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Comparison of PCR and pp65 Antigenemia Assay with Quantitative Shell Vial Culture for Detection of Cytomegalovirus in Blood Leukocytes from Solid-Organ Transplant Recipients

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This study compared PCR and an assay for cytomegalovirus (CMV) pp65 antigenemia (CMV-vue; INCSTAR Corp.) with a quantitative shell vial culture (QSVC) technique for the detection of CMV in serial blood specimens from 46 solid-organ transplant recipients. In a comparison based on 535 specimens tested by PCR and QSVC, CMV was detected by PCR in 41 and by QSVC in 37 of 43 recipients at risk of CMV infection. The mean number of days after transplantation of initial detection of CMV was 29.9 for PCR and 34.0 for QSVC ($P = 0.01$). The antigenemia assay was performed on 395 specimens, including 304 of those also tested by PCR. In these specimens, CMV was detected by the antigenemia assay, QSVC, and PCR in 30, 32, and 35 (respectively) of 38 patients at risk, with no statistically significant difference in the time to detection. Each of the assays detected CMV in similar proportions of patients with and without clinically significant CMV infection. PCR stayed positive longer after transplantation than the other assays but frequently returned to negative when more than 6 months had elapsed after transplantation. The antigenemia assay and PCR stayed positive longer after institution of antiviral therapy than QSVC. PCR can provide highly sensitive detection of CMV viremia, but a PCR assay for CMV is not yet available in kit form. The pp65 antigenemia assay and shell vial culture are quantifiable and comparable in sensitivity. Either is recommended for rapid detection of CMV in blood specimens from solid-organ transplant recipients.

Cytomegalovirus (CMV) infection is a frequent complication of solid-organ transplantation, especially in the period 1 to 4 months after transplantation (21). The diagnosis is best made by demonstration of viremia, which corresponds more closely with clinically significant CMV disease than the presence of virus in other readily accessible specimens (8, 10, 19). The shell vial assay, which yields results within 24 to 48 h, has greatly improved the clinical utility of viral blood cultures compared with that of traditional viral cultures, which may require 14 days or more before viral growth is apparent (15). Recently, two alternative methods, the detection of CMV antigens directly in circulating leukocytes by immunoperoxidase or immunofluorescence staining (4, 12, 14, 26, 27) and the detection of CMV DNA by PCR (5, 6, 9, 11, 14, 17, 20, 23, 25), have also been used for the rapid detection of CMV in blood. In addition to being rapid, the antigenemia assay has the potential advantage of providing quantitative data regarding the proportion of circulating leukocytes expressing CMV antigens. PCR is of interest because of its potential for extreme analytic sensitivity.

As part of an effort to improve the diagnosis of CMV infections, we have developed a quantitative modification of the shell vial assay (7). The result, expressed as infectious centers (ICs) per 100,000 leukocytes, provides a measure of the level of infectious CMV in the peripheral blood and can be used to follow the course of CMV viremia in recipients of solid-organ transplants. In the present study, we have used the quantitative shell vial assay as the basis for evaluating both PCR and a commercially available CMV antigenemia assay (CMV-vue; INCSTAR Corp., Stillwater, Minn.) for the detection of CMV in blood specimens from solid-organ transplant recipients. The assays were performed on sequential specimens from individual organ transplant recipients, obtained after transplantation, and thus the expected time course of CMV viremia following organ transplantation could be used to assist in evaluating the performance of each of the assays.

MATERIALS AND METHODS

Subjects and specimens. Subjects in this study were 46 patients who underwent solid-organ transplantation at the Washington University Medical Center during the period from July 1990 to September 1991. The study was carried out with the approval of the Washington University Human Studies Committee. The organs transplanted were 33 lungs (6 donor CMV seropositive [D+]/recipient CMV seronegative [R−], 13 D+/R+, 12 D−/R+, and 2 D−/R−), 8 kidneys (all D+/R−), 3 livers (2 D+/R− and 1 D−/R−), and 2 hearts (both D+/R−). All subjects who were CMV seropositive before transplantation or who received an organ from a seropositive donor were considered to be at risk for CMV after transplantation. Patients undergoing kidney or heart transplantation were also part of a study comparing the effectiveness of acyclovir plus intravenous immunoglobulin with that of acyclovir alone for the prevention of CMV infection in D+/R− recipients (3).

Specimens were sought weekly from lung transplant patients for the first 12 weeks after transplantation and then at 3, 6, and

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12 months after transplantation. Additional specimens were obtained for evaluation of clinical findings suggestive of CMV disease. Specimens from kidney, liver, and heart transplant recipients were obtained less frequently. Blood specimens were drawn into sterile heparinized tubes and transported within approximately 4 h to the Virology Laboratory of the Department of Pediatrics, Washington University School of Medicine. Quantitative shell vial culture (QSVC) was performed on all 670 specimens collected for this study, while PCR and the antigenemia assay were performed on 535 and 395 specimens, respectively. The specimens tested by PCR included all those obtained from the first 23 patients enrolled in the study. Because the primary focus of this study was on the early detection of CMV viremia, PCR testing on the 23 patients enrolled later was omitted for specimens obtained after the first one which was positive by QSVC. Neither the titer measured by QSVC nor the results of the antigenemia assay or PCR were used in patient management. During the study, antiviral therapy for CMV consisting of ganciclovir or foscarnet was used to treat episodes of CMV infection that were judged to be clinically significant on the basis of the severity of symptoms such as fever and malaise or histopathologic evidence of CMV in a biopsy specimen. Three D+/R− patients received prolonged courses of prophylactic antiviral therapy.

Preparation of blood leukocyte specimens. To prepare blood leukocytes for use in the three assays, 6 ml of heparinized blood was diluted to 9 ml with phosphate-buffered saline before the addition of 1 ml of 5% dextran (molecular mass, 250 kDa; Pharmacia, LKB Biotechnology [Piscataway, N.J.], or INCSTAR) in 0.9% NaCl. After mixing, the blood suspension was allowed to incubate at 37°C for 10 to 15 min. The leukocyte-rich upper layer was removed, the cells were pelleted, and the remaining erythrocytes were lysed with 3 ml of a commercial ammonium chloride lysing solution (INCSTAR). The remaining leukocytes were then washed twice with Eagle's minimal essential medium supplemented with 10% fetal bovine serum and counted electronically with an S-Plus-Jr cell counter (Coulter Electronics, Hialeah, Fla.).

QSVC. For the quantitation of CMV viremia, a modification of the centrifugation shell vial technique was used as previously described (7). Briefly, two to four commercially prepared MRC-5 fibroblast shell vials (Viro Med, Minnetonka, Minn.) were inoculated with 0.25 ml each of the prepared leukocyte suspension. The number of leukocytes inoculated into each shell vial was usually between 1×10^6 and 2×10^6. Rarely (27 of 670 specimens), the recovery of leukocytes was insufficient to inoculate this number and a smaller number (always at least 2×10^5) was inoculated. Immunofluorescent antibody staining of the fibroblast cells to detect the 72-kDa CMV immediate-early (IE) antigen was performed after approximately 40 h of incubation with DuPont monoclonal antibody 9221 (DuPont, Doraville, Ga.) (24). Stained coverslips were examined at magnification ×200 with a Leitz epifluorescence microscope, and the total number of fluorescent foci from each of the shell vials was counted, divided by the total number of leukocytes inoculated into all of the shell vials, and expressed as the number of ICs per 10^5 leukocytes inoculated.

PCR. PCR for the detection of CMV in blood specimens was done by a modification of the procedure of Jiwa et al. (17). After the isolation of blood leukocytes as described above, 1×10^6 leukocytes were transferred to a microcentrifuge tube and pelleted by a brief centrifugation. In a few samples (17 of 535), mostly from patients with low leukocyte counts, a smaller number of cells was used. After discarding the supernatant, 150 μl of absolute methanol was added. The specimens were then stored in methanol at 4°C until needed. To prepare the specimen for PCR, the methanol was evaporated and 50 μl of 0.04 M NaOH was added to the dried cell pellet. Specimen tubes were boiled for 10 min, after which approximately 30 μl of 0.1 M Tris-HCl (pH 7.6) was added to bring the pH of the specimen to 9.0. An 8-μl aliquot of the processed specimen was added to the PCR mixture prepared as described by Jiwa et al., with the exception that the final concentration of the 50-μl PCR mixture was 10 mM Tris-HCl (pH 8.6). To decrease the amplification of nonspecific products, components and reaction tubes were kept at 4°C during the preparation of the reaction mixture. Just before the amplification reaction, tubes were placed in the thermal cycler (Perkin-Elmer) and preheated to 95°C for 5 min, after which the PCR was run for 32 cycles, each consisting of 1 min at 95°C followed by 3 min at 65°C. After amplification, 15 μl of the product was analyzed on a 3% 3:1 NuSieve (FMC BioProducts, Rockland, Maine) agarose gel containing ethidium bromide (0.5 μg/ml) and photographed with UV illumination. All specimens were run twice by the same amplification protocol but with two separate sets of CMV-specific primers. One reaction utilized primers described by Jiwa et al., which amplify a 146-bp segment of the major IE gene of CMV (17). The other reaction used primers that amplify a 136-bp segment of the CMV gp64 late antigen (23). With dilutions of a plasmid containing the CMV IE gene (gift of P. Olivo, Department of Medicine, Washington University School of Medicine), the sensitivity of the assays was determined to be 100 copies of the respective target sequences. To avoid false-positive reactions due to contamination, all recommended procedures for avoiding contamination were strictly observed (18). Additionally, before use, all tubes, pipette tips, and reagents except for primers and *Taq* polymerase were exposed to 300,000 μJ of 254-nm UV irradiation by using a UV nucleic acid linker (Stratalinker UV Crosslinker; Stratagene, La Jolla, Calif.). Each assay run included a positive control tube and a negative control tube. In the positive control tube, the plasmid containing the IE gene (for the IE gene assay) or a lysate of MRC-5 cells infected with a laboratory strain of CMV (for the late gene assay) was substituted for the patient specimen. The negative control tube contained water in place of the patient specimen. PCR runs were considered valid only if the results of the positive and negative control tubes were appropriate.

CMV antigenemia assay. The CMV antigenemia assay was performed on 395 leukocyte specimens by using prototypes of the CMV-vue kit (INCSTAR). The instructions of the manufacturer were followed, except that for 92 specimens, a different method of applying leukocytes onto microscope slides was used. For these specimens, three microscope slides were prepared by cytocentrifuging 50 μl of a leukocyte suspension containing 150,000 cells prepared as described above onto glass slides, which were then stored at −70°C until they were stained. Two of these slides were stained with CMV monoclonal antibodies (see the description below), and one was used as a conjugate control in which the CMV antibodies were omitted. For 337 specimens (including 34 specimens also prepared by cytocentrifugation), the procedure currently recommended by the manufacturer was followed. Briefly, following the preparation of the leukocyte samples as described above, 25 μl of a leukocyte suspension containing 2×10^6 cells per ml (50,000 cells) was applied to each of three wells on slides supplied with the kit. After allowing the cells to settle and attach for 15 min at room temperature, the slides were fixed in acetone and then stored in a desiccator at 4°C until staining. The slides were stained according to the manufacturer's instructions by using a mixture provided with the kit of two murine monoclonal

antibodies specifically reactive for the CMV protein pp65 as the primary antibody. The CMV monoclonal antibodies were omitted in staining one of the three wells prepared with each specimen so that this well could serve as a conjugate control. Following washing, the slides were subjected to reaction with a horseradish peroxidase-conjugated anti-mouse antibody as the secondary antibody. After additional washing and reaction with the chromogen-substrate reagent, the slides were examined by light microscopy. Cells, usually polymorphonuclear leukocytes, exhibiting dark brown to red-brown nuclear or perinuclear staining were counted. Two wells were examined for each sample. The results from slides prepared by either method were expressed as positive cells per 10^5 leukocytes examined. Preliminary studies indicated that for the 34 specimens stained by both methods of slide preparation, there was no significant difference in the results according to the method of slide preparation. For these specimens, only the result from the CMV-vue slide was considered in the analysis of results.

Statistical analysis. The significance of differences in the number of patients in whom CMV was detected first by PCR and QSVC were determined by the Sign test (2). The significance of differences in the mean number of days before initial detection of CMV viremia in the same comparisons was determined by the paired *t* test. A one-way analysis of variance with a repeated measures design was used to test the significance of differences in the mean number of days before initial detection of CMV viremia in specimens tested by PCR, the antigenemia assay, and QSVC (2). The correlation between ICs per 10^5 leukocytes as measured by QSVC and positive cells per 10^5 leukocytes as measured by the antigenemia assay was determined by using StatView version 4.0 (1). Differences in individual mean levels of viremia or antigenemia of patients with significant or insignificant CMV infection were evaluated by the Wilcoxon test.

RESULTS

Specimens tested. QSVC for CMV was performed on all 670 blood specimens from 46 solid-organ transplant recipients included in the study. Of these specimens, 151 (22.5%) were positive, with titers ranging from 0.02 to 53.9 ICs per 10^5 leukocytes. For 36 of the 670 specimens (5.4%), both shell vials exhibited toxicity to the fibroblast monolayer and could not be evaluated.

Comparison of PCR and QSVC. In a preliminary study, PCR using both primer sets was performed on DNA extracted from leukocytes of 10 healthy volunteers, including 6 who were CMV seropositive. None of the specimens were positive for CMV DNA (data not shown).

PCR was performed on 535 of the blood specimens which had been analyzed by QSVC. In this comparison, specimens were considered positive by PCR when CMV DNA was detected with either one or both of the primer pairs used. Of the 535 specimens, 118 were positive by QSVC and 274 were positive by PCR, of which 78% were positive with the IE gene primers and 92% were positive with the late gene primers. The results of PCR in relation to the level of viremia determined by QSVC are shown in Table 1. PCR was positive in 113 (96%) of the 118 specimens that were positive by QSVC and 146 (38%) of the 384 specimens that were negative by QSVC. The five specimens that were positive by QSVC and negative by PCR all had CMV titers of <0.1 IC per 10^5 leukocytes.

The abilities of PCR and QSVC to document CMV infection were compared for all 40 patients at risk of CMV other than the 3 D+/R− patients who received prophylactic antiviral therapy. PCR detected CMV DNA (one or more specimens positive) in all 40 patients, compared with 37 (93%) in whom CMV infection was detected by QSVC. Of the 40 patients, 31 had clinically significant infection, as defined by the need for antiviral therapy. PCR detected CMV DNA in all the patients examined, whether or not they had significant infection, while QSVC was positive for 29 (94%) of those with significant infection and 10 (91%) of the 11 whose CMV infection was not clinically significant.

TABLE 1. Comparison of PCR results with QSVC results for detection of CMV in leukocytes of solid-organ transplant recipients

QSVC result (ICs/10^5 leukocytes)	PCR (no. [%] of specimens)	
	Tested	Positive
Negative	384	146 (38)
0.02–0.1	44	39 (89)
≥0.1–1.0	50	50 (100)
≥1.0	24	24 (100)
Toxic	33	15 (45)
Total	535	274 (51)

The specificity of PCR was evaluated in several ways. First, 38 specimens from patients not at risk of CMV were tested, and all were negative. Second, results from specimens obtained during the first 2 weeks after transplantation were examined. None of 28 specimens obtained during the first week after transplantation and only 2 of 21 specimens obtained during the second week after transplantation were positive. In comparison, 56% of all specimens obtained more than 2 weeks after transplantation were positive. Finally, the four patients who were positive by PCR but never positive by QSVC were evaluated for other evidence of CMV infection. Two of the patients showed other evidence of CMV infection, consisting of either a positive conventional tube culture of the blood for CMV or a positive bronchoalveolar lavage culture and/or lung biopsy indicative of CMV pneumonitis. The presence or absence of CMV infection was indeterminate in the other two patients, but one of them had a febrile illness consistent with acute CMV infection. For this patient, CMV infection was not documented by culture or serology, but only four specimens, all from within the first 41 days after transplantation, were available for study. The fourth patient, who was seronegative and received a transplant from a seropositive donor, received a prolonged course of prophylactic ganciclovir therapy. Consecutive specimens from days 25, 29, and 32 after transplantation were positive with only the late gene primers. The CMV antigenemia assay (see the comparison of results below) was positive at a low level in only the specimen from day 32.

For patients who were positive by both PCR and QSVC, a comparison of the times to first positivity was carried out. This comparison was limited to the 35 patients who were positive by both assays for at least one specimen obtained during the first 6 months after transplantation. PCR detected CMV first in 17 patients, compared with first detection in 2 patients by QSVC and simultaneous detection in 16 patients by both assays ($P = 0.001$; Sign test). In order to compare the mean numbers of days before the first positive result by both tests, four patients who received ganciclovir before the first positive QSVC were excluded. For the remaining 31 patients, the mean number of days to initial detection was 29.9 for PCR, compared with 34.0 for QSVC ($P = 0.01$; paired *t* test).

Comparison of antigenemia assay with PCR and QSVC. The CMV antigenemia assay was performed on 395 specimens, including 304 that were also analyzed by PCR and were part of the group of specimens described above. The specimens tested

TABLE 2. Comparison of pp65 antigenemia assay results with QSVC results for detection of CMV in leukocytes of solid-organ transplant recipients

QSVC result ($ICs/10^5$ leukocytes)	Antigenemia assay (no. [%] of specimens)	
	Tested	Positive
Negative	282	39[a] (14)
0.02–0.1	33	12[b] (36)
≥0.1–1.0	45	30[b] (67)
≥1.0	22	20[b] (91)
Toxic	13	4 (31)
Total	395	105 (27)

[a] 22 (73%) of 30 specimens tested by PCR were positive.
[b] All 44 specimens also tested by PCR were positive.

by the antigenemia assay were from 40 patients, of whom 38 were at risk for CMV infection. The results of the antigenemia assay in relation to the level of viremia determined by the QSVC are shown in Table 2. A total of 143 specimens were positive by either assay, including 62 that were positive by both assays, 38 that were positive by QSVC and negative by the antigenemia assay, and 43 that were positive by the antigenemia assay and negative (39 specimens) or toxic (4 specimens) by QSVC.

A significant correlation (correlation coefficient, 0.7; $P = 0.001$) was present between the level of viremia measured by QSVC in the 395 specimens and the level of antigenemia measured by the antigenemia assay in the same specimens. The number of positive cells per 10^5 leukocytes measured by the antigenemia assay averaged more than 30-fold higher than the number of infectious foci per 10^5 leukocytes detected by QSVC in the same specimen, although there was wide variation in the ratio of positive cells to ICs in individual specimens.

Most of the specimens that yielded discrepant results had low levels of viremia and/or antigenemia (Table 2). PCR was performed on 34 of the specimens that were positive only by QSVC and 29 of the specimens that were positive only by the antigenemia assay; CMV DNA was detected in 85 and 69% of these specimens, respectively. Of the 38 specimens positive only by QSVC, 2 (5%) were obtained while the patient was receiving antiviral therapy (ganciclovir or foscarnet), compared with 11 of the 39 specimens (28%) that were positive only by the antigenemia assay. Only two of the specimens that were positive by the antigenemia assay and not by QSVC had fewer than the optimal number of cells inoculated into the QSVC assay.

The abilities of each assay to detect CMV in individual patients were compared by using specimens from 34 patients at risk for CMV infection, excluding those who received prophylactic antiviral therapy. The antigenemia assay detected CMV in at least one specimen for 27 (79%) patients, compared with 31 (91%) for QSVC and 34 (100%) for PCR. Of the 34 patients at risk, 28 had clinically significant CMV infection. The antigenemia assay detected CMV in 82% of those with significant infection and 67% of the six with insignificant infection, compared with QSVC detection of CMV in 89% of the patients with significant infection and 100% of the patients with insignificant infection.

The times to positivity for each of the assays were compared by using the specimens from the 18 patients who had series of specimens that were suitable for this analysis because each of the assays was positive at least once before the patient received ganciclovir or foscarnet. In this three-way comparison, the numbers of patients whose PCR, antigenemia assay, or QSVC was the first assay to turn positive (either by itself or simultaneous with one or both of the other assays) were 15, 10, and 9, respectively. The mean days to first detection were 29.6 for PCR, 31.1 for the antigenemia assay, and 33.6 for QSVC. These differences were not statistically significant ($P = 0.13$; analysis of variance).

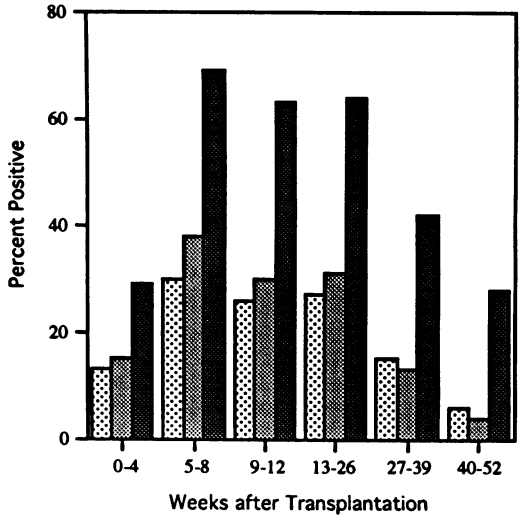

FIG. 1. Percentage of leukocyte specimens tested that were positive for CMV by QSVC, antigenemia assay, and PCR during different intervals after solid-organ transplantation. ▨, QSVC; ▩, antigenemia assay; ■, PCR.

Duration of positivity and effect of antiviral therapy. The proportion of specimens that were positive during successive intervals after transplantation and the effect of antiviral therapy were evaluated by using the results of all tests performed (QSVC, $n = 670$; PCR, $n = 535$; antigenemia assay, $n = 395$). The proportion of specimens positive was maximal for each assay during the interval 5 to 8 weeks after transplantation and then declined steadily (Fig. 1). PCR was positive for a higher proportion of specimens than either of the other assays during each interval, while the proportions of specimens positive by QSVC and antigenemia assay were similar for all intervals examined (Fig. 1). The effect of antiviral therapy (ganciclovir or foscarnet) was analyzed by using the 133 specimens obtained from 33 patients while they were receiving one of these drugs. The results are shown in Fig. 2. QSVC became negative most rapidly, with a positivity rate of only 6% for specimens obtained after 7 to 13 days of antiviral therapy. PCR stayed positive for the longest period, with a positivity rate of 43% for specimens obtained after more than 13 days of antiviral therapy. Similar results were found when these analyses were restricted to specimens on which all three assays were performed (data not shown).

DISCUSSION

This study compared three different assays designed for the rapid detection of CMV in blood. We found that all three of the assays, QSVC, PCR, and the pp65 antigenemia assay, achieved rapid and sensitive detection of CMV. PCR was the most sensitive of the three, allowing recognition of CMV in blood specimens from a larger number of patients and at an earlier time after transplantation than the other assays. PCR also stayed positive for the longest time after transplantation. QSVC and the antigenemia assay were comparable to one

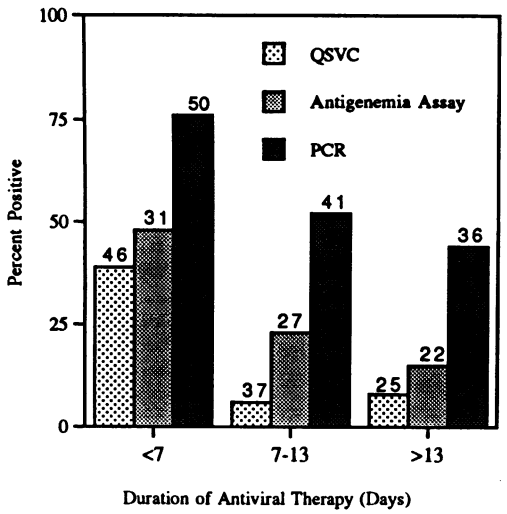

FIG. 2. Percentage of leukocyte specimens tested that were positive for CMV by QSVC, antigenemia assay, and PCR after different periods of antiviral therapy. The number of specimens tested by each assay during each interval is shown by the number over the corresponding bar.

another both in the number of patients for whom CMV was detected in blood and in the time to detection. When antiviral therapy was used, QSVC tended to become negative first, while PCR stayed positive the longest.

The evaluation of new diagnostic techniques which may be more sensitive than existing techniques can be problematic. Ideally, additional information should be used to resolve discrepancies that occur when the new test yields a positive result for a specimen that is negative by the existing standard. Our approach was to use sequential specimens from patients undergoing solid-organ transplantation. The pretransplant CMV serostatus of the recipient was known, as was that of the transplant donor, allowing us to classify recipients as at risk for CMV (D+ or R+) or not at risk (D− and R−). CMV viremia could be anticipated for most or all of the recipients at risk, beginning several weeks after transplantation (19, 21). Not surprisingly, we found a large number of specimens that were positive by PCR but not by QSVC or by the antigenemia assay. The finding that none of these discrepancies occurred in specimens in which CMV viremia would have been considered unlikely (specimens obtained from patients not at risk or from patients at risk but before the risk period after transplantation) supported the specificity of PCR. All but two of the patients for whom such discrepancies occurred had positive cultures for CMV at other times in their clinical course, and those two remaining patients had either inadequate follow-up to determine whether CMV infection was truly present or prolonged prophylactic antiviral therapy which may have caused the cultures to be negative.

A critical issue with regard to PCR detection of an infectious agent such as CMV which may be latent in healthy individuals is whether the exquisite sensitivity of PCR will detect latent infection, rendering the test useless for the diagnosis of active infection. This fear was not realized in the present study. Three findings suggest that PCR as performed in this study was indicative of active CMV infection. First, CMV DNA was not detected in healthy CMV-seropositive volunteers. Second, in CMV-seropositive recipients, PCR was uniformly negative for specimens from the first week after transplantation and was positive on only two specimens from the second week. Third, many patients who had definite evidence of active CMV infection on the basis of PCR, antigenemia assay, and QSVC during the second or third month after transplantation became PCR negative during the period 6 to 12 months after transplantation. Similar findings have been reported by others (5, 14, 17, 20, 23), although detection of CMV DNA by other PCR protocols has also been reported in a small number of blood samples from healthy seropositive or seronegative individuals or from allogeneic bone marrow transplant recipients before transplantation (11). The number of cells analyzed is a critical determinant of the sensitivity of an assay to detect a cell-associated virus, and it is likely that the number of cells used for PCR analysis in the present study accounts for the ability of PCR to distinguish active from latent infection.

Our data provide a detailed view of the potential role of PCR in the diagnosis of posttransplant CMV infection, in comparisons with both QSVC and the pp65 antigenemia assay. PCR appears to have two advantages in this setting. The first is the ability to recognize CMV activation several days to 1 week before either QSVC or the antigenemia assay. This capability might make PCR appropriate for use as the trigger for starting preemptive antiviral therapy in patient groups previously defined as being at high risk of clinically significant CMV infection (16, 22). Although PCR as employed in the present study would not decrease the number of patients treated compared with that resulting from an approach based on treating all patients at risk (16, 22), it would allow the timing of such therapy to be individualized for each patient. While this approach is attractive, its utility must be verified in an appropriate clinical trial. The second advantage of PCR is also related to the very high sensitivity of PCR. The finding that PCR was positive in virtually all situations in which there was evidence of CMV activation suggests that a negative result could be uniquely useful in ruling out active CMV infection. Unfortunately, as also described previously for liver transplant recipients (11), the sensitivity of PCR also limits its utility. Because PCR was positive for all patients at risk (excluding those who received prophylactic antiviral therapy), qualitative PCR as performed in this study obviously does not differentiate between clinically significant and insignificant infections. It is possible that a quantitative PCR assay would be more useful in making this distinction. Alternatively, it has been suggested that detection of CMV DNA in plasma (25) or serum (6) might indicate clinically significant infection.

A number of studies have demonstrated the utility of the pp65 antigenemia assay for the diagnosis of CMV infection after solid-organ transplantation (5, 12–14, 26–28). In these studies, the antigenemia assay has been more sensitive than shell vial culture. Two studies have compared PCR and the antigenemia assay and have found PCR more sensitive (5, 14). The present study used a commercial version of the pp65 antigenemia assay. Our data confirm the greater sensitivity of PCR and indicate that the commercial pp65 antigenemia assay and QSVC were of equivalent sensitivity for the recognition of active CMV infection. The substantial number of discrepancies between QSVC and the antigenemia assay were mostly in specimens with low levels of viremia or antigenemia and suggest that either assay may not consistently detect low levels of CMV. It seems most likely that the difference from previously published studies in the relative sensitivities of the antigenemia assay and the shell vial assay is the result of the enhanced sensitivity of the shell vial assay as performed in this study. The present study did confirm the previous finding (14) that the antigenemia assay stays positive longer than culture after the institution of antiviral therapy. This phenomenon

accounted for some but not all of the discrepancies between QSVC and the antigenemia assay.

A potential advantage of both QSVC and the antigenemia assay is that these assays can provide quantitative measures of the level of CMV viremia or antigenemia. While several early studies suggested a clear relationship between the level of antigenemia and the presence of clinically significant CMV infection (13, 14, 27), more recent studies have shown that at least some patients can be asymptomatic with high levels and others can be symptomatic with low levels (26). The findings of the present study were similar. Although there was a trend towards higher levels of antigenemia and viremia in those with symptomatic infection, the levels overlapped, and the differences between the two groups were not statistically significant. The question of whether measuring the level of CMV viremia enhances the clinical value of a CMV detection assay requires further study, and relevant investigations are underway in our laboratory and elsewhere. At a minimum, it is likely that a quantitative assay could provide an early warning of the presence of antiviral-drug resistance if the level of viremia or antigenemia failed to decline after the start of antiviral therapy.

Laboratories now have the opportunity to choose among three rapid assays with demonstrated sensitivity for the recognition of active CMV infection. The shell vial assay is the most familiar to laboratories and can be modified to provide quantitative data if desired. The pp65 antigenemia assay is now commercially available and provides information equivalent to that provided by the shell vial assay. Its other advantages include a very rapid turnaround time, quantifiability, the need for fewer leukocytes than the shell vial culture, and the need for no cell culture or sophisticated laboratory equipment. Its major disadvantage is the technical skill required for accurate reading of the slides. PCR is the most sensitive assay but probably has the lowest predictive value for recognizing clinically significant infection. Currently, CMV PCR is not commercially available in kit form and its format does not lend itself to processing a large volume of specimens. It is likely that PCR will not become widely used in clinical laboratories until instrumentation has been developed to allow processing of a large volume of specimens without danger of cross-contamination. It is also likely, but not yet proven, that quantitative modifications of PCR will enhance its clinical utility. For the present, laboratories can conveniently choose between the shell vial assay and the pp65 antigenemia assay for rapid diagnosis of CMV infections.

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