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Kinetics of Specific Immunoglobulins M, E, A, and G in Congenital, Primary, and Secondary Cytomegalovirus Infection Studied by Antibody-Capture Enzyme-Linked Immunosorbent Assay

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Antibody-capture enzyme-linked immunosorbent assay (ELISA) using enzyme-labeled cytomegalovirus (CMV) nuclear antigen is a reliable and easily performed test suitable for routine use. As the serologic response to CMV infection may, however, vary considerably among patients, we have studied the kinetics of CMV-specific immunoglobulin M (IgM), IgE, IgA, and IgG antibodies in 352 sera from 61 patients by using antibody-capture ELISA and complement fixation (CF) tests. In a CMV mononucleosis group (n = 17), most patients had antibodies of all four immunoglobulin classes, but antibody levels decreased rapidly, with half the patients having a borderline-positive or a negative reaction for all classes, except IgG, 2 months after the appearance of symptoms. Twelve patients with a primary CMV infection after renal or bone marrow transplantation also developed all immunoglobulin-class antibodies. In only two patients did CMV IgM and IgE antibodies precede seroconversion of CF antibodies, and in one patient, these antibodies lagged months behind. Most patients had all classes of CMV antibodies, except IgA, for a year or more. Among 10 transplant patients with a secondary CMV infection, 50% had long-lasting IgM antibodies, and very few had IgE or IgA antibodies, but all had IgG antibodies to CMV. In 13 infected infants, the CMV-specific serologic response was also characterized by long-lasting IgM, IgE, and IgG antibodies. Two patients did not develop detectable IgM antibodies, and one of these did not show IgE antibodies either. The IgA response in infants as a whole was lacking; a few, however, were borderline positive. Of the nine acquired immunodeficiency syndrome patients with CMV infection studied during their last year of life, only one had antibodies in all four classes, the rest had only CF antibodies, and all except for one had IgG-class antibodies. All sera studied were also tested against a control antigen produced from noninfected cell nuclei. It was found that some patients developed antibodies to nuclear antigens in parallel with the rise in specific antibodies. The nonspecific antibodies occurred in all four classes, but most often they were of the IgM class. Addition of unlabeled control antigen to the conjugates was not always sufficient to abort this nonspecific reaction.

Antibody-capture enzyme-linked immunosorbent assay (ELISA) using enzyme-labeled cytomegalovirus (CMV) antigens has been found especially valuable in diagnosing infections with CMV, as it is possible to detect specific antibodies in the different immunoglobulin classes (immunoglobulin M [IgM], IgE, IgA, and IgG) in patients (3, 11–13, 21, 22, 27–29). By simultaneously testing for both IgM- and IgE-class antibodies to CMV, we have recently found that the serologic diagnosis of neonatal CMV infection was improved considerably, indicating the value of detecting specific antibodies of other immunoglobulin classes than IgM and IgG in CMV-infected patients (13).

In contrast to the indirect tests (4–7, 9, 10, 16–18, 20, 23, 24, 26), in which antigen is fixed to the solid phase and which are used to detect antibodies of the different immunoglobulin classes to CMV, the antibody-capture ELISA gives no problems with false reactions caused by rheumatoid factors or competitive IgG antibodies. Furthermore, enzyme assays are preferable to radioimmunoassays (RIA) (5, 16–18, 20, 23, 25, 26), as the high costs of equipment and reagents, the short shelf life of reagents, and concern for radioactivity are well-recognized limitations of RIA. Immunofluorescence tests (IFT) (6, 14, 17, 24, 25) are too laborious to be used routinely.

Earlier studies using different techniques, RIA, ELISA, or IFT, have shown a great variance in the kinetics of immunoglobulin-class antibodies to CMV in different groups of patients. Most of these studies have been based on titration of sera (5, 6, 9, 14, 16, 18, 21, 26), which is less attractive than one-dilution tests (4, 5, 7, 10, 11–13, 20, 22, 23, 25, 27, 28) for routine diagnostic purposes.

As the antibody-capture ELISA is a one-dilution test, we found it of interest to report the kinetics of the specific IgM, IgE, IgA, and IgG antibodies in various groups of CMV-infected patients. These groups include congenitally and neonatally infected infants, adults with CMV mononucleosis, renal transplant (RAT) patients, bone marrow transplant (BMT) patients with either a primary or secondary CMV infection, and CMV-infected patients with acquired immunodeficiency syndrome (AIDS). By incorporating a control test using enzyme-labeled control antigen made from uninfected cell nuclei, it was found that some patients developed antibodies to nuclear antigens in parallel with the rise in specific antibodies. The nonspecific antibodies might occur in all four immunoglobulin classes, but most often they were of the IgM class.

MATERIALS AND METHODS

Patients. A total of 352 serial sera were available from 12 BMT patients, 10 RAT patients, 13 infants, 17 patients with CMV mononucleosis, and 9 patients with AIDS. Of the transplant patients, 12 probably had a primary CMV infection with seroconversion of complement-fixing (CF) antibodies, whereas 10 had a significant rise (greater than fourfold)

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in CF antibodies, indicating reactivation of a latent CMV infection or a reinfection. CMV was isolated from the urine samples of 16 of the 22 transplant patients, and 6 to 12 sera were studied from each patient. Five of the infants had CMV isolated within the first week of life, indicating congenital infection, whereas the rest had CMV isolated within the first 4 months of life. It was impossible to decide whether the latter had a congenital or acquired CMV infection. A total of four to eight sera were studied from each infant. The patients with CMV mononucleosis were previously healthy persons in whom CMV IgM and CMV CF antibodies had been found. At least two blood specimens taken at an interval of at least a month were available. Clinically, the patients had a self-limiting disease characterized by long-lasting fever, malaise, and lymphocytosis. Most had evidence of liver involvement with or without vomiting or diarrhea. Seven patients had a significant rise in CF antibodies, and the rest exhibited a rise to a CF titer of 32 or more. Infectious agents other than CMV were excluded, and all patients reacted negatively in the mononucleosis agglutination test for heterophile agglutinins (Cellognost-Mononucleosis) (Behringwerke AG). A total of two to four sera per patient were studied. The nine patients with AIDS all had signs of CMV infection during their last year of life, as CMV was isolated either from urine, saliva, or sputum, and all sera examined had CF titers between 8 and ≥256. The number of sera available per patient was between 4 and 13.

Diagnostic methods. Methods for isolating CMV in human lung cell (HEL) cultures and determination of CF antibodies have been published previously (3).

Antigens. CMV antigen was prepared from the nuclei of HEL cells infected with the Ad-169 strain of human CMV essentially as reported previously (12). Briefly, when a nearly complete cytopathic effect was observed, cells were trypsin dispersed and suspended in hypotonic buffer at 0°C for 30 min. Nonidet P-40 was added, and the cells were disrupted in a tight-fitting Dounce homogenizer. When the nuclei were largely freed from the cytoplasm, the suspension was sedimented by centrifugation, and the pellet was suspended in cold phosphate-buffered saline (PBS). The nuclei were separated from cellular debris by centrifugation (1,200 × *g* for 15 min at 4°C) through a layer of 17% Ficoll 400 in PBS. The pellet was suspended in 0.01 M sodium carbonate buffer (pH 9.5). The suspension was sonicated and clarified by centrifugation (5,000 × *g* for 10 min). The supernatant was used as CMV antigen for enzyme labeling. By the CF test, it was found that the CMV antigen contained 32 antigen units before labeling. Control antigen was prepared from the nuclei of uninfected HEL cells by the same procedure.

Antigen-labeling procedure and determination of optimal dilutions of conjugates and unlabeled antigen. Five-milligram samples of CMV and of control antigens were labeled separately with horseradish peroxidase by the periodate method, essentially as described earlier (12), and were used as conjugates. The dilution of CMV conjugate for each immunoglobulin test was determined by checkerboard titration with positive and negative sera. The control conjugate was used in the same concentration as the CMV conjugate in the four immunoglobulin tests. The CMV conjugate and control conjugate matched in reactivity when tested with sera, giving spurious IgM results (heterophile agglutinin-positive sera and sera with antinuclear antibodies [11]). Further, the reactivity of control conjugate with CMV-negative sera was lower than the cutoff values of the various immunoglobulin tests with CMV conjugate. The amount of control antigen added to the conjugates was also determined by checkerboard titration with five specimens with spurious reactions which could be suppressed by the addition of unlabeled antigen. The optimal amount varied from batch to batch, but the batch used in this study was stronger than previous ones (12, 13), as it was effective at a concentration of 33 μg of conjugate per ml.

Antibody-capture ELISA. The antibody-capture ELISA for detection of CMV IgM, IgE, IgA, and IgG antibodies was carried out essentially as described previously (12) for the detection of CMV IgG antibodies. Briefly, individual wells of ELISA plates (Immunoplate I [Nunc, Copenhagen, Denmark]) were coated with 0.1 ml of anti-human IgM, IgE, IgA, or IgG (μ, ε, α, and γ chain specific, respectively [Dakopatts, Copenhagen, Denmark]). The antibodies were used at a dilution of 1:3,200, 1:1,000, 1:1,000, and 1:500, respectively, in carbonate buffer (pH 9.6). After 1 h of incubation at 37°C, the wells were washed in PBS containing 0.05% Tween 20 at pH 7.4 (PBS-T). Then 0.1 ml of serum, diluted 1:10 (IgM, IgE, and IgA) or 1:100 (IgG) in PBS-T with 2% fetal calf serum, was added to four wells per immunoglobulin class studied.

After incubation for 2 h at 37°C, the plates were washed in PBS-T. Two of the four wells received 50 μl of CMV conjugate per well, and the other two wells received 50 μl of control conjugate per well. The conjugates were diluted 1:1,600, 1:2,400, 1:1,600, and 1:1,200 for use in the IgM, IgE, IgA, and IgG tests, respectively, in PBS-T with 2% fetal calf serum and 33 μg of unlabeled control antigen per ml. After incubation overnight at 4°C, the wells were washed in PBS-T. Then 50 μl of substrate solution (2 mg of *ortho*-phenylenediamine per ml of 0.1 M citrate phosphate buffer [pH 5.0] with 0.75 μl of 35% H_2O_2) was added to each of the wells. After 10 min of incubation in the dark at room temperature, the reaction was stopped by adding 0.1 ml of 1 M H_2SO_4 to each well. A_{490} was determined by spectrophotometry (Immunoreader NJ-2000 [Nunc]) with A_{620} as the reference. Wells containing only 0.1 ml of H_2SO_4 were used as blanks. Thereafter, the CMV-specific activity of each immunoglobulin class was calculated as the mean absorbance of the duplicate test with CMV conjugate. The cutoff level was determined as the mean absorbance plus 3 standard deviations for sera from 61 CMV-negative blood donors tested with CMV conjugate.

The cutoff level was an absorbance of 0.2, 0.1, 0.07, and 0.06 for IgM, IgE, IgA, and IgG antibodies, respectively. In addition, the reactivity of a specimen with the control conjugate had to be below the cutoff level with CMV conjugate for the reaction to be considered specific. As mentioned in Results, a number of patients had a simultaneous rise in reactivity against both the CMV conjugate and control conjugate. However, we considered these reactions specific for CMV antibodies if the reactions occurred in parallel with a significant rise in CF antibodies or if the specimens contained specific antibodies in the IgM or the IgE class. Within-run and between-day coefficients of variation were 4 and 15%, respectively, as determined with a CMV antibody-positive serum.

RESULTS

To diminish the influence of test variation on the results, only one batch each of CMV conjugate, control conjugate, and unlabeled control antigen was used throughout this study. Furthermore, all samples from individual patients were tested in parallel with CMV and control conjugate in all four immunoglobulin classes in the same run, and the

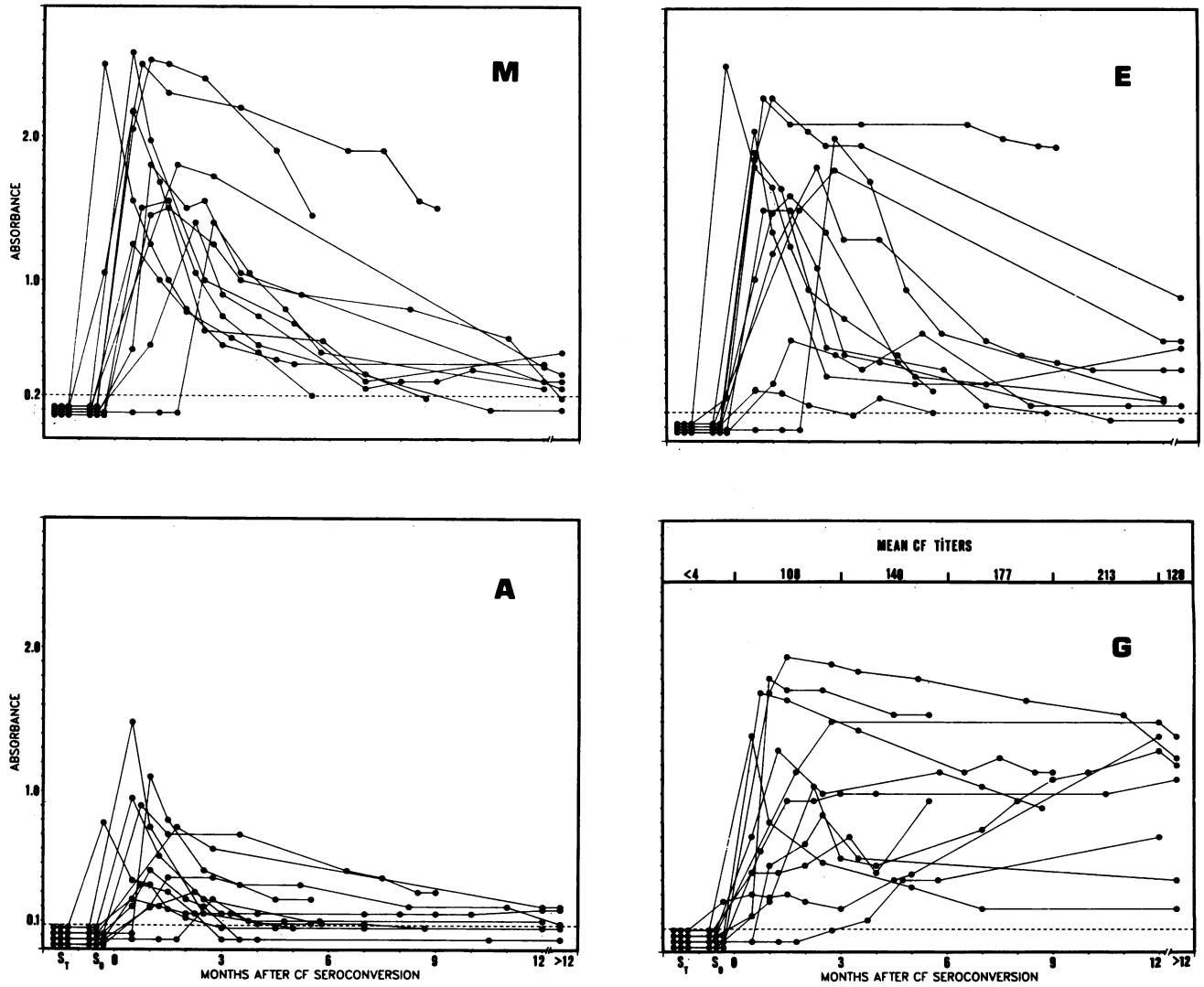

FIG. 1. CMV-specific antibodies of the IgM (M), IgE (E), IgA (A), and IgG (G) classes in sera from seven BMT and five RAT patients with a primary CMV infection. The first serum (S_T) was obtained at the time of transplantation, and the second (S_O) was obtained just before seroconversion in CF titer. The sera studied between S_T and S_O have been omitted, as they reacted negatively in all tests. The cutoff level (-----) is indicated.

reactivity on individual plates was controlled through the use of one positive and one negative control serum on each plate. During the same day, patients from different groups were studied, and the results are presented on the figures using the same scale.

Serologic responses in transplant patients. Figure 1 shows the antibody response in five RAT and seven BMT patients with a primary CMV infection. All patients were negative for antibodies to CMV in all four immunoglobulin classes at transplantation. A rise in antibodies was not seen until the last serum sample obtained just before seroconversion in CF antibodies. At that time, two patients had developed IgM- and IgE-class antibodies to CMV and one of these patients had developed IgA- and IgG-class antibodies as well. This group of patients as a whole developed a strong serologic response, and CMV antibodies were found in all four immunoglobulin classes in all patients. The maximum response occurred in most patients simultaneously with the rise in CF antibodies, except for one patient in whom CF antibodies were detectable 6 weeks before any specific immunoglobulin-class antibodies were found. About 6 months after CF seroconversion, 11 patients (92%) were still positive for IgM and IgE antibodies to CMV, whereas only 7 (58%) had IgA antibodies. Serum samples obtained 1 year or more after seroconversion were only available from eight patients, but six (75%) and seven (88%) of these still contained IgM and IgE antibodies, respectively, and four (50%) were positive for IgA antibodies to CMV. All patients remained CMV IgG positive throughout the study, many with a considerably higher reactivity than in the other immunoglobulin classes.

The kinetics of specific immunoglobulin-class antibodies in five RAT and 5 BMT patients with a secondary CMV infection is seen in Fig. 2. A low positive response of IgG antibodies was present in 80% of the patients at the time of transplantation, and a few were also positive for CMV IgM and IgE. After the significant rise in CF antibodies, five (50%) patients developed a moderate-to-high response in CMV IgM antibodies and all became IgG positive; however, only two did so with a high response. The specific IgE antibody response was generally lacking or very weak,

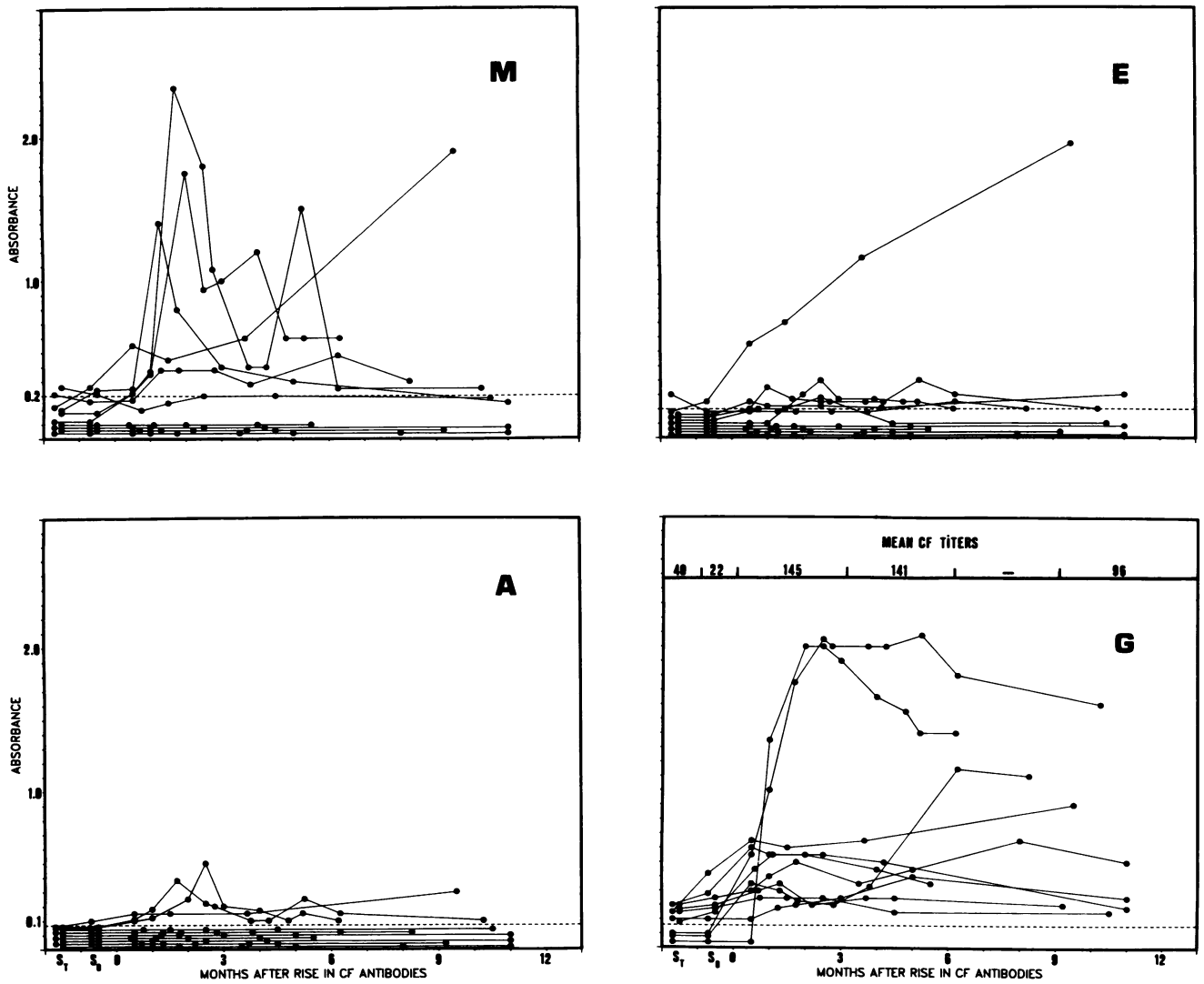

FIG. 2. CMV-specific antibodies of the IgM (M), IgE (E), IgA (A), and IgG (G) classes in sera from five BMT and five RAT patients with a secondary CMV infection. The first serum (S_T) was obtained at the time of transplantation, and the second (S_O) just before the significant rise in CF titer. The sera studied between S_O and S_T have been omitted, as they reacted in between the reaction of S_T and S_O or lower in all tests. The cutoff level (-----) is indicated. −, Too few specimens were taken to permit calculation of mean CF titer.

except in one patient. CMV IgA antibodies with low reactivity were only seen in three (30%) patients. At 6 months after the significant rise in CF titer, a few patients still were CMV IgM positive, only one was IgE positive, and three were IgA positive. All patients, however, were CMV IgG positive but with great individual variances in the response.

Antibody responses in previously healthy patients with CMV mononucleosis. A total of 42 sera were collected from 17 patients with CMV mononucleosis. Although the study period for these patients was rather short, a clear picture of the serologic responses in this group of patients was seen (Fig. 3). The initial sera from all patients, taken 2 to 4 weeks after the onset of symptoms, contained CMV antibodies of all four classes. The reactivity was moderate to high in the IgM and IgE classes but was low to moderate in the IgA and IgG classes. Upon follow-up, reduced reactivities in all four immunoglobulin classes were seen in most patients. Sera were available from seven patients 2 to 3 months after the appearance of symptoms, and at that time, three patients had lost their antibodies in the IgM and IgE classes and only one was moderately positive in the IgA class. CMV IgG-class antibodies were still measurable with antibody-capture ELISA. Sera taken after 3 months or later from four patients showed reactivities in the IgM, IgE, and IgA classes beneath or at cutoff levels. However, these sera still contained some CMV IgG-class antibodies. Although seven patients had a significant rise in CF antibodies and a further three had CF titers of 128 or more, the mean CF titer in this group of patients was considerably lower than that in transplanted patients (Fig. 1 and 2).

Antibody responses in AIDS patients with CMV infection. A total of 59 sera collected during the last year of life from nine AIDS patients were available for the study of immunoglobulin-class antibodies to CMV. They all had active CMV infection during this period, as CMV was isolated from all of the patients in titers from 10 to 10^4 PFU/ml from urine, saliva, or sputum. All sera contained CF antibodies to CMV in titers from 8 to ≥256, and in all sera from eight of the patients, CMV IgG-class antibodies were also present. In the last patient, all sera were without any demonstrable immu-

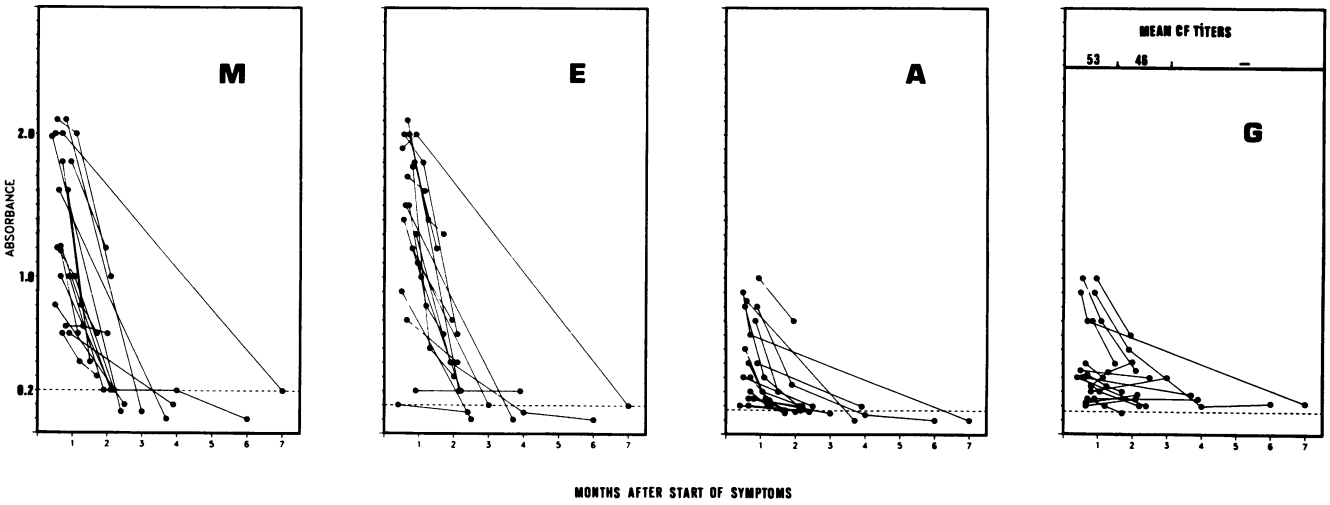

FIG. 3. CMV-specific antibodies of the IgM (M), IgE (E), IgA (A), and IgG (G) classes in sera from 17 patients with CMV mononucleosis. The cutoff level (-----) is indicated. –, For the calculation of mean CF titer, too few specimens were taken.

noglobulin-class antibodies to CMV. CMV antibodies of the other classes were only found in one patient, who during the terminal 6 months of life, seroconverted in CMV IgM- and IgE-class antibodies simultaneously, with a significant rise in CMV IgG-class antibodies and CF antibodies. Only one other patient had a significant rise in CMV IgG-class antibodies. In several patients, a decline in CMV antibodies was seen in the terminal period. Mean CF titers were rather stable during the period in this patient group, falling from 113 to 60 during the last 6 months.

Antibody responses in infants with congenital or acquired CMV infection. In summary (Fig. 4), it was found that 12 (92%), 11 (85%), 5 (38%), and 13 (100%) of the infants developed CMV antibodies of the IgE, IgM, IgA, and IgG class, respectively, during the period studied. Positive reactions in the group as a whole were seen early; only one of the patients became CMV IgA antibody positive after the fourth month of life. The decrease in the immunoglobulin-class antibodies occurred during follow-up, except in the IgG class in most patients. At the age of 1 year, one (13%) and four (50%) of the eight patients studied still had CMV antibodies of the IgM and IgE classes, respectively. At that time, none had CMV IgA antibodies and only five (63%) had CMV IgG antibodies. Positive reactions in the IgA test generally were very low, whereas moderate-to-high reactivity was seen in the other three immunoglobulin-class tests.

Reactivity in sera against control conjugate. Reactivity against control conjugate above cutoff levels of the various immunoglobulin-class tests was seen in 31 (9%) of the 352 sera studied. The sera were from 15 patients, 8 of whom had a primary CMV infection after transplantation and 1 of whom had a secondary posttransplant CMV infection. The other six patients were CMV mononucleosis patients. Reactivity above cutoff levels with control conjugate was only seen in sera which also reacted positively in the same immunoglobulin class with CMV conjugate. The rise of these antibodies against control conjugate occurred simultaneously with the rise against CMV conjugate but declined earlier, usually 2 to 8 weeks after the rise. High reactivity against control conjugate, i.e., a reactivity at the same level as measured against the CMV conjugate, was seen in only 12 sera, all from BMT patients. In 10 of the sera, the nonspecific reaction was only seen in the IgM test, and in 2, it was seen in both the IgM and the IgE tests. The reactivity against control conjugate in the 19 other sera was just above cutoff levels. In summary, 28 of 31 sera showed reactivity against control conjugate in the IgM test, whereas only 9, 3, and 3 showed this reactivity in the IgE, IgA, and IgG tests, respectively.

DISCUSSION

Infection with CMV is very common in transplant patients immunosuppressed with azathioprine or cyclosporine A in combination with prednisone (3, 4, 6, 15–18, 20, 22, 25, 29). Thus, about 90% of RAT patients have evidence of infection postoperatively, and about 10% may have clinically evident infection (3). CMV pneumonia, a life-threatening infection, may occur in about 20% or more of BMT patients (6, 22). In patients with AIDS, CMV infection is one of the important opportunistic infections and may cause death in these patients (7). Furthermore, CMV can infect the fetus (1, 13) and cause severe congenital disease; CMV infection also occurs later in life, clinically most often evident as CMV mononucleosis (8, 18). In addition, subclinical infections are common.

Much laboratory diagnosis of CMV infection is still based on serology. Antibodies appear, on average, 17 days after the onset of CMV-related syndromes (17). The total amounts of CMV antibodies can be measured by the CF test, and specific antibodies of the different immunoglobulin classes, mainly IgM or IgA, may be detected by RIA, IFT, or ELISA. RIA are less attractive for routine diagnostic purposes, since, according to Stagno et al. (23), the isotope labeling must be performed every 3 to 4 weeks to detect CMV IgM antibodies in adult sera, and the labeling must only be few days old to detect low levels of CMV IgM in cord sera. IFT need an automatic reader to be convenient in the routine laboratory, and these readers are just being developed. Therefore, the only attractive method for routine determination of CMV antibodies of the various immunoglobulin classes has been ELISA, although this method has been found to be less sensitive than RIA for the detection of IgM antibodies to CMV (23).

The CF test for detecting CMV antibodies has been miscredited due to a little less sensitivity than IFT and

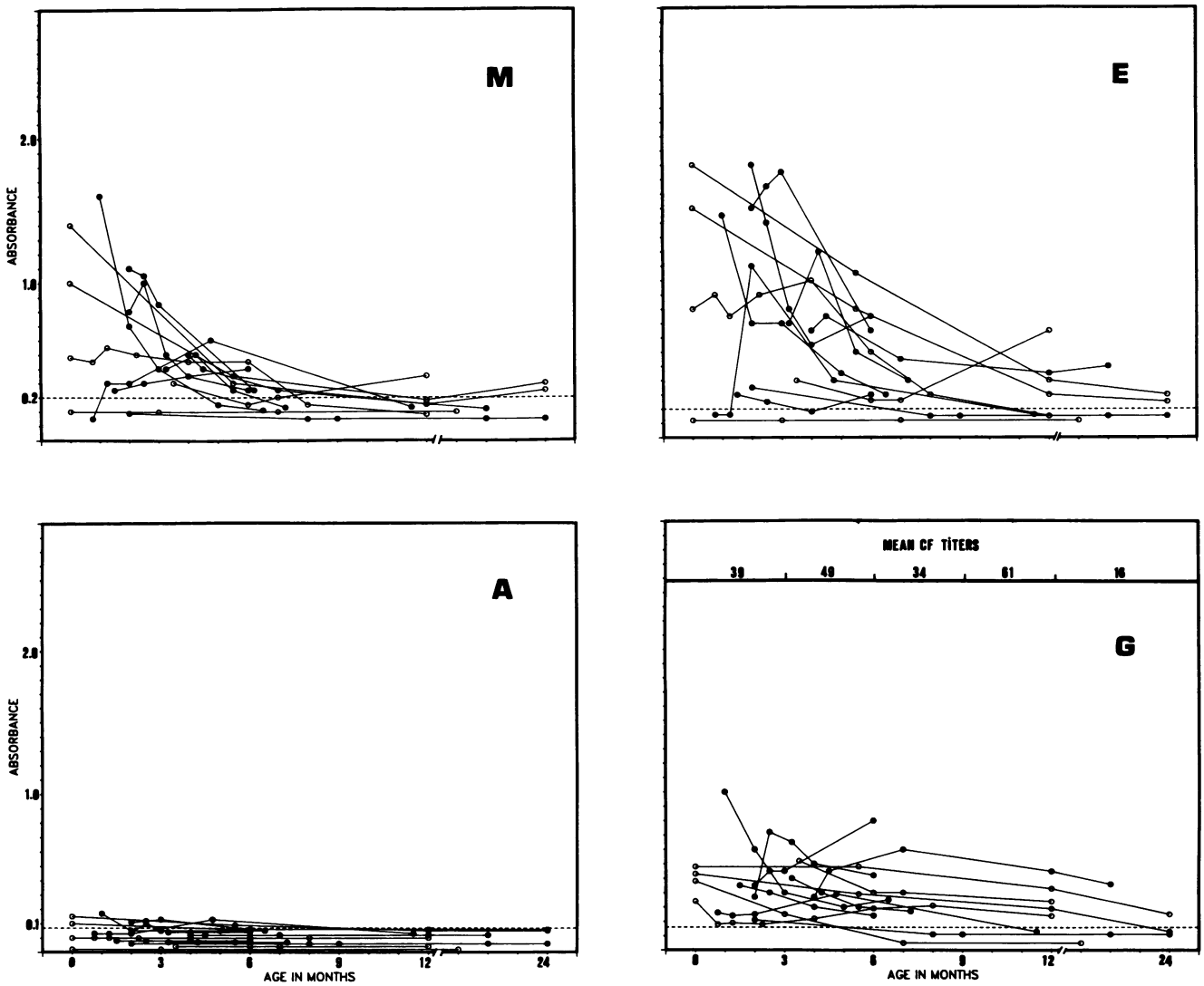

FIG. 4. CMV-specific antibodies of the IgM (M), IgE (E), IgA (A), and IgG (G) classes in sera from 13 neonates with congenital (○) or acquired (●) CMV infection. The cutoff level (-----) is indicated.

ELISA, but the method is still widely used (8, 17, 18, 24, 25). As a diagnostic tool, we have found our own CF test, based on home production of antigen, to be very suitable for monitoring transplanted patients, because preillness sera are often available. The method is less usable for detecting CMV disease in neonates, because of passively transferred maternal CMV IgG antibodies in about 50% of the newborns (1). It has also been found to be less sensitive in diagnosing acquired CMV infections in previously healthy persons, because the preillness sera usually are lacking and significant rises in CF antibodies or elevated CF titers (≥128) occur in less than half of those patients. Furthermore, while monitoring BMT and RAT patients, we have, albeit rarely, found patients who do not develop CF antibodies to CMV, even though they react in other CMV tests or excrete CMV. Therefore, additional tests for the determination of antibodies to CMV, especially acute-phase antibodies, are necessary to increase the diagnostic potency.

This study was performed with an antibody-capture ELISA using enzyme-labeled CMV antigen, described about 8 years ago (21). It has been found very useful for detecting IgM, IgE, and IgA antibodies to CMV (3, 11, 13, 21, 27, 28), and we found that it could also be used for the detection of CMV IgG-class antibodies in infected patients (12). For epidemiological studies, however, the CMV IgG ELISA, as described, is not sensitive enough, as indirect ELISAs, IFT, and CF tests are more sensitive. CMV IgD-class antibodies can also be detected with this technique (results not published), but as the response in CMV-infected patients seems to be weak, we have not included IgD studies in this report. However, in rubella, IgD antibodies occur regularly (26).

To reduce variance, this study was carried out with just one set of conjugates and one batch of unlabeled antigen. Production of antigens and the labeling procedure is not difficult or time consuming (12), and 1 ml of conjugate lasts for about 32,000 wells when diluted 1,600 times. The total amount of conjugate for this study was thus about 2 ml, with 1 ml of each. The CMV conjugate and control conjugate contained the same amount of antigen measured as protein and could be used in identical concentrations. The two

conjugates correspond in strength to the conjugates we have produced earlier, but the unlabeled antigen was three times stronger than the ones we have used in previous studies (12, 13).

In transplant patients with a primary infection, we found that all four immunoglobulin-class antibodies to CMV rose in all patients, often simultaneously with CF antibodies. Only two patients had detectable immunoglobulin-class antibodies before CF seroconversion, and the CF antibodies of one patient appeared 6 weeks before any specific antibodies could be detected by ELISA. This emphasizes the importance of using several antibody tests for diagnosing CMV infection. The immunoglobulin-class antibodies persisted in most patients throughout the study period (almost a year), although a certain decline in the response was seen. All patients with CMV mononucleosis also had raised CMV IgM-, IgE-, IgA-, and IgG-class antibodies, but the decline in response was rapid, with some having a negative or borderline-positive response 2 to 3 months after the onset of symptoms. These observations correspond well with previous findings with indirect ELISAs, RIA, and IFT (4, 9, 17, 20, 24–26, 29). We also found that half the patients with a secondary posttransplant CMV infection had raised specific IgM antibodies, but some of these patients may also produce CMV antibodies of the IgE class (Fig. 2). Only one patient, however, developed high reactivity in the IgE test. Therefore, as suggested by van Loon et al. (27), it is not always possible to distinguish between primary and secondary CMV infection on the basis of the presence of CMV IgE antibodies in a patient. In secondary CMV infections, the immunoglobulin-class antibodies persisted for several months; IgA-class antibodies, however, were weak or lacking. By monitoring the serologic response in infants, we also found persistence of IgM, IgE, and IgG antibodies to CMV in many patients. The IgA response in this group was weak and, in many patients, was lacking. Previous studies on specific IgA production in CMV-infected infants do not seem to have been published, but in Epstein-Barr virus infection, too, the IgA response is low or lacking in infants (14).

AIDS patients were included in this study because the severe immunosuppression caused by viral infection in the lymphocytes might influence the serologic response to CMV during the terminal stage when elevated levels of antibodies to CMV are found (7). The serologic picture was rather uniform, with all patients having CF antibodies to CMV. A significant rise and fall in antibodies of all classes to CMV and CF antibodies was only seen in one patient, although all patients had a CMV infection during that period, as demonstrated by isolation of CMV, often in high titers. Many of them probably also had viremia, but blood samples were not received from these patients for cultivation, because the patients were hospitalized in another part of the country. Viremia is often accompanied by CMV IgM antibodies in both primary and secondary CMV infection in transplant patients (3, 18). Therefore, compared with the findings in transplant patients, a defect in CMV IgM antibody production may be present in AIDS patients, although this needs further study.

Nonspecific reactions, i.e., reactivity in sera against cellular antigens in the CMV conjugate, may of course occur (4, 11, 18, 25, 26). To a wide extent, these nonspecific reactions can be depressed by adding unlabeled control antigen, harvested from noninfected HEL cell nuclei, to the conjugates. In our experience, however, it is necessary to run in parallel a test for specificity using a control conjugate. Concerning nonspecific reactions in CMV-infected patients, we found them especially in the IgM test and in sera from BMT patients, in which reactivity with control conjugates might be of the same order of magnitude as the one measured with CMV conjugate. Nonspecific reactions were rare in the IgE test, as only two sera were found to react strongly against control conjugate. Nonspecific reactions in the IgA and IgG tests were also very few and weak and can thus be ignored in most patients. Reactivity against control conjugate was never seen in CMV-infected infants (13) and in sera from AIDS patients but occurred also in sera from a few adults with CMV mononucleosis. In these patients, however, the reactivity against control conjugate in each case was much weaker than the reactivity against CMV conjugate, and thus it is of minor importance. Reactivity to control conjugates was restricted to a short period of time during which the patients seroconverted or had a significant rise in CMV CF antibodies. We therefore interpreted the reactivity to control conjugate as being caused by autoantibodies, which are known to be produced during the immunologic response to CMV (2).

Previous studies on the IgM-capture ELISA have shown that false-positive reactions may be seen in sera which are Paul-Bunnell positive or contain antinuclear antibodies (11). Although these sera also react against control conjugate, they constitute a diagnostic problem in the serologic laboratory. It is important to study whether these sera would also cause a false-positive reaction in the IgE, IgA, and IgG tests. If not, it could be a further stimulus to run a test for another immunoglobulin-class antibody to CMV in parallel with the IgM ELISA. For the confirmatory test, we would prefer an IgE test because of its higher sensitivity for detecting CMV antibodies in infants (13).

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