Semiquantitative Measurement of Cytomegalovirus DNA in Lung and Heart-Lung Transplant Patients by in Vitro DNA Amplification*

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We report the cases of two lung transplant recipients (one heart-lung and one single lung) who eventually developed cytomegalovirus (CMV) pneumonitis after documentation of increasing CMV DNA titers in sequential bronchoalveolar lavage (BAL) specimens by polymerase chain reaction (PCR) amplification. To our knowledge, this is the first report that semiquantitation of PCR-amplified DNA can detect an increase in CMV DNA titer in BAL specimens prior to the onset of clinical symptoms or detection of infection by traditional techniques in lung transplant patients. The results obtained in these two cases suggest that DNA titer measurement on sequential BAL samples may differentiate latency from active viral replication and, thus, provide an opportunity for clinical intervention before the development of overt clinical symptoms.

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| BAL = bronchoalveolar lavage; CMV = cytomegalovirus; DEAFF = detection of early antigen fluorescent foci; PCR = polymerase chain reaction |

Cytomegalovirus (CMV) pneumonitis is a frequent and often serious complication of lung and heart-lung transplantation. The reported incidence among heart-lung recipients varies from 7 percent to 80 percent.1 Cytomegalovirus pneumonitis is traditionally diagnosed in lung transplant patients by identification of the typical viral inclusions in biopsy specimens or cytology specimens, by viral culture, or by a rise in anti-CMV antibody titers.2 With these techniques, patients are often clinically ill for days or weeks before a definitive diagnosis is made.

Polymerase chain reaction (PCR) amplification provides an exquisitely sensitive and specific method of detecting selected DNA target sequences and has been used to demonstrate selected pulmonary infections.3,4 However, in the case of latent DNA viruses like CMV, the mere finding of CMV DNA cannot be unequivocally associated with the eventual development of disease secondary to CMV. Using PCR amplification, we report the first documentation (to our knowledge) of increasing CMV DNA titers in sequential bronchoalveolar lavage (BAL) specimens from lung transplant recipients (one single lung and one heart-lung) prior to development of CMV pneumonitis. 

CASE REPORTS

Sequential BAL specimens from the transplanted lung(s) were obtained for DNA extraction, cytology, and shell vial and routine viral cultures in two patients considered at high risk for the development of CMV infection following lung or heart-lung transplantation. Transbronchial biopsy specimens were obtained simultaneously. Both patients received standard immunosuppression as per our protocol that has been described elsewhere.5

CASE 1

A 21-year-old white man underwent combined heart-lung transplantation for end-stage respiratory failure from cystic fibrosis. Preoperatively, the recipient was seronegative for previous CMV exposure. The donor was seropositive for previous CMV infection. He received CMV prophylaxis with IgIV (Sandoglobulin IV) representing purified IgG from pooled donors and was treated for acute lung allograft rejection on day 14 with good response. He was discharged home on day 30 but was readmitted to the hospital for fever and cough with increasing pulmonary infiltration on day 35. Transbronchial biopsy specimen on day 35 showed CMV pneumonia. He was treated with ganciclovir and with IgIV (Sandoglobulin IV) with good clinical response. One BAL sample from each transplanted lung was obtained on days 7, 14, 22, 30, 35 (one BAL sample obtained from left lung only due to technical problems), and 39 after transplantation.

CASE 2

A 46-year-old white woman underwent left single lung transplantation with closure of a large patent ductus arteriosus for Eisenmenger's complex. Her preoperative serum samples demonstrated high-titer IgG antibodies to CMV as did serum samples from the donor. She was treated prophylactically with IgIV (Sandoglobulin). After treatment for acute lung allograft rejection on day 4 and again on day 21, fevers and pulmonary infiltration with respiratory failure persisted. Transbronchial biopsy specimen on day 34 revealed CMV pneumonia. She was treated with ganciclovir and IgIV but developed profound neutropenia after 10 days of treatment, leading to

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its interruption and intermittent dosing thereafter. She continued her downhill course and died of respiratory failure on the 64th postoperative day. Autopsy showed extensive gram-negative pneumonia, focal necrosis of the host lung, and rare CMV inclusions. One BAL sample from the transplanted lung was obtained on days 21, 26, and 34 after transplantation.

METHODS

DNA from BAL samples was extracted by a standard phenol/chloroform procedure and quantitated by fluorescent dye method. Synthetic oligonucleotides were prepared (by Genetic Designs Incorporated, Houston) and sequences were derived from HCMV pp71, a late antigen phosphoprotein. The primers and probes used for this study flank primer sequences previously tested in our laboratory. The PCR was performed using previously described techniques. A total of 35 cycles were performed for the PCR.

Approximately 10 µl of the final reaction was examined by gel electrophoresis with ethidium bromide staining to determine if a band was present at 500 base pairs, indicating amplification of the specific CMV DNA had occurred. The results were further verified by dot blot hybridization with a probe specific to the internal sequence of the amplified DNA.

Semi-quantitative assessment of viral load in the BAL samples was obtained by determining the serial dilution of BAL DNA at which the appropriate band was no longer detectable on a gel. One microgram was used for the initial amplification of the DNA. The dilutions were prepared by addition of the patient DNA to 10 ng of CMV-negative human genomic DNA as a carrier to block nonspecific binding of patient DNA to surfaces. The DNA "titer" was expressed as the negative log of the highest dilution that produced a specific band detectable by ethidium bromide staining on an acrylamide gel.

The BAL samples were cultured in human foreskin fibroblast cultures according to standard tissue culture technique and in shell vial cultures using a monoclonal antibody to detect CMV at 24 and 48 h after inoculation. Ten hematoxylin-eosin-stained sections of each transbronchial biopsy specimen taken at the time of lavage were examined for CMV intranuclear and intracytoplasmic inclusions. Papanicolaou-stained smears of the lavage fluids were screened for CMV inclusions. The results of these traditional methods of diagnosis were recorded as negative or positive for CMV. The results of PCR amplification were compared with the results of viral cultures and cytologic studies obtained from aliquots of the same BAL specimens, with the histologic features of the simultaneously obtained biopsy specimens, and with the presence of concurrent clinical symptoms.

RESULTS

In case 1, PCR convincingly detected CMV DNA in a routine serial BAL specimen on day 14 following transplantation, three weeks prior to detection by traditional methods of diagnosis (Fig 1). Shell vial cultures and routine cultures showed identical positive results except that the results from the shell vial cultures were available earlier. The DNA titer showed a gradual rise in viral load and an apparent plateau on day 22 after transplant (Fig 2). This rise and plateau was also reflected in the increase in the relative intensity of the PCR-amplified band on the gel (Fig 1). Clinical symptoms of CMV pneumonitis and identification of viral inclusions on BAL specimens did not occur until day 35 after transplant, two weeks after the plateau in viral load was detected by PCR.

In case 2, PCR initially detected CMV DNA in a BAL specimen obtained on day 21 after transplantation while the patient was being treated for rejection. Viral cultures obtained on days 26 and 34 were positive by shell vial culture at 24 h and became positive on routine culture 14 and 8 days later, respectively.
inclusions were first identified on BAL smears on day 34 after transplant. Although the CMV DNA titer increased during this time, clinical symptoms were not preceded in this case by any apparent plateau in viral load (Fig 3).

**Discussion**

Both transplant rejection and opportunistic infection continue to limit the success of lung and heart-lung transplantation. Most deaths in these patients are due to opportunistic infection rather than transplant rejection. However, infection, including CMV pneumonitis, results from the immunosuppression necessary to prevent and/or treat rejection. Augmented immunosuppression is often required early after lung transplantation since acute rejection is most common at one to three weeks following transplant. Cytomegalovirus pneumonitis, when it occurs, becomes clinically manifest two or three weeks later.

The reliability of PCR in detecting CMV DNA in patients with CMV infection documented by established methods has been confirmed previously. When compared with tissue culture, detection of CMV by PCR in urine specimens from newborns provided a sensitivity of 93 percent, a specificity of 100 percent, and a predictive value of a positive result of 100 percent. Several techniques are now available for rapid viral culture. Although further study is needed, our two cases suggest that detection of viral DNA titers in BAL samples is at least as rapid as detection of infection by the shell vial technique. Others have directly compared PCR with another rapid culture technique (detection of early antigen fluorescent foci [DEAFF]) and have found the latter to be less sensitive. In a study of 24 bone marrow transplant and 52 kidney transplant patients, PCR confirmed the presence of CMV DNA in the peripheral leukocytes of patients with CMV viremia diagnosed by DEAFF. Viremia was detected in four of 13 patients by PCR one to two weeks prior to DEAFF and in six of 13 patients for one to five weeks after it was no longer detectable by DEAFF.

Our two cases confirm that CMV DNA can be detected in BAL samples with PCR during the initial stages of acute infection and before the onset of symptoms or identification of the infection by standard methods. More important, the results in these two cases suggest that viral load can be assessed by measuring the DNA titer. Due to viral latency, a simple positive/negative result by PCR will not provide the discrimination necessary to make decisions regarding institution of antiviral therapy. Potentially, using a measure of viral load such as demonstrated herein might make it possible to establish the degree of viral burden associated with the development of clinical symptoms in the majority of cases. Although further study is needed, the ability to assess viral load in a rapid and routine manner in lung and heart-lung patients by this technique could permit administration of antiviral drugs prior to overt clinical illness when therapy may be more efficacious.

**References**

ABIM Clinical Cardiac Electrophysiology Examination

The American Board of Internal Medicine has announced the registration period for this exam is January 1-April 1, 1992, and the examination will take place November 4. For information and application forms, contact the Registration Section, ABIM, 3624 Market Street, Philadelphia 19104 (800:441-2246).
