JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1981, p. 416-422
0095-1137/81/030416-07$02.00/0

Vol. 13, No. 3

Direct Enzyme-Linked Immunosorbent Assay That Uses Peroxidase-Labeled Antigen for Determination of Immunoglobulin M Antibody to Cytomegalovirus

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A direct enzyme-linked immunosorbent assay was developed for the measurement of immunoglobulin M (IgM) antibody to cytomegalovirus (CMV). Wells of microtiter plates were coated with anti-human IgM. Each patient's serum was added at a dilution of 1:100, and IgM from the serum was allowed to react with anti-human IgM. The amount of CMV-specific IgM antibody bound was determined by measuring the intensity of color change after the addition of peroxidase-labeled CMV antigen and substrate. Nuclei of infected cells served as an antigen source. CMV IgM could be detected only in IgM fractions of sera from patients with a recent CMV infection. Rheumatoid factor did not cause false-positive results. No cross-reactions were observed when paired sera from 22 patients with herpes simplex or varicella and single sera from 12 patients with suspected infectious mononucleosis were tested by the direct enzyme-linked immunosorbent assay. Each of 17 patients with a seroconversion for CMV antibody showed CMV-specific IgM antibody. In six of these patients the antibody was detected in the initial serum. The direct enzyme-linked immunosorbent assay for CMV IgM is a specific and sensitive test for the diagnosis of recent CMV infections and possesses distinct advantages over indirect tests.

Cytomegalovirus (CMV) infections have been routinely identified by detection of antibody titer rises in paired sera from patients by means of the complement fixation (CF) or indirect hemagglutination test or by isolation of the virus from urine or throat washes. Since virus-specific immunoglobulin M (IgM) is found in congenitally infected infants and in currently or recently infected adults, a prompt serological diagnosis can be facilitated by detection of IgM antibody to CMV. Several methods have been developed for determination of CMV-specific IgM, including the indirect immunofluorescence (IF) test (8, 15), the enzyme-linked immunosorbent assay (ELISA) (1, 21), and the radioimmunoassay (13). A major problem with these indirect tests using whole sera is the occurrence of false results. First, rheumatoid factor (RF) may cause false-positive results by reacting with antigen-IgG complexes, and, second, IgG antibody may cause false-negative results by inhibiting the binding of IgM. These obstacles have been overcome by using sucrose density gradient centrifugation and testing the IgM fraction by the IF test (21), by absorption of RF with insolubilized gamma globulin (3), and by testing whole serum in the IF test for IgM antibody after absorption of IgG with anti-human γFc (9). However, these methods are laborious, and IgG is not always completely removed by a single absorption procedure.

A simple method for separating IgM from IgG has been recently described by Krech and Wilhelm (14) for rubella and by Duermeyer and van der Veen (4) for hepatitis A using an anti-human IgM solid-phase immunosorbent. We developed a direct ELISA for detection of CMV IgM by application of this principle in conjunction with the use of enzyme-labeled antigen (H. Schmitz, personal communication; 17). Sera from patients were added to anti-human IgM-coated wells in plastic plates. CMV-specific IgM was demonstrated by adding, subsequently, peroxidase-labeled CMV antigen and substrate. This paper presents the results of experiments to examine the specificity and sensitivity of the test.

MATERIALS AND METHODS

Sera. Sera were obtained from 55 patients with clinical signs suggestive of CMV infection; 27 of them had, in addition, renal transplants, 7 had recently received multiple blood transfusions, and 3 suffered from malignancies. Of the 55 patients, 51 were adults 18 to 72 years of age. Four patients were children 3, 4, 9, and 15 years of age, none of whom had been congenitally infected with CMV. In addition, sera were obtained from 34 patients clinically suspected of having herpes simplex or varicella-zoster infection or infectious mononucleosis. Furthermore, 36 sera with

various levels of RF and with or without CMV-specific IgG antibody were selected from sera submitted for testing for RF.

Antigens. The AD-169 strain of human CMV was used to prepare antigen for both ELISA and CF. For ELISA a CMV nuclear antigen was prepared from a CMV-infected human diploid fibroblast cell strain (Flow 2002; Flow Laboratories, Inc., Rockville, Md.) essentially according to the procedure described previously (21, 22). Briefly, when a nearly complete cytopathic effect was observed, cells were trypsin dispersed, suspended 1:10 in hypotonic buffer containing 50 mM NaCl–20 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.4)–4 mM $MgCl_2$–1 mM trisodium ethylenediaminetetraacetate, and incubated at 0°C for 30 min. After addition of 0.35% Nonidet P-40 and disruption of the cells in a tight-fitting Dounce homogenizer (monitored by periodic phase-contrast microscopic examination), the nuclei were diluted with cold phosphate-buffered saline (PBS) to a concentration of approximately 10×10^7/ml. Thereafter, they were separated from cellular debris by centrifugation at $1,500 \times g$ for 15 min at 4°C through a layer of 22.5% Ficoll 400 in PBS. The pellet of partially purified nuclei was resuspended in PBS. After sonication, the disrupted material was clarified by centrifugation for 10 min at $5,000 \times g$. The supernatant was used as viral antigen in the indirect ELISA for IgG or IgM antibody to CMV. The protein content as determined by the Lowry method was approximately 0.4 mg/ml.

The same procedure was applied to prepare peroxidase-labeled antigen for the direct ELISA for IgM antibody, except for the last step. The pellet of nuclei was resuspended in a smaller volume of PBS giving a final protein concentration of 3 to 5 mg/ml. Two methods were applied for labeling CMV antigen with horseradish peroxidase (HRPO; RZ 3.2, Sigma Chemical Co., St. Louis, Mo.). Method A was the periodate method described by Wilson and Nakane (24). Briefly, 4 mg of HRPO was dissolved in 1 ml of distilled water, followed by the addition of 0.2 ml of freshly prepared 0.1 M $NaIO_4$. This solution was stirred for 2 h at room temperature and subsequently dialyzed against 1 mM sodium acetate buffer (pH 4.4) overnight at 4°C. Next, the pH of the HRPO-aldehyde solution was raised to 9.0 to 9.5 by the addition of 0.02 ml of sodium carbonate buffer (0.01 M; pH 9.5), and, simultaneously, a solution of 5 mg of CMV antigen in 1 to 1.5 ml of sodium carbonate buffer (0.01 M; pH 9.5) was added. The reaction mixture was stirred for 2 h at room temperature. Next, 0.1 ml of a freshly prepared $NaBH_4$ solution (4 mg/ml) was added, and the mixture was left for 18 h at 4°C. After extensive dialysis against PBS (pH 7.2) containing 0.02% NaN_3, bovine serum albumin was added to a final concentration of 1%.

In method B, CMV antigen and HRPO were coupled with *N*-succinimidyl 3-(2 pyridyldithio)propionate (SPDP; Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. HRPO (10 mg/ml) and CMV antigen (3 to 5 mg/ml) were dissolved in 0.1 M sodium phosphate buffer (pH 7.5) containing 0.1 M NaCl. Next, SPDP was dissolved in ethanol (8 mg/ml) and rapidly added with stirring to the HRPO and antigen solution to a final concentration of 0.15 mg of SPDP per mg of protein. The reaction was continued for 30 min at room temperature with occasional stirring. Thereafter, excess reagent and low-molecular-weight reaction products were removed by extensive dialysis. The antigen solution was dialyzed against 0.1 M sodium phosphate buffer (pH 7.5) containing 0.1 M NaCl, and the HRPO solution was dialyzed against 0.1 M sodium acetate buffer (pH 4.5) containing 0.1 M NaCl. Next, the 2-pyridyl disulfide groups in the HRPO solution were reduced by reaction with dithiothreitol (0.05 M final concentration) for 20 min at room temperature. After removal of excess reducing reagent and pyridine 2-thione by gel filtration with Sephadex G-25 and 0.1 M sodium phosphate buffer (pH 7.5) containing 0.1 M NaCl, the thiol-containing and 2-pyridyl disulfide-containing solutions were mixed with stirring for 2 h at room temperature. After extensive dialysis against PBS (pH 7.2) containing 0.02% NaN_3, bovine serum albumin was added to a concentration of 1%. No significant differences were observed in the quality of conjugates prepared by the two methods. The optimal dilution of labeled antigen as determined by block titration was 1:200. The antigen was stored at 4°C. No decrease in activity of the labeled antigen was found after storage for at least 6 months. Viral antigen for the indirect ELISA was divided into small volumes and stored at −70°C. The optimal concentration of antigen for the indirect ELISA as determined by block titration was 1:100. This dilution was used in all tests. CMV antigen for CF was prepared by glycine extraction of CMV-infected human diploid fibroblasts by the method of Kettering et al. (11).

ELISA procedures. (i) Direct ELISA-IgM. Wells of microtitration plates (Cooke M 29AR) were coated with 0.2 ml per well of rabbit anti-human IgM (μ-chain specific· Dakopatts, Copenhagen, Denmark) at a 1:400 dilution in buffer (pH 9.0) containing 10 mM tris(hydroxymethyl)aminomethane-hydrochloride, 150 mM NaCl, and 2 mM trisodium ethylenediaminetetraacetate. After overnight incubation at 4°C, the plates were washed four times with PBS containing 0.05% Tween 20 (PBS-Tween) and shaken dry. Next, 0.1 ml of test serum at a 1:100 dilution in PBS-Tween with 1% bovine serum albumin was added to each of two wells. After incubation at 37°C for 2 h in a humidified atmosphere, the plates were washed four times with PBS-Tween. A 0.1-ml amount of labeled antigen at a 1:200 dilution in PBS-Tween with 1% bovine serum albumin was then added to each well and incubated for 1 h at 37°C in a humidified atmosphere. After washing four times with PBS-Tween, substrate solution (0.1 ml/well) was added. The substrate solution was prepared immediately before use by dissolving 1 mg of orthophenylenediamine per ml in 0.1 M PBS (pH 6.0) followed by the addition of 0.1% of 30% H_2O_2. After 30 min of incubation in the dark at room temperature, the reaction was stopped by adding 0.2 ml of 3N H_2SO_4. The optical density of the reaction, providing a measure for the amount of specific IgM antibody present, was determined by spectrophotometry (Titertek Multiskan; Flow Laboratories Ltd., Irvine, Scotland) with the buffer control as a blank. The results are expressed as the ratio of the absorbance of

the patient's serum to the negative control serum (P/N ratio). Tests with 180 negative sera indicated that the mean level of ratios (absorbance of test serum/absorbance of negative control serum) was close to 1.0. This indicates that the negative control serum was adequate to serve as representative of negative sera. A P/N ratio of 2.0 lies approximately three standard deviations above the mean value obtained from the negative sera and, thus, might be considered to indicate a specific reaction. However, 2 of 14 sera selected for the presence of high levels of IgM-RF and CMV-IgG antibody showed P/N ratios close to 2.0, namely, 1.9 and 2.3; the IgM fractions of these sera were negative in the direct and indirect ELISA-IgM (see below). Therefore, we decided to consider a P/N ratio higher than 3.0 as positive, thereby increasing the specificity of the reaction. Application of this criterion did not affect the efficacy of the direct ELISA-IgM in detecting positive sera during the acute phase of illness in the present study. All of the first serum samples collected from the 55 patients clinically suspected of having CMV infection showed a P/N ratio either lower than 2.0 or higher than 3.0.

(ii) Indirect ELISA for IgG and IgM antibody. The indirect ELISA was performed essentially as described previously (23). A 0.2-ml amount of CMV antigen suspension at a 1:100 dilution of 0.05 M carbonate-bicarbonate buffer (pH 9.6) was added to each well of microtitration plates. Sera were examined at a 1:100 dilution in PBS-Tween with 2% fetal bovine serum. Peroxidase-conjugated anti-human IgM (μ-chain specific) or IgG (γ-chain specific) were purchased from the Institut Pasteur (Paris, France) and were used at a 1:1,000 dilution in PBS-Tween with 1% bovine serum albumin or 2% fetal bovine serum, respectively. The results are expressed as P/N ratios as described in (i).

RF assays. IgM-RF was determined by a direct ELISA developed in our laboratory (submitted for publication), which was performed essentially as the direct ELISA-IgM technique described above. Briefly, wells of flat-bottomed microtitration plates (Cooke M 29AR) were coated with rabbit anti-human IgM. Subsequently, sera from patients were added at a 1:20 dilution. After incubation at 37°C for 2 h, the plates were washed, and peroxidase-conjugated rabbit IgG was added. After incubation for 2 h, substrate solution containing orthophenylenediamine was added, and the mixture was incubated in the dark at room temperature for 30 min, after which the reaction was stopped by the addition of H_2SO_4. The results were expressed as absorbance at 492 nm. In addition to ELISA, a latex fixation test adapted to microtitration plates was used for estimation of RF (12).

Other serological tests. The CF procedure was carried out by the microtechnique of Casey (2). IgM antibody to Epstein-Barr virus was determined by an indirect IF technique (20) with P_3HR_1 cells fixed to microscope slides and commercial goat anti-human IgM conjugated with fluorescein isothiocyanate (Hyland Laboratories, Costa Mesa, Calif.) at a 1:80 dilution in PBS (pH 7.2).

Sucrose density gradient centrifugation. A 0.2-ml amount of undiluted serum was layered on top of a gradient of 10 to 30% (wt/wt) sucrose. After centrifugation at 286,500 × g (R_{max}) for 25 h at 4°C in the SW41 Ti rotor of a Beckman L5-65 centrifuge, 20 fractions, approximately 0.6 ml each, were collected from the bottom of the centrifuge tube. To detect the separate Ig classes in the fractions, a control serum with high IgG and IgM antibody titers for toxoplasma was fractionated routinely, and the serum fractions were tested by IF for IgG antibody and IgM antibody to toxoplasma. IgM antibody (without traces of IgG antibody) was found in fractions 2 to 8.

RESULTS

Specificity for IgM class of antibody. Fractions of nine sera obtained after sucrose density gradient centrifugation were examined by the direct ELISA-IgM and the indirect ELISA-IgG. The sera had CF antibody titers between 128 and 2,048. Three of the sera were positive in the direct ELISA-IgM. The IgM fractions of these three sera were positive in the direct ELISA-IgM, whereas the IgG fractions of these sera and all fractions of the six remaining sera were negative, indicating that specific IgG antibody did not give false-positive reactions. Results of representative experiments are illustrated in Fig. 1 and 2. Similar results were obtained when the gel filtration procedure described previously (18) was applied for the separation of IgM and IgG. The IgM fractions of two CMV-specific IgM-positive sera obtained in this way were found to be positive in the direct ELISA-IgM, whereas the IgG fractions of these sera were negative.

Effect of RF. RF did not give false-positive results in the direct ELISA-IgM. Five sera (no. 3 to 7) with high CMV antibody levels in ELISA-IgG and moderate or high levels of RF in the latex fixation test and the ELISA test for IgM-

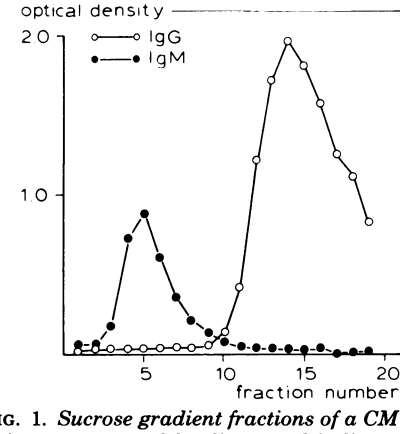

FIG. 1. *Sucrose gradient fractions of a CMV IgM-positive serum tested by direct and indirect ELISA for CMV IgM and IgG antibody, respectively. The serum had a CF antibody titer of 1:128.*

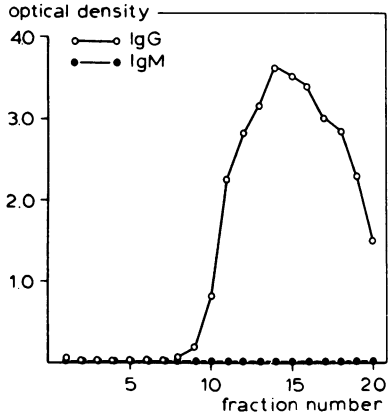

FIG. 2. *Sucrose gradient fractions of a CMV IgM-negative serum tested by direct and indirect ELISA for CMV IgM and IgG antibody, respectively. The serum had a CF antibody titer of 1:2,048.*

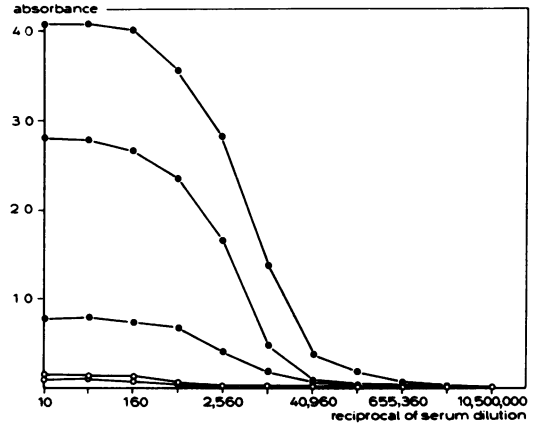

FIG. 3. *Titration of three positive (●) and two negative (○) human sera in direct ELISA for CMV IgM antibody.*

TABLE 1. *Lack of effect of rheumatoid factor on the direct ELISA for IgM antibody to CMV*

Serum no.	CMV IgG level[a] (ELISA)	RF		CMV IgM level[a]	
		Latex fixation	ELISA[b]	Indirect ELISA	Direct ELISA
1	0.4	1,280	0.12	0.9	0.8
2	0.5	2,560	1.11	0.6	0.7
3	52.6	320	0.18	8.2	0.6
4	73.0	1,280	0.57	8.3	1.1
5	88.1	2,560	0.29	21.0	1.5
6	67.5	10,240	1.07	101.6	1.9
7	81.3	20,480	0.87	74.0	2.3

[a] P/N ratio at a 1:100 serum dilution. A ratio higher than 3.0 was considered positive.

[b] Absorbance at a 1:20 dilution. Mean value and standard deviation of 29 RF-negative (latex fixation, <10) sera were 0.027 and ±0.011, respectively. An absorbance of 0.06 (three standard deviations above the mean) or higher was considered positive.

RF were negative in the direct ELISA-IgM for CMV (Table 1). In contrast, the same sera were positive when tested by the indirect ELISA-IgM. The latter results evidently represented false-positive reactions due to IgM-RF reacting with specific IgG, since negative results were obtained when IgM fractions of these sera were examined by the indirect ELISA-IgM (data not shown). Furthermore, sera with RF but without CMV-specific IgG (no. 1 and 2) were negative in both the indirect and direct ELISA tests for IgM antibody.

Dose-response relationship of the direct ELISA-IgM. A dose-response test was performed in serial fourfold dilutions on three positive and two negative sera (Fig. 3). All three positive sera, including the one with a relatively low IgM antibody level, showed a sigmoid curve.

TABLE 2. *Absence of IgM antibody to CMV in paired or single sera from 34 patients with herpes simplex virus, varicella-zoster virus, or Epstein-Barr virus infections*

Infecting virus	No. of patients	CMV IgM level[a] (direct ELISA)	
		Mean	Range
Herpes simplex virus	9[b]	1.0	0.5–1.8
Varicella-zoster virus	13[b]	0.7	0.3–1.7
Epstein-Barr virus	12[c]	0.9	0.6–1.3

[a] Mean and range of P/N ratios at a 1:100 serum dilution. A ratio higher than 3.0 was considered positive.

[b] Significant CF antibody rises in paired sera.

[c] Single sera positive for IF-IgM antibody to Epstein-Barr virus.

It seems likely that the leveling of the curve at high serum concentration reflected saturation of IgM binding sites on the anti-human IgM-coated plates. It is further seen that the test was highly sensitive, detecting IgM antibody at serum dilutions ranging between 1:10,240 and 1:163,840.

Cross-reactivity with other herpesviruses. To test the specificity of the direct ELISA-IgM for detecting CMV-specific IgM antibody, 22 sets of paired sera from patients with a fourfold or greater rise in CF antibody titer to herpes simplex virus or varicella zoster virus and single sera from 12 patients with IF-IgM antibody to Epstein-Barr virus were examined in the direct ELISA-IgM for CMV (Table 2). No CMV-specific IgM antibody was demonstrable in these sera.

Diagnosis of current CMV infections. Acute- and convalescent-phase sera from 55 patients with a fourfold or greater rise in CF antibody to CMV were tested by the direct ELISA

for CMV-specific IgM antibody and by the indirect ELISA for specific IgG antibody (Table 3). IgM antibody to CMV was found in one or both serum specimens from all patients with seroconversions and from 12 of 38 patients with antibody in their initial sera. In six instances IgM antibody was demonstrated in acute-phase sera without detectable antibody in CF and indirect ELISA-IgG.

Table 4 illustrates the antibody response in five patients with a serologically confirmed CMV infection from whom three to five serum specimens were obtained sequentially for up to 102 days after the onset of illness. IgM antibody was detected in sera taken 9 days after the onset of symptoms and reached a peak or plateau by the time CF antibody titers were still increasing. No IgM antibody was found in sera from patient no. 5, who probably had a reactivation or reinfection with CMV. In six other patients (data not shown) who were followed for a longer period, IgM antibody was still detectable in sera for up to 3 to 5 months after the onset of symptoms.

TABLE 3. *IgM antibody to CMV in acute- or convalescent-phase sera (or both) from 55 patients with seroconversions or CF antibody titer rises to CMV as measured by direct ELISA-IgM*

Patient category	No. of patients with IgM antibody to CMV	
	Positive	Negative
Seroconversion[a]	17 (6)[b]	0
Titer rise	12 (7)	26

[a] First serum was negative for CMV antibody in CF and indirect ELISA-IgG.

[b] The number of patients with IgM antibody in first serum is indicated within parentheses.

DISCUSSION

Techniques for the detection of CMV-specific IgM antibody facilitate the early diagnosis of CMV infections since the presence of specific IgM is an indication of current or recent infection. The direct ELISA technique described in this study is an efficient method for demonstrating CMV IgM. Advantages of the test are that laborious procedures for separation of IgM from IgG, such as gradient centrifugation (21) or absorption with anti-γFc (9), are circumvented, and that, unlike the IF test, the direct ELISA provides an objective quantitation of results. Recent studies (20) show that human IgM without antiviral activity can bind to the cytoplasm

TABLE 4. *Comparison of CMV antibodies in serial serum specimens from five patients with CMV infections as measured by CF, indirect ELISA-IgG, and direct ELISA-IgM*

Patient no.	Age (yr)	Days after onset of symptoms	CF	CMV IgG level[a] (ELISA)	CMV IgM level[a] (ELISA)	Symptoms or diagnosis
1	15	2	<2	0.6	1.8	Fever, malignancy
		9	4	9.9	31.7	
		23	32	10.9	10.2	
		30	ND[b]	10.0	3.3	
2	35	9	<2	1.7	18.2	Fever, malaise, renal transplant
		17	<2	6.7	27.4	
		27	16	24.5	24.7	
		49	128	30.4	30.2	
3	18	0[c]	<2	0.3	1.3	Fever, renal transplant
		7[c]	<2	6.5	67.6	
		12[c]	4	12.1	71.8	
4	27	30	16	25.5	45.0	Mononucleosis
		36	32	30.7	37.9	
		82	32	35.9	9.5	
		102	32	32.6	1.3	
5	62	−2	ND	28.0	0.7	Received 17 units of blood
		15	16	41.4	0.8	
		17	64	62.9	0.7	
		25	256	76.5	0.8	
		32	1024	ND	1.3	

[a] P/N ratio at a 1:100 serum dilution. A ratio higher than 3.0 was considered positive.

[b] ND, Not done.

[c] Day of onset unknown. Figures indicate days after collection of first serum specimen.

of CMV-infected cells, which may result in the occurrence of nonspecific reactions in IgM antibody assays. This difficulty was overcome by employing nuclei of infected cells instead of complete cell extracts as antigen (21, 22). The requirement of coupling antigen to peroxidase may be considered a disadvantage of the direct ELISA. However, no major problems were encountered in the preparation of peroxidase-labeled antigen. Furthermore, a large quantity of labeled antigen sufficient for thousands of tests may be prepared at one time since the antigen can be stored for at least 6 months without detrimental effects.

The direct ELISA-IgM proved to be highly specific. Thus, there was no evidence of false-positive results in sera with high levels of RF and high CMV-specific IgG antibody titers. Evidently, the reaction is performed on a sufficiently "purified" IgM preparation. Lack of interference by RF has also been reported in tests for rubella and hepatitis A IgM antibody using the same procedure for obtaining IgM antibody (4, 5, 14, 19). Furthermore, the direct ELISA-IgM appeared to be specific for CMV antigen. Paired sera from patients with rises of antibody titer to herpes simplex virus or varicella-zoster virus were examined by this test (Table 2). Most of these patients were children, and the acute-phase sera showed no homologous antibody or only a low antibody titer. Thus, these infections probably represented primary infections. None of the patients showed CMV-specific IgM antibody. These results agree with those of other workers (9, 15). Similarly, we found no CMV IgM in sera from patients with clinically suspected infectious mononucleosis, who had a positive IF-IgM test for Epstein-Barr virus. In contrast, other investigators (7, 9) using the IF-IgM test reported a high incidence of cross-reactions between CMV and Epstein-Barr virus. Possible explanations for the discrepancy with our results may be that different techniques and antigen preparations were used in the latter studies, and that nonspecific reactions caused by RF were not eliminated in one of the studies (7). Since the direct ELISA-IgM for CMV does not cross-react with Epstein-Barr virus-positive sera, there is no chance of falsely identifying infectious mononucleosis as CMV infection, which may be of importance in view of the overlapping symptomatology observed in patients with CMV mononucleosis and infectious mononucleosis.

The finding that CMV-specific IgM antibody could be detected at serum dilutions as high as 1:163,840 points to the sensitivity of the direct ELISA-IgM. Furthermore, all 17 patients with a seroconversion for CMV antibody showed IgM antibody. Although the absence of antibody in the first serum does not necessarily mean that the infection is a primary one, since the antibody titer from a previous infection may have fallen to an undetectable level, it is reasonable to assume that the vast majority of patients, if not all of them, with seroconversions were experiencing primary infections. Of 38 patients with antibody in their initial sera, 12 developed CMV-specific IgM antibody. It may be assumed that most of these patients had a reactivation of a previous CMV infection or a reinfection with CMV. Other investigators (1, 6, 16) have also observed that a proportion of patients with evidence or reactivation or reinfection with CMV did produce IgM antibody.

CMV-specific IgM antibody was detected in 6 of the 17 acute-phase sera which were negative for CF antibody and specific IgG antibody as determined by the indirect ELISA, thus providing further evidence of the sensitivity of the test. This finding also indicates that detection of IgM antibody has a role in making a prompt diagnosis of CMV infection. The present study and previous reports (9, 10) showed that CMV-specific IgM antibody may persist in some patients for up to 2 to 5 months after infection. Thus, the mere finding of IgM antibody does not accurately indicate the time of the onset of infection. Detection of IgM antibody can, however, be considered a presumptive sign of a recent infection acquired or reactivated within the previous 2 to 5 months. The presence of IgM antibody in the absence of CF and specific IgG antibody is strong evidence of current infection.

ACKNOWLEDGMENTS

We thank Ria Heeren, Jan Zoll, and Dirk Luyt for their skillful technical assistance.

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