Low Predictive Value of Polymerase Chain Reaction for Diagnosis of Cytomegalovirus Disease in Liver Transplant Recipients

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Received 9 December 1991/Accepted 14 April 1992

The polymerase chain reaction (PCR) and viral culture techniques were prospectively compared for the detection of cytomegalovirus (CMV) in blood samples from 24 liver transplant recipients. Nine patients had one or more episodes of viremia, seven of which were clinically symptomatic infections. All samples in which CMV was isolated by culture were positive by the PCR. However, the PCR result was also positive for one or more samples from 11 patients who never developed CMV-related symptoms. Although the PCR is a very sensitive technique for CMV detection in blood samples from liver transplant recipients, it is not useful as a marker of symptomatic CMV disease.

Cytomegalovirus (CMV) is the single most frequent infectious agent in liver transplantation (11). In transplantation patients, CMV not only is a major cause of morbidity and mortality but also can be associated with an immunosuppressive state, superinfection by other opportunistic microorganisms, and allograft rejection (7). Viremia is a prominent feature of CMV infection and, unlike urinary or pharyngeal shedding, is frequently associated with clinical symptoms and organ involvement (6, 11). Therefore, an early and sensitive diagnosis of CMV viremia may be of clinical importance in that it may trigger a search for organ invasion and potential early antiviral therapy. Of the different diagnostic methods available to detect CMV viremia, viral culture by the conventional and/or shell vial technique is the standard procedure. Polymerase chain reaction (PCR) technology, which consists of an in vitro primer-directed amplification of a specific DNA region, is an exquisitely sensitive technique and has been shown to be of clinical and diagnostic value in the identification of several viral infections (5, 8). The diagnostic role of the PCR for CMV detection is currently being evaluated for several immunosuppressive states (1, 3, 4, 10); however, the clinical relevance of the PCR in CMV disease in liver transplant recipients has not been studied. Because of the need for an early, rapid, and sensitive diagnosis of CMV in blood samples, we decided to test whether the PCR could have clinical value in the diagnosis of CMV viremia and symptomatic illness compared with the viral culture technique. For this comparison, we designed a prospective study in which blood samples were obtained pre- and posttransplantation and processed for CMV detection by both the PCR and viral culture. During follow-up, CMV infection was defined as the presence of a positive culture in any sample. CMV disease included a viral syndrome (a positive blood leukocyte culture and fever, malaise, and usually leukothrombocytopenia) or histological evidence of tissue invasion by CMV or both.

Twenty-four liver transplant patients were studied, with a mean follow-up of 81 days (range, 35 to 180 days). Blood samples for culture and the PCR were obtained on days 0, 7, 14, 21, 28, 60, 120, and 180 posttransplantation and for any suspicious episode of CMV infection. A pharyngeal swab and urine samples were also simultaneously cultured for CMV. Donor and recipient pretransplant serum samples were tested for anti-CMV immunoglobulin G by a commercial enzyme immunoassay (Sigma, St. Louis, Mo.). Conventional viral culture and rapid culture by the shell vial technique were performed as previously described (9). For the PCR reactions, 7.5 × 10⁴ leukocytes were lysed at 56°C for 60 min in 10 mM Tris-HCl (pH 8.3)–50 mM KCl–2.5 mM MgCl₂–0.15% Nonidet P-40 and Tween 20–60 μg of proteinase K per ml. After heat inactivation, DNA amplification of a region of CMV IE1 gene was performed with 0.5 μM of specific primers (4) and 1.25 U of Taq polymerase (Perkin-Elmer, Norwalk, Conn.) in the same buffer described above at a final MgCl₂ concentration of 1.5 mM. The samples were then heated at 95°C for 1 min and subjected to 35 cycles at 95°C for 1 min and at 60°C for 1 min, with a final extension segment at 60°C for 10 min. Ten microliters of the resulting product (50 μl) was electrophoresed in 4% agarose (NuSieve, 3%; SeaKem, 1%; FMC), stained, and photographed. The total processing time was 7 h. Human fibroblasts infected with CMV AD169 and leukocytes from CMV-seropositive and -seronegative healthy blood donors were used as controls. The minimal level of CMV DNA in our samples detected by the PCR, with the linearized cosmids pCM1058 containing the HindIII E fragment of AD169 (2), was in the range of 10² to 10³ DNA copies.

One hundred forty-five blood samples from 24 patients were tested. The results are summarized in Table 1. CMV was cultured in 11 blood leukocyte samples from nine patients. All samples in which CMV was cultured were positive by the PCR. CMV DNA was also detected by the PCR in 32 blood leukocyte samples from nine patients without viremia. None of the CMV-seropositive and -sero-

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Our results suggest that the PCR may not be a useful method for the diagnosis of CMV disease: of the 18 patients with a positive PCR result, 11 never developed symptomatic infection, whereas 7 of the 9 viremic patients were symptomatic. Therefore, culture of CMV from blood leukocytes is a better marker than the PCR for symptomatic infection associated with organ invasion. Seven of the nine patients with positive PCR results and negative blood leukocyte cultures had evidence of CMV infection at other sites in an asymptomatic form, indicating that it is unlikely that these PCR results were false positive.

In conclusion, our study showed that even though the PCR of CMV DNA may be a very sensitive technique for CMV viremia detection, it is not useful as a marker of clinically symptomatic CMV infection, as a large number of the PCR-positive patients never developed clinical disease. Our data are in accord with those of Gerna et al. (3) in that the detection of low levels of viral replication by a very sensitive technique, as is the PCR, could take place in the absence of clinical evidence of infection. Nonetheless, factors such as quantification of the number of DNA copies, identification of the different types of cells with a positive PCR result, or amplification of CMV RNA by the PCR may add additional valuable information that needs to be evaluated further to determine its clinical usefulness.

This work has been financed by a grant (FIS 90/0171) from the Fondo de Investigación Sanitaria.

REFERENCES


