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NOTES

Prospective Study of Cytomegalovirus Antigenemia in Allograft Recipients

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A recently published technique for direct detection of cytomegalovirus (CMV) antigenemia was tested prospectively in 27 transplant recipients. Eighteen patients developed active CMV infections and 10 of the 18 experienced CMV syndrome. All of the infections were detected by classical virus isolation and/or serologic techniques. No antigenemia was demonstrated.

Because of the effectiveness of cytomegalovirus (CMV) hyperimmune globulins and antiviral drugs in the prophylaxis and treatment of CMV disease in allograft recipients (9), rapid, sensitive, and early detection of CMV has become important in the management of these patients. In many centers, patients are monitored for laboratory evidence of CMV infection following transplantation. However, the time-consuming nature and relative lack of sensitivity of classical methods for the detection of CMV are well documented, and multiple techniques must be used for maximum efficacy of monitoring, since no single standardized test can be relied upon for the earliest possible detection of CMV infections in all patients (5). Though polymerase chain reaction technology provides rapid and sensitive detection of the presence of viral genomes (3), its usefulness as a single monitoring test has not yet been demonstrated, and its application is, in any case, restricted by high cost and technical complexity.

In contrast, a recently published method (12, 13) for the detection of CMV antigenemia in peripheral leukocytes has been proposed for the sensitive and early detection of CMV disease. The method consists of detecting CMV immediate-early antigen in slide preparations of polymorphonuclear leukocytes (PMNLs) by using mouse monoclonal antibodies (MAbs) in an immunoperoxidase technique. The advantages of this method include comparatively low cost, technical simplicity, and speed; the originators of the technique also found it to be more sensitive than the detection of viremia and proposed that it could be used to monitor the effectiveness of antiviral therapy. These authors and other workers who used immunofluorescence (IFA) instead of immunoperoxidase (6) both observed a correlation between the degree of antigenemia in peripheral leukocytes and the onset and severity of CMV syndrome.

Using the IFA technique, we have studied this method prospectively, in parallel with current methods, to determine its effectiveness in detecting CMV infection in transplantees. Specimens of clotted and heparinized blood and of urine were collected prior to transplantation, biweekly for 3 months following transplantation, and monthly thereafter for 6 to 12 months. Specimens were collected more frequently, several times per week, if or when CMV disease was suspected. A total of 134 heparinized blood specimens from 27 allograft recipients (16 renal, 10 cardiac, 1 bone marrow) were examined directly for antigenemia and cultured as described below; 221 urine specimens from the same patients were cultured.

PMNLs were isolated by the method of van der Bij et al. (13), spotted on multiwell slides, and fixed by the method of Jiwa et al. (4). IFA staining was done in parallel, using the following reagents: (i) pooled MAbs C-10 and C-11 (Biotest AG, Dreiech, Germany), specific for two immediate-early antigens of molecular weight 70,000 with fluorescein-conjugated goat anti-mouse immunoglobulin (Zymed Laboratories Inc., San Francisco, Calif.) (optimal dilutions were established by checkerboard titrations on AD169-infected cells); (ii) the Syva Microtrak CMV Culture Identification Test, which consists of pooled MAbs specific for an immediate-early antigen of molecular weight 72,000 and an early antigen of molecular weight 50,000, with fluorescein-conjugated goat anti-mouse immunoglobulin G antibodies. The staining procedure was as specified in the kit protocol. Test controls consisted of CMV-infected and uninfected fibroblasts. For each specimen, approximately 2.5×10^5 PMNLs were stained, using each set of MAbs. Shell vial cultures of PMNLs and urine specimens were done in duplicate in human fetal lung fibroblasts as described by Smith and Espy (8), except that specimens were diluted 1:5 to avoid toxicity and staining was done at 3 days postinoculation, using the Syva Microtrak CMV Culture Identification Kit. In addition, duplicate tube cultures were inoculated and spin-amplified as described previously (5). Isolation of CMV was confirmed by IFA, using the Syva kit.

Sequential serum specimens were tested in pairs for CMV antibody, using either latex agglutination (CMV Scan Latex Agglutination Test; Becton Dickinson Microbiology Systems, Cockeysville, Md.) or immunofluorescence for IgG or IgM antibodies or both (CMV Test and CMV IgM Test; Gull Laboratories Inc., Salt Lake City, Utah).

Active CMV infection was defined as isolation of CMV from one or more specimens and/or seroconversion (primary infections) or a greater than fourfold rise in IgG antibodies (secondary infections) (13). The definition of CMV syn-

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drome was that used by van den Berg et al. (11), that is, active CMV infection with unexplained fever for 3 or more days and one or more of the following: arthralgia, leukopenia, thrombocytopenia, liver enzyme rises, and unexplained pneumonitis.

Of the 27 patients, 10 experienced CMV syndromes; 5 were primary and 5 were secondary infections. Of the 10 cases, 5 (3 primary and 2 secondary infections) received ganciclovir therapy. Nine of the ten symptomatic patients seroconverted or showed a significant increase in CMV antibody level and excreted CMV in urine; the 10th, a bone marrow recipient, excreted virus but was not tested serologically.

Eight patients experienced active CMV infection but were asymptomatic. One showed an increase in antibody level and excreted virus, two were positive only by serology, one excreted virus but showed no serologic response, and four excreted virus but were inadequately sampled serologically. Positive virus isolation results were obtained 3 to 24 weeks posttransplant (median, 6 weeks), while positive serology results were obtained 4 to 16 weeks posttransplant (median, 8 weeks).

Of the 134 heparinized blood specimens, 96 were from the 18 patients who experienced CMV infection; 54 were collected from the 10 symptomatic patients before and during the acute stage of the disease. Neither antigenemia nor viremia was demonstrated in these specimens.

These results differ from those obtained in other studies (2, 11, 14) which demonstrate that the direct detection of antigenemia is an early and sensitive indicator of symptomatic CMV infection. In a prospective study of 2,894 specimens from 104 transplantees, however, other workers found that the direct detection of antigenemia was of limited value for the early detection of CMV infection (1).

Several elements of the present study may not be comparable to some of the published work and may help to explain the difference in results. Though most of our patients were tested several times per week while experiencing symptoms of infection, our routine testing was less frequent than in some studies (11). As well, in the present study more than half of the specimens were processed the day after collection, due to transportation delays. These delays in processing, plus a suboptimal incubation period for conventional tube cultures (3 weeks), diminished the likelihood of detecting CMV viremia in our study. The effect of processing delays on the detection of antigenemia is less clear: while some reports refer to processing within 3 to 5 h of specimen collection (13), a recent publication by some of the same authors indicates that antigenemia can be detected in specimens held overnight before processing (10). Since several studies have shown that direct detection of antigenemia is up to twice as sensitive as virus culture (12, 14), we expected to find some evidence of antigenemia in these patients despite the suboptimal transport and culture conditions.

In the five patients with CMV syndrome who received ganciclovir, early treatment following onset of symptoms may have interfered with detection of both antigenemia and viremia (6). However, antigenemia has been shown to precede and/or coincide with the onset of clinical symptoms (10, 12) and to persist in some patients for a short time after the start of ganciclovir treatment (2). This makes it difficult to ascribe failure to detect antigenemia in those patients to ganciclovir treatment.

Finally, if, as has been proposed, there is an inverse relationship between levels of antigenemia and host immunocompetence (10), one could speculate that differences in immunosuppressive regimens might result in rates of antigenemia varying among medical centers and levels of antigenemia varying among different patient groups. For instance, since completion of this study, we have detected CMV antigenemia in AIDS patients, in specimens processed essentially as described above but in all cases within 5 h of collection (7).

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