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GUIDANCE OF GANCICLOVIR THERAPY WITH pp65 ANTIGENEMIA IN CYTOMEGALOVIRUS-FREE RECIPIENTS OF LIVERS FROM SEROPOSITIVE DONORS[1]

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Cytomegalovirus (CMV*), the first reported transplant-associated opportunistic virus (*1*), has remained the most frequent single cause or co-cause of infections throughout the ensuing three decades (*2*). Because prophylactic drug and hyperimmune globulin therapy has not been effective in these CMV-infected patients (*2-4*), Rubin (*5*) suggested withholding treatment until the phase of rapid viral replication (preemptive therapy). Early diagnosis of CMV infection, upon which this strategy depends, can be accomplished in the presymptomatic phase (*6, 7*) with a rapid quantitative direct demonstration of CMV antigen pp65 in cytospin preparations of peripheral blood leukocytes (PBL) (*8, 9*). This method has aided in the management of 20 CMV seronegative recipients of livers from seropositive donors, a circumstance that carries an 85-90% risk of virus transmission of CMV disease, presumably from migratory monocytes from the graft (*10*) that are known to harbor the latent virus in healthy seropositive individuals (*11*).

The 20 recipients under tacrolimus/prednisone immunosuppression were followed for 308±100.5 days (range, 63-520 days) after transplantation; a death at 63 days unrelated to CMV accounted for the only follow-up of <3 months. Heparinized blood samples were obtained by schedule during the first 3 months or when clinically indicated during this period and later. The pp65 antigen was detected in duplicate leukocyte spots (2×10^5 cells in 200 μl/glass slide) with the technique of Van der Bij et al. (*8*), using immunofluorescence (*12*) instead of immunoperoxidase staining (*9*). The anti-pp65 mouse monoclonal antibody was clone 1C3 (Biosoft, Argene, France). Fluorescein-isothiocyanate-labeled goat anti-mouse Fab2 fragment (Cappel, Durham, NC) was applied as the conjugate. Slides were counterstained with Evans blue dye and observed under 400× magnification for the typical green nuclear fluorescence.

A conventional shell vial culture assay was done on the same blood specimens. Anti-CMV antibodies (IgG and IgM) were measured before and after transplantation using a semiautomated immunofluorescence test (FIAX test system, Whittaker Bioproducts, Inc., Walkersville, MD). IgG titers >20 were considered positive. CMV IgG was also the routine test used for donor screening.

Intravenous ganciclovir therapy (Cytovene; Syntex Pharmaceutical Ltd., Palo Alto, CA) was started only with the first detection of pp65 antigenemia, and continued until pp65 clearance. The ganciclovir dose was governed by creatinine clearance: >50 ml/min → 5 mg/kg of ganciclovir twice daily, 10-50 ml/min → 5 mg/kg every 24-48 hr, and <10 ml/min → 5 mg/kg every 48-96 hr.

As expected, 17 (85%) of the 20 patients had positive pp65 tests (CMV antigenemia) after a median posttransplant interval of 34 days (range, 9-83 days). Positive shell vial CMV cultures (viremia) were obtained at the same time as the first antigenemia diagnosis in only 5 of these 17 cases.

Twelve of the 17 patients were asymptomatic when the first positive pp65 test was obtained, including a recipient who was diagnosed only 9 days after transplantation (Fig. 1). The pp65 levels were generally low: 20±28 (SD) per 2×10^5 leukocytes. However, 6 of the 12 had further rises in antigenemia for 1 to 12 days after therapy was started, to a median peak of 73 stained PBLs (range, 29-446 PBLs), before antigenemia began to clear. An example is shown in Figure 1. Ganciclovir instituted at the time of the first antigenemia prevented clinical disease in 11 of the 12 infected patients; the exceptional patient had a transient fever. The five other patients had CMV hepatitis at the time antigenemia was detected 12-83 days after surgery (median, 34 days). The median number of pp65-positive cells was 475 (range, 177-872).

Antigenemia was cleared with intravenous ganciclovir therapy in all 17 patients but recurred in 10 (59%) patients

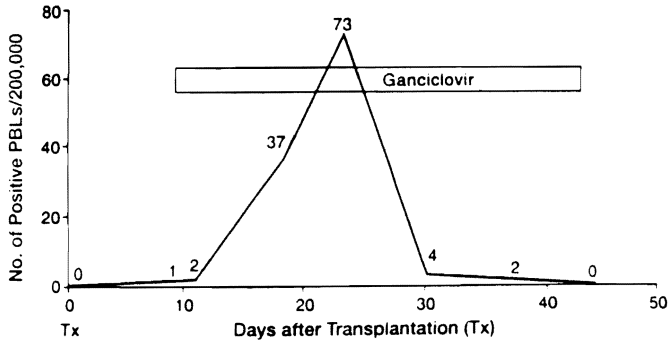

FIGURE 1. Preemptive ganciclovir treatment guided with CMV antigenemia in an asymptomatic, previously seronegative liver transplantation recipient whose donor was CMV positive.

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* Abbreviations: CMV, cytomegalovirus; PBL, peripheral blood leukocyte.

5-174 days (median, 57 days) after the drug had been discontinued. Recurrence was independent of IgG conversion ($P=0.26$). The only patient who was symptomatic with recurrence had 430 pp65-stained cells, which cleared after resumption of ganciclovir. The nine asymptomatic patients had 3-101 pp65-stained cells. Ganciclovir retreatment promptly cleared the antigenemia in the recipient with 101 stained cells. Retreatment was withheld in the other eight patients whose samples had <100 stained cells, and the antigenemia resolved spontaneously in all.

In these cases, the antigen assay was useful as a signal to begin preemptive antiviral treatment, as envisioned by Rubin (*2*), as a means to decide whether to reinstitute ganciclovir in the event of a recurrence (previously emphasized by The et al. [*6*]) and as a guide to individualize the duration of a ganciclovir course in all of these circumstances. The assay also can be an important differential diagnostic tool. The persistence of symptoms despite clearance of the CMV pp65 antigen is an unambiguous warning of co-infection by another pathogen. Conversely, failure of antigen clearance should raise suspicion of ganciclovir-resistant CMV mutants. The pp65 findings are specific. With a novel method for quantitation of CMV DNA in leukocytes, a highly significant correlation was demonstrated between leukoDNAemia and the presence and quantity of pp65 antigenemia (*13*).

The sensitivity and quantitation nature of the pp65 test allowed new observations about response to therapy, such as the reason for increases in pp65 levels seen during the first few days after beginning ganciclovir in half of the preemptively treated patients. This may have reflected a delay in suppression of viral replication or, alternatively, phagocytosis by PBLs of degrading CMV matrix protein from lysed, infected leukocytes (*14*) or from the ballooned and infected endothelial cells that shed into the circulation (*15*).

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