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Early Detection of Active Cytomegalovirus (CMV) Infection after Heart and Kidney Transplantation by Testing for Immediate Early Antigenemia and Influence of Cellular Immunity on the Occurrence of CMV Infection

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To determine the incidence of active cytomegalovirus (CMV) infection after organ transplantation and its relationship with the immune system, 55 renal and 14 cardiac transplant recipients were closely monitored for active CMV infection (expression of CMV immediate early antigen in granulocytes—antigenemia—and positive cultures) and immune parameters. All 19 CMV-seronegative recipients with seronegative donors remained seronegative, showing that no CMV transmission occurred by leukocyte-depleted blood products. Primary CMV infection occurred in 4 of 11 (36%) patients with positive donors and was symptomatic in 1 (9%) patient. Active CMV infection was found in 29 of 39 (74%) seropositive patients and was symptomatic in 3 (8%) patients. CMV antigenemia was always the first indication of active CMV infection (antigenemia, on average, at day 45 ± 15; immunoglobulin G rise at day 71 ± 36; and positive cultures at day 70 ± 17). Cellular immunity, as measured by lymphocyte proliferation (LPT), proved to be of importance in the prevention of active CMV infection, as 14 of 15 patients with negative LPT obtained active CMV infections with antigenemia and positive cultures, whereas 1 of 10 patients with positive LPT did so ($P < 0.0001$).

Active cytomegalovirus (CMV) infections frequently occur after heart and kidney transplants and can be the cause of serious disease. The incidence varies between 40 and 90% (1, 5, 7, 8, 10, 14, 29). The source of the infection can be reactivation of an endogenous latent CMV strain, reinfection or primary infection with a donor CMV strain (1, 2, 10), or both, since CMV can be transmitted by the transplanted organ.

Active CMV infection and CMV disease only develop in immunocompromised hosts, such as organ transplant recipients treated with immunosuppressive therapy. The cause of active CMV infection is thought to be due mainly to the decrease in cellular immunity, especially the cytotoxic T-cell function, which is influenced by immunosuppressive therapy (6, 20).

Recently, a new method of detecting an active CMV infection has been developed by using monoclonal antibodies against immediate early antigens (IEAs) of CMV in peripheral blood polymorphonuclear leukocytes (PMNs). This antigenemia test seems to be more sensitive than culture of buffy-coat cells from viremic patients (26).

In this study we evaluated the antigenemia test, together with quick cultures of urine, saliva, and buffy-coat cells, in the detection of active CMV infection in heart and kidney transplant recipients in relation to humoral (immunoglobulin M [IgM] and IgG antibodies) and cellular immunity (lymphocyte proliferation). The antigenemia test was shown to provide an earlier indication of an active CMV infection than were cultures or a rise in IgG antibodies.

Cellular immunity, as measured by lymphocyte proliferation, appeared to play an important role in preventing generalized and more serious CMV infections, as documented by antigenemia combined with positive cultures and the presence of CMV disease.

MATERIALS AND METHODS

Patients. (i) Kidney transplant recipients. From May 1988 to October 1989, a total of 55 patients received a kidney transplant and were included in this study (organ survival, more than 3 months). CMV-seronegative patients receiving a kidney from a CMV-seronegative donor were monitored serologically. The immunosuppressive regimen consisted of cyclosporine A and steroids or imuran and steroids. Rejections were treated with high-dose steroids; persistent rejections were treated with anti-T-cell globulin (ATG; RIVM, Bilthoven, The Netherlands) or anti-CD3 monoclonal antibody (OKT3).

(ii) Heart transplant recipients. A total of 15 patients received a heart transplant. One patient was excluded from this study because she died within 1 month after transplantation. The maintenance immunosuppressive therapy consisted of steroids, imuran, and cyclosporine A. Rejections were treated with steroids (grade II), OKT3, or ATG (persistent grade II, grade III).

Cells and virus. Human diploid fetal fibroblasts (FFs) were cultured in RPMI 1640 medium (GIBCO, Uxbridge, England) supplemented with glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum (Flow Laboratories, Irvine, England).

CMV strain AD 169 was cultured in FFs. The culture medium was replaced at least once a week. When extensive cytopathic effects occurred, the cells were trypsinized

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(0.05% trypsin in phosphate-buffered saline [PBS]), and antigen was prepared from the cells by repeated freezing and thawing (CMV late antigen [LA]).

Isolation of mononuclear cells and PMNs. Heparinized peripheral blood was obtained from the transplant recipients at least every 2 weeks up to 3 months after organ transplantation. Mononuclear cells (MNCs) were isolated by Ficoll (Pharmacia Fine Chemicals, Piscataway, N.J.) density centrifugation. The bottom fraction, which contained erythrocytes and PMNs, was used to isolate the PMNs. For this purpose, the cells were suspended in PBS containing 1.25% dextran and incubated for 20 min. After sedimentation, the top-layer cells were washed, the remaining erythrocytes were lysed, and the PMNs were washed again. These cells were used in the culture assays and for the detection of antigenemia.

Lymphocyte proliferation assay to CMV. Isolated MNCs were suspended in RPMI 1640 medium containing 15% pooled human type AB serum and supplemented with glutamine, penicillin, and streptomycin. The pooled human serum was from CMV-positive blood donors. A total of 10^5 MNCs were stimulated with 10^4 CMV-infected FFs. Uninfected FFs were used as controls. The specificity of CMV-infected FFs was tested by comparison with cultures stimulated by herpes simplex virus- or varicella zoster virus-infected FFs or *Candida* antigen (3).

Proliferation was measured by determining the incorporation of [^{3}H]thymidine. The MNCs were pulsed on day 5. The cells were harvested 16 to 18 h later, and the incorporation of [^{3}H]thymidine was measured in a liquid scintillation counter. Lymphocyte proliferation tests with a stimulation index of at least 3 (control/uninfected fibroblasts) and with a net pulse of at least 1,000 cpm more than that of the control were considered to be positive.

Quick cultures of urine, saliva, and buffy-coat cells. FFs were seeded into 96-well flat-bottom culture plates. After 3 to 4 days (confluent monolayer), urine, saliva (diluted 1:1 with culture medium containing fetal bovine serum), and PMNs obtained from the patients at least every 2 weeks were inoculated into the wells. PMNs were used because they proved to be the cells that can cause a CMV-positive culture (5; unpublished data). Before inoculating the wells, the PMNs were frozen (−80°C) and thawed to minimize the destruction of the monolayer of FFs by the PMNs. The wells were inoculated with 5×10^6 to 10×10^6 PMNs per six wells. The plates were centrifuged (12, 24), and the monolayers were incubated for 2 h at 37°C. After the incubation the plates were washed with culture medium containing fetal bovine serum and cultured for another 3 to 4 days at 37°C in a 5% CO_2 in air atmosphere.

For the detection of CMV infection in the FFs, the DEAFF method was used (9, 23). The wells were fixed with cold methanol for 10 min, washed with PBS, and incubated for 2 h with a monoclonal antibody against the CMV IEA (Biosoft, Paris, France) that was diluted 1:200 in RPMI 1640 medium with 1% bovine serum albumin–0.1% azide. This monoclonal antibody is directed against a CMV IEA of 72 kilodaltons, which appears in FFs about 1 h after infection, and the intensity peaks at approximately 96 h after infection. After the plates were washed, a 1:100 dilution of fluorescein-conjugated goat anti-mouse immunoglobulin (Becton Dickinson & Co., Mountain View, Calif.) was added to the wells and incubated for 1 h. The plates were washed with PBS, 100 μl of PBS containing 0.1% azide was added to the wells, and positive cultures were detected with a fluorescence microscope.

IEA test on PMNs for the detection of antigenemia. Isolated PMNs from the peripheral blood of the patients were suspended in RPMI 1640 medium containing 1% human serum. Cytospin preparations were made (1.5×10^5 cells per cytospin). The cytospin preparations were dried in air and fixed with acetone. Infection of the cells was detected with monoclonal antibody anti-IEA (CMV-C10 and CMV-C11; Biotest, Dreieich, Federal Republic of Germany) and peroxidase staining. At least two cytospin preparations per patient were incubated every 2 weeks with a combination of two monoclonal antibodies against CMV IEA for 1 h at room temperature. One preparation served as a control and was incubated with PBS. The monoclonal antibodies were kindly provided by T. H. The (University Hospital, Groningen, The Netherlands). Both monoclonal antibodies are directed against a 70-kilodalton IEA. Virus specificity was tested on herpes simplex virus type 1-infected Vero cells, herpes simplex virus type 2- and varicella zoster virus-infected FFs, and lymphoblastoid cell lines expressing Epstein-Barr virus nuclear (Raji) or virus capsid (P3HR1) antigens. No cross-reactivity was observed (27, 28).

After the first incubation, the slides were washed and incubated with a horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (TAGO, Burlingame, Calif.) for 1 h at room temperature. The slides were washed and stained with 3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, Mo.) in acetate buffer (pH 4.9) with 0.015% H_2O_2. Counterstaining was performed with Mayer hematoxylin (Riedel-de Haen).

Determination of the IgG and IgM levels in serum. The IgG and IgM levels against CMV LA in serum were determined by an enzyme-linked immunosorbent assay (ELISA; Sorin Biomedica, Saluggia, Italy) and in an ELISA with CMV LA-coated plates as described previously (16). In preliminary experiments, these assays showed similar results; therefore, only the results obtained by ELISA (Sorin) are presented here. A significant rise in IgG levels in serum was defined as at least a fourfold rise in the CMV LA ELISA, which corresponded with a rise in IgG in serum of at least 25 arbitrary units by ELISA (Sorin).

Diagnosis of active CMV infection and CMV disease. Active CMV infection was diagnosed when the patients had a positive serology together with positive cultures or IEA antigenemia. Viremia implied positive buffy-coat cell cultures. CMV disease was diagnosed when the patients had active CMV infection and suffered at the same time from one or more of the symptoms mentioned by Smiley et al. (22).

Statistical evaluation. Statistical evaluation of the differences between the groups was performed by the Fisher exact test (Tables 1 to 3 and Fig. 2) or Student's *t* test (Fig. 1).

RESULTS

CMV infection and detection by culture and antigenemia. The results of the measured CMV parameters in all kidney and heart transplant recipients who were followed for at least 3 months are presented in Table 1.

(i) Kidney transplant recipients. All 10 CMV-seronegative kidney transplant recipients with a CMV-seronegative donor remained CMV seronegative. The same was true for six CMV-negative recipients with a donor from whom no serum was available or with a very low IgG anti-CMV LA titer, indicating, presumably, that CMV was passively acquired. Of the 29 kidney transplant recipients who were CMV seropositive before organ transplantation, 22 patients (76%) developed an active CMV infection, as detected by positive

TABLE 1. Results of CMV infection parameters in different patients

Type of organ transplant and serology (donor/recipient)	No.	No. of patients with active CMV infection[a]					No. of patients with CMV disease
		Total	Antigenemia	Culture	Culture and antigenemia	Viremia	
Kidney							
+/+	9	6	1	3	2	1	0
−/+	16	13	11	2	0	0	0
?/+[b]	4	3	1	0	2	1	1
+/−	10	3	0	0	3	2	1
?/−	6	0	0	0	0	0	0
−/−	10	0	0	0	0	0	0
Heart							
+/+	3	3	0	0	3	1	1
−/+	5	3	0	0	3	3	1
?/+	2	1	0	0	1	0	0
+/−	1	1	0	0	1	1	0
−/−	3	0	0	0	0	0	0
Combined heart and kidney							
+ or −/+	39	29 (74)					3 (8)
+/−	11	4 (36)					1 (9)
− or ?/−	19	0					0

[a] CMV infection was determined by CMV antigenemia, positive cultures from urine or saliva or both or by viremia. Values in parentheses are percentages.
[b] A question mark indicates that either no donor serum was available or the IgG levels were low and, possibly, positive because of the blood transfusions (passively acquired).

cultures, antigenemia, or both. In 13 patients, only antigenemia was detected as a sign of a CMV reactivation. In 8 of these 13 patients, antigenemia was monitored by a significant rise in IgG levels in serum, but cultures of urine, saliva, and buffy-coat cells remained negative throughout the follow-up period. Only 7 of the 39 patients at risk for CMV disease had both antigenemia and positive cultures and only 4 had viremia. No significant differences were found between seropositive patients with negative or positive donors.

A primary CMV infection occurred in 3 of the 10 (30%) CMV-negative recipients with a CMV-positive donor. Primary infection could be detected 14, 29, and 56 days after organ transplantation. In all three patients, antigenemia was the first indication of the CMV infection, even before the appearance of IgM or positive cultures.

(ii) Heart transplant recipients. All three CMV-seronegative heart transplant recipients with a CMV-seronegative donor remained CMV seronegative. Of the 10 CMV-positive heart recipients, 7 (70%) patients developed active CMV infection and all 7 patients had both antigenemia and positive cultures.

Two CMV-negative patients had transplanted hearts from CMV-positive donors. One patient died 1 month after the transplant (no CMV infection was detected), and one patient developed a primary CMV infection that was first detected by positive cultures of saliva and buffy-coat cells followed by IgM antibodies at day 58 after the transplant. No serious symptoms occurred.

In 14 kidney and heart transplant recipients who had both antigenemia and positive cultures of urine or saliva, antigenemia appeared, on the average, 45 ± 15 days after the organ transplants, positive cultures appeared at day 70 ± 18 ($P < 0.005$ compared with antigenemia), and IgG rise to CMV LA appeared at day 71 ± 38 ($P < 0.05$ compared with antigenemia) (Fig. 1).

Only 4 patients had CMV disease (three patients with interstitial pneumonia, one patient with fever and leukopenia only); all four patients had strongly positive antigenemia, and three of these patients had viremia. All four patients recovered, two of them after ganciclovir treatment.

Serology. None of the 19 seronegative patients with negative or presumably negative donors showed seroconversion. Of the 11 seronegative patients with positive donors, only 4 showed an active CMV infection (antigenemia and positive cultures; Table 1) and seroconversion. One patient who received hyperimmunoglobulin (Cytotect; Biotest, Dreieich, Federal Republic of Germany; 1 ml/kg, five times during 7 weeks) had no IgM antibodies or direct IgG anti-CMV antibodies. Antigenemia appeared before IgM antibodies did in two kidney transplant recipients with primary CMV infection. Of the secondary infections or reactivations, one kidney and three heart transplant recipients had positive IgM anti-CMV LA titers. Of these patients, two had CMV-positive donors and two had CMV-negative donors.

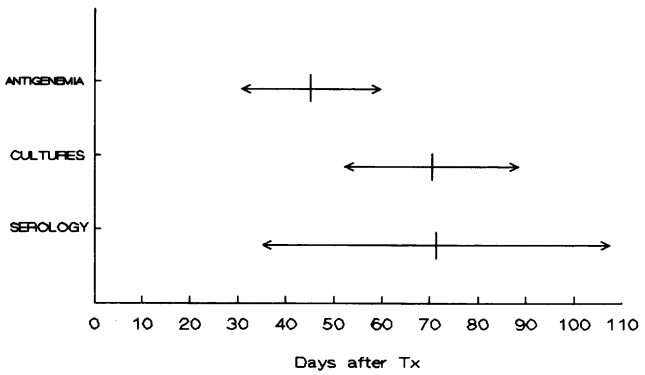

FIG. 1. Detection of active CMV infection over time after organ transplantation (Tx). All 14 patients had antigenemia, positive urine or saliva cultures, and an increase in IgG anti-CMV levels. No significant differences were observed between the heart and kidney recipients. Statistical evaluations were as follows: antigenemia versus positive cultures, $P < 0.005$; antigenemia versus increase in IgG, $P < 0.05$.

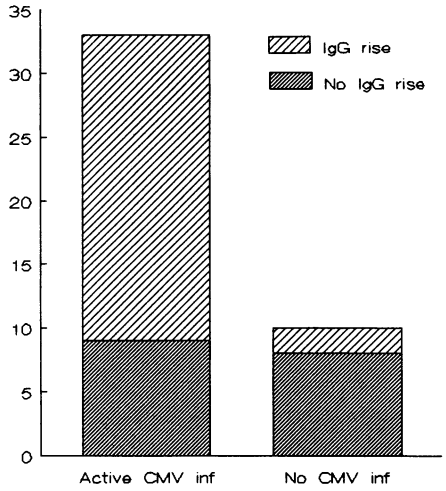

FIG. 2. IgG anti-CMV LA serology. IgG serologies from CMV-positive recipients and CMV-negative recipients who had a primary infection were evaluated. Results for 11 heart and 32 kidney recipients are presented. Infection was detected by antigenemia, positive cultures, or both. For the comparison of the two groups (active CMV infection [inf] and no CMV infection), $P = 0.004$.

Of the 33 heart and kidney recipients with an active CMV infection, 24 (73%) patients had a significant rise in IgG levels in serum. Of the 10 patients with no active CMV infection (as determined by antigenemia, positive cultures, or both), 2 (20%) had a quick rise in IgG levels in serum (Fig. 2).

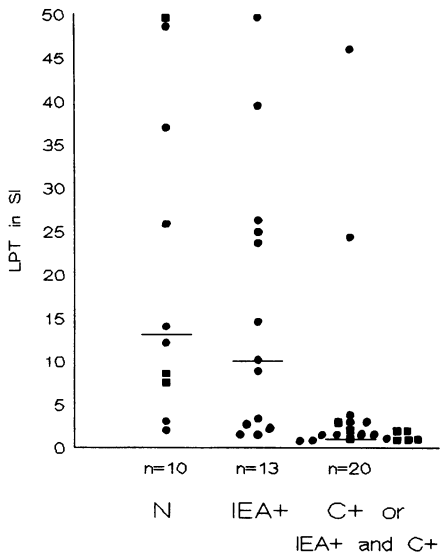

FIG. 3. Lymphocyte proliferation (LPT) in kidney and heart recipients. CMV-positive recipients and CMV-negative recipients who had primary infections were evaluated. Results are expressed as a stimulation index (SI; control/uninfected FFs). Proliferation was measured after transplantation and before active CMV infection. For kidney recipients (●), values are medians at day 30 (range, 12 to 128 days); for heart recipients (■), values are medians at day 31 (range, 26 to 65 days). N, No infection; IEA, IEA antigenemia; C, culture positive. ——, median value.

TABLE 2. Lymphocyte proliferation in kidney and heart transplant recipients combined

Infection status	No. of patients		
	Proliferation	No proliferation	Total
Active infection with antigenemia and cultures	1	14[a]	15
No active infection	9[b]	1[b]	10
Total	10[b]	15[b]	25

[a] Including four patients with primary infections. Without the four patients with primary infections, $P = 0.0003$.
[b] $P < 0.0001$.

Lymphocyte proliferation to CMV-infected FFs. (i) Kidney transplant recipients. Of seven kidney transplant recipients with antigenemia and positive cultures, six (86%) had no lymphocyte proliferation to CMV-infected FFs after the transplant and before active CMV infection was detected (Fig. 3). Of the six patients without active CMV infection, five patients had a high lymphocyte proliferation to CMV-infected FFs after the kidney transplant (83%).

CMV disease appeared only in patients without lymphocyte proliferation to CMV-infected FFs after kidney transplantation but before the onset of CMV disease.

(ii) Heart transplant recipients. Of 11 heart transplant recipients, 8 had no lymphocyte proliferation to CMV-infected FFs after the transplant. Of these patients, one had a primary infection and seven had CMV reactivation or reinfection. All seven of these patients had positive cultures and antigenemia; five had viremia and two had symptoms of CMV disease. Three patients had high lymphocyte proliferation, and in all three of these patients, neither antigenemia nor positive cultures were detected ($P = 0.006$).

(iii) Heart and kidney transplant recipients combined. Of 15 transplant recipients who had an active CMV infection with antigenemia and positive cultures, 14 had no lymphocyte proliferation to CMV-infected FFs (Table 2). Of 10 patients without active infection, 9 had a high lymphocyte proliferation to CMV-infected FFs ($P < 0.0001$). When primary infections were excluded, since CMV-negative patients did not show proliferation to CMV-infected FFs, the results were still significant ($P = 0.0003$).

Treatment with OKT3, ATG, or both. OKT3 or ATG treatment was given to 5 of the 11 heart transplant recipients. Of these five patients, four had an active CMV infection after treatment, and lymphocyte proliferation to CMV was negative. One patient was treated with ATG, but lymphocyte proliferation to CMV remained positive and no CMV infection was detected.

Of 32 kidney recipients (CMV positive and CMV negative with a primary infection), 8 were treated with ATG, OKT3, or both to prevent rejection. Of these eight patients, six had no lymphocyte proliferation to CMV but had an active CMV infection; two of these six patients had CMV disease. One patient received OKT3, proliferation to CMV became negative, but no infection was detected. One patient had high levels of proliferation to CMV after ATG treatment but no infection (Table 3).

Differences between heart and kidney transplant recipients. Twenty-nine CMV-positive kidney recipients were included in this study (Table 1). Of these 29 patients, 22 (76%) developed an active CMV infection. In the CMV-positive heart transplant recipients, 7 of 10 patients had an active CMV infection (70%; differences not significant). All 7 heart

TABLE 3. ATG or OKT3 administration and lymphocyte proliferation to CMV

Type of organ transplant	No. of patients[a]	
	Anti-CD3	No anti-CD3
Heart	5	6
Kidney	8	24
Total	13	30
Lymphocyte proliferation to CMV, negative	11	10
Lymphocyte proliferation to CMV, positive	2	20

[a] Included in this assessment were at-risk patients: CMV-positive recipients and CMV-negative recipients with primary infections. For lymphocyte proliferation versus anti-CD3 treatment, $P = 0.02$.

recipients had antigenemia as well as positive cultures of urine or saliva, whereas only 4 of the 22 kidney recipients (18%) were positive by both tests ($P = 0.0002$). Antigenemia occurred in 13 of the 22 kidney recipients (59%), and positive cultures were detected in only 5 patients (23%).

Of the 29 CMV-positive kidney recipients and the 3 kidney recipients who had a primary CMV infection, 18 of the 32 patients (56%) had high lymphocyte proliferation to CMV-infected FFs after the transplant. In the heart recipients, 3 of 11 patients (27%) had high lymphocyte proliferation to CMV-infected FFs after the transplant ($P = 0.004$ compared with the kidney recipients).

DISCUSSION

In this study 55 kidney and 14 heart transplant recipients were extensively followed for parameters of CMV infection for at least 3 months after organ transplantation. Several parameters were measured at least every 2 weeks, including immunity of the patients to CMV (serology and lymphocyte proliferation to CMV LA), detection of active CMV infection (IEA antigenemia and cultures of urine, saliva, and buffy-coat cells), and symptoms of CMV disease. Evaluation of the results can lead to an estimation of the risk factors that are involved in the development of active CMV infection and disease. The results also show the accuracy of the methods available for detection of infection.

All 13 CMV-negative kidney and heart recipients with a CMV-negative donor remained CMV negative. As these patients received only strongly leukocyte-depleted blood products (filtered erythrocytes and centrifuged platelets), we conclude that strong leukocyte depletion is a safe method for the prevention of primary CMV infections by blood products; this has been our experience with another patient group (patients with leukemia who received bone marrow transplants) as well (4). It also indicates that the occurrence of CMV infection in the other patients was not influenced by the transfusion of blood products.

Another six CMV-negative kidney recipients with donors of unknown serology also remained CMV negative. For these donors, either there was no serum available ($n = 3$) or the IgG anti-CMV LA titer was low and was, possibly, passively acquired ($n = 3$). Therefore, other methods of determination of whether a donor is CMV positive or negative can be valuable, for instance, performing a polymerase chain reaction on donor material (13, 17) or detection of IEA and early antigen (25) in the donor organ.

Of the 10 CMV-seronegative kidney recipients with a CMV-seropositive donor, only 3 (30%) developed a primary CMV infection. This incidence is low compared with those reported previously (Smiley et al. [22], 61%; Metselaar et al. [15], 89%; Grundy et al. [10], 79%; Betts et al. [1], 78%; Ho et al. [11], 83%). Chou and Norman (2) have established the importance of donor factors on the transmission of CMV with the transplanted kidney. Toorkey and Carrigan (25) found CMV IEA-positive cells in six of nine kidneys from normal CMV-seropositive donors. The low incidence of primary infection in our study can only be explained by coincidence. Hyperimmunoglobulin treatment or immune suppression did not influence the incidence of primary CMV infection (data not shown). In CMV-seropositive donors, either CMV was not present in the donor kidney or it was not able to reactivate and cause infection. In view of the results of hyperimmunoglobulin treatment, it seems important that differentiation between these possibilities be done in the future.

In the heart transplant recipients, infection was always detected by antigenemia together with positive cultures of urine or saliva (8 patients), whereas in the kidney recipients, infection was detected by antigenemia alone in 13 of the 25 recipients, by antigenemia together with positive cultures in 7 patients, and by positive cultures only in 5 patients. The differences between the heart and kidney recipients are probably due to differences in immune suppression. Heart transplant recipients were treated with triple therapy (steroids, imuran, and cyclosporine A) and needed anti-CD3 treatment more often, whereas kidney recipients were treated with steroids together with cyclosporine A or imuran, which was reflected in the lower percentage of patients with lymphocyte proliferation to CMV-infected FFs in the heart transplant group (3 of 11 patients) compared with that in the kidney transplant group (18 of 32 patients).

In 13 kidney recipients, mild antigenemia (<100 positive PMNs per 10^6 cells) was detected as an indication of an active CMV infection, and in 8 of these 13 patients, infection was confirmed by a significant rise in IgG levels in serum. In the 14 kidney and heart recipients who had antigenemia together with positive cultures, antigenemia was the earliest parameter that became positive. Also, of the two kidney and two heart recipients with CMV disease, CMV disease was accompanied in three of them by a high number of CMV IEA-positive PMNs (>100/10^6 cells). CMV disease was present at the time of antigenemia in all four patients, and positive cultures were detected a few weeks later.

Schirm and colleagues (21, 26) and Revello et al. (19) have correlated antigenemia and viremia. In our study, antigenemia appeared earlier, more often, and for a longer period of time than viremia did; and these parameters were not correlated. These data suggest the greater sensitivity of IEA antigenemia detection. The appearance of IEA antigenemia without viremia can also be explained by an increase in the expression of immediate-early genes in PMNs, which may occur without viral replication and dissemination. The absence of positive cultures can be explained in this way.

However, a high number of CMV IEA-positive PMNs (>100/10^6 cells) is correlated with CMV disease and viremia, and these findings, to some extent, support the data obtained by Schirm and colleagues (21, 26) and Revello et al. (19).

In our study, antigenemia appeared to be a very valuable test for the detection of an active CMV infection, because antigenemia is the first parameter of an active infection, and strong antigenemia is correlated with CMV disease.

For prevention of an active CMV infection and CMV disease, cellular immunity is thought to be the most impor-

tant factor of the host defense mechanisms. In particular, the action of cytotoxic T cells to CMV-infected target cells has been studied by Quinnan et al. (18) and Rook et al. (20). A relationship between cytotoxic T-cell activity against CMV and protection against CMV infection in vivo has been postulated in renal transplant recipients (20). We measured lymphocyte proliferation to CMV-infected fibroblasts as an indication of T-cell immunity to CMV after and before organ transplantation and during and after CMV infection in transplant recipients. A total of 15 heart and kidney recipients had CMV infection (with and without CMV disease), as detected by antigenemia together with positive cultures. Of these patients, 14 (93%) had no lymphocyte proliferation to CMV-infected FFs after transplantation and before the detection of infection. Of the 10 patients without active infection, only 1 patient had no lymphocyte proliferation. These results indicate that the absence of cellular immunity, as measured by lymphocyte proliferation to CMV-infected FFs, is an important risk factor for developing an active CMV infection and illustrates the importance of helper-T-cell responses, in addition to the well-recognized role of CMV-specific cytotoxicity, in recovering from opportunistic CMV infections. Anti-CD3 treatment in most of the patients leads to negative lymphocyte proliferation to CMV. Serious CMV infections occurred only in patients treated with OKT3 or ATG. However, CMV infections also occurred in patients who were not treated with ATG or OKT3, but these were of moderate severity or without symptoms. Strong proliferation to CMV-infected FFs in vitro is an indication of cellular immunity to CMV in vivo and is strongly correlated with protection against CMV reactivation or reinfection in CMV-positive transplant recipients.

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