with pneumonia was 1:64,812. This might suggest that, in fact, EBs are superior to purified MOMP for measuring IgM antibody by EIA, or alternatively, it could reflect the use of different reagents or different concentrations of reagents used in the different EIAs.

Comparison of MIF and EIA IgM antibody titers revealed a sensitivity and specificity of 100% for infants and 86 and 76% for adults, respectively. The sensitivity and specificity of the EIA for sera from adults were determined with sera from individuals attending a sexually transmitted disease clinic who are at high risk of chlamydial infection and may not be representative of other populations. Analysis of IgM serology and culture results indicated a stronger correlation between the presence of chlamydial IgM antibody and the recovery of *C. trachomatis* from infants than from adults. EIA detected IgM antibody in only 19 of 27 (70.4%) serum samples from adults with genital tract infections, whereas, it detected IgM antibody in 17 of 17 infants with extraocular infection (Table 2 and 3). We have shown previously (24) that RF can cause false-positive IgM results but that RF can be removed by treatment with anti-human IgG antibody. We have not detected RF in any of the 58 infant serum samples included here. In a previous study (18) only 1 of 60 serum samples from infants contained measureable RF, suggesting that RF false-positive results may not be a significant problem in testing infant sera. At present we do not know why the sensitivity and specificity of the EIA is better for sera from infants than that from adults. The existence of blocking factors in adult sera could contribute to the decreased sensitivity, while the occurrence of low or nondetectable levels of RF, which is difficult to remove from adult sera by our pretreatment, could produce the decreased specificity. Alternatively, false-positive antibody results may follow infection with Epstein-Barr virus, a known polyclonal B-cell activator, or other B-cell activators unrelated to *C. trachomatis*. Further studies will be required to determine the source of false positivity.

The presence of high levels of chlamydial IgM antibody is not 100% specific for diagnosing chlamydial pneumonia in infants. Schachter et al. (18) noted that some infants who persistently shed chlamydia from the rectum had MIF IgM antibody titers of >1:32. In addition, some infants with pneumonia due to viruses or other bacteria with asymptomatic shedding of chlamydia from the nasopharynx have been shown to lack IgM antibody in serum (18). Pneumonia of nonchlamydial etiology, together with asymptomatic carriage of chlamydia in the nasopharynx, occurs in about 3% of cases of pneumonia in infants under the age of 6 months (19). Clinically inapparent but persistent nasopharyngeal colonization by chlamydia has been recognized as a common sequelae to chlamydial inclusion conjunctivitis (1). The addition of chlamydial IgM testing in the diagnosis of pneumonia in infants may provide a useful adjunct in helping to discriminate among the various etiologic agents of pneumonia. We investigated five infants born to chlamydia culture-positive women who did not yield *C. trachomatis* from any site (including the eye, nasopharynx, or rectum) but who had chlamydial IgM antibody detected by EIA. The presence of IgM antibody in culture-negative individuals may indicate reduced viability of *C. trachomatis* during handling and transportation of specimens (9).

Serology has been shown to have limited usefulness in diagnosing chlamydial genital tract infections in adults (17). Using the MIF test, Schachter et al. (17) showed that only 6 of 19 (32%) of chlamydia-positive women compared with 14 of 75 (19%) of chlamydia-negative women attending a sexually transmitted disease clinic had IgM antibody. In this study employing an EIA we detected IgM antibody in only 19 of 27 (70%) adults attending a similar clinic. The low prevalence of IgM antibody in culture-positive individuals likely is due to repeated infection with *C. trachomatis* and indicates the need for isolating the organism in a population at high risk for sexually transmitted diseases.

ACKNOWLEDGMENTS

This study was supported in part by grants from the Physicians Services Incorporated Foundation and St. Joseph's Hospital Foundation (J.B.M. and M.A.C.) and by Public Health Service grant EY-02216 from the National Institutes of Health (J.S.).

We gratefully acknowledge the excellent technical assistance of Patricia Hart, Anna Pietruszkiewicz, and Chantal Johanns and the secretarial assistance of Colleen Saunders and Yvonne Christieson.

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