Increased intestinal permeability during cytomegalovirus infection in renal transplant recipients

Abstract  Cytomegalovirus (CMV) infections in renal transplant recipients can affect the gastrointestinal tract, but significant clinical manifestations are seldom seen. We hypothesize that subclinical involvement of the gastrointestinal tract may be quite frequent during CMV infection. In order to study this, we measured intestinal permeability by calculating the urinary lactulose mannitol (LM) excretion ratio after oral administration of lactulose and mannitol (normal < 0.030) in patients with symptomatic and asymptomatic CMV infection. A total of 111 patients were enrolled in the study, 104 of whom were tested on postoperative day (POD) 10. Twenty-nine patients developed CMV infection, 12 of whom could be studied with the permeability test (median POD 40). Another nine patients without CMV infection were also studied at day 40 and served as controls. The LM ratio increased significantly during CMV infection compared to measurements before active infection (median 0.060 vs. 0.030, P < 0.01) and was significantly higher during the infection than in the control group (median 0.007, P < 0.01). No correlation could be found between the LM ratio and viral load, humoral response to the virus, or symptomatology of infection. We conclude that an increased intestinal permeability is found in a substantial number of patients with an active, albeit asymptomatic, CMV infection after renal transplantation. Pathophysiological mechanisms and clinical implications remain speculative but will be subject to further study.

Key words  CMV, renal transplantation, intestinal permeability. Renal transplantation, CMV, intestinal permeability. Permeability, intestinal, CMV. Intestinal permeability, renal transplantation, CMV.

Introduction  Cytomegalovirus (CMV) infection is the most frequent infectious complication after renal transplantation. Although CMV infections after renal transplantation are frequently seen, a substantial number of them are asymptomatic. When CMV infection causes disease, most patients exhibit a so-called self-limiting CMV syndrome consisting of spiking fever, arthralgia, leukocytopenia, thrombocytopenia, and elevated serum liver enzymes. Less common manifestations involve the gastrointestinal tract, the lungs, the eyes, the kidney, the heart, and the nervous system. Clinical manifestations of gastrointestinal involvement include ulcerative lesions anywhere along the gastrointestinal tract, intestinal pneumatosis [13], pancreatitis, and hepatitis.

We hypothesize that there is frequent subclinical organ involvement during CMV infection in renal transplant patients. This would be in accordance with the systemic nature of this type of infection. For example, van
Son et al. [14] have already demonstrated that when subjected to sensitive pulmonary function tests, a majority of patients with an active CMV infection have pulmonary dysfunction, even without pulmonary symptoms. Thus far, subclinical involvement of the gastrointestinal tract during CMV infection has not been studied.

The epithelium of the gastrointestinal tract has important transport functions, but the barrier function with respect to luminal molecules is at least as important. Intestinal permeability relates to the barrier function, and the permeation of marker molecules is used to measure the permeability. Most intestinal permeability tests are based on quantitation in the urine of marker molecules ingested orally. However, not only the permeability of the intestinal surface but many other factors, such as gastric emptying, dilution by secretions, and renal clearance, determine the excretion of marker molecules. These factors can be eliminated by using two markers that differ in permeability but are affected equally by all of the other factors. This principle is called differential permeability testing. The excretion ratio of these two markers has been shown to be a reliable test for intestinal permeability. This method of measuring intestinal permeability is widely accepted and used for testing mucosal dysfunction, making more invasive diagnostic procedures unnecessary [15]. In this study we evaluated the subclinical involvement of the gastrointestinal tract in renal transplant recipients with CMV infection by determining intestinal permeability with lactulose and mannitol as markers.

**Materials and methods**

One hundred eleven patients transplanted between 1990 and 1993 were included in the study. Sixty five were men. The mean age was 44 years (range 18-68 years). The median dialysis period pretransplantation was 32 months (range 0-144 months). There were 101 first transplantations, 9 second, and 1 third. Six transplantations were living related. Fifty-three percent of the patients were seropositive for CMV before transplantation. Patients were considered seropositive when IgG antibodies against CMV late antigen (CMV LA) were present. No CMV prophylaxis (acyclovir, gancyclovir, or anti-CMV immunoglobulins) was given. Initial immunosuppression consisted of cyclosporin A and low-dose prednisolone. Patients with a second or third transplant received induction therapy with monoclonal antibodies (OKT3), followed by triple therapy (azathioprine, cyclosporin A, and low-dose prednisolone). All patients gave informed consent before participating in the study.

A differential permeability test was performed to measure intestinal permeability. We used lactulose (342 Da, 0.52 nm) and mannitol (182 Da, 0.40 nm) as markers.

After an overnight fast of at least 6 h and with an empty urinary bladder, patients drank 100 ml of water containing 10 g lactulose and 0.5 g mannitol. The osmolality of the solution was 255 mosmol/l. No oral intake was allowed during the first 2 h after ingestion of the test solution, and subsequently milk or sugars were not allowed until 5 h after commencing the test. Urine was collected during a period of 5 h after the test solution had been ingested. The volume of the urine collected was recorded and an aliquot was refrigerated at -20°C until the time of analysis.

The lactulose and mannitol concentrations in the urine were measured by gas liquid chromatography, as described by Laker [9] and Laker and Mount [10], respectively, with minor modifications. Briefly, the samples were mixed with internal standard solution (a-methyl glucose), washed, and dried. Pyridine/hydroxysil (Chromopack, Middelburg, The Netherlands) was added and the specimens were heated at 60°C for 2 h. Samples were analyzed in a Packard 428 chromatograph (Packard-Becker, Delft, The Netherlands) on a 200-cm column of 3% OV-1 (Chromopack, Middelburg, The Netherlands), operated at 190°C for 7 min and at 250°C for 6 min; the temperature was elevated from 190°C to 250°C in 12 min. Mannitol, 1 mmol/l, and lactulose, 1 mmol/l, were used as standard solutions (Janssen Pharmaceutica, Belgium).

The lactulose mannitol excretion ratio (LM ratio) was calculated by dividing the urinary lactulose excretion by the urinary mannitol excretion, expressed as percentages of the ingested doses. Normal values are below 0.030 and are independent of renal function. Especially in our patients, who had a broad range of glomerular filtration rates, it was necessary to be very sure about the independence of renal function, as reviewed in the literature [4, 15]. For this reason, we measured the LM ratio in 10 non-transplanted patients without gastrointestinal diseases from the outpatient renal clinic and with glomerular filtration rates below 25 ml/min; these ratios were in the normal range.

Diagnosis of active CMV infection was made using the CMV antigenemia assay, as described by van der Bij et al. [1, 2] and reviewed by Chou [3] and by Ljungman and Griffiths [11] during the Fourth International CMV Workshop (Paris, 1993). In short, peripheral blood leukocytes were isolated, cytocentrifuged, and incubated with a mixture of monoclonal antibodies directed against a 65-66 kD CMV antigen, followed by immunoperoxidase staining. The number of antigen-positive cells per 50,000 leukocytes was counted in triplicate. The antigenemia assay was performed at least once weekly starting on postoperative day (POD) 12. In all patients the antigenemia was followed by either seroconversion (primary infection) or a significant rise (reactivation) in CMV IgG antibodies. IgM and IgG CMV antibodies were measured quantitatively by ELISA using late stage CMV-infected fibroblasts as antigen [5].

Intestinal permeability was measured with the LM test at POD 10 to avoid possible bias of the postoperative state. Those values are referred to as baseline values. During active CMV infection, diagnosed by a positive antigenemia assay, the intestinal permeability was measured again (median POD 40). In five patients we were able to perform more than one permeability test during CMV infection, and in the analysis we used the result of the test with the highest LM ratio. Since we measured the LM ratios during CMV infection on median POD 40, we also measured LM ratios on median POD 40 in a control group of renal transplant recipients without CMV.

Statistical analysis was performed using the Wilcoxon signed rank test and the Mann-Whitney U-test. Differences in creatinine clearances were evaluated with Student's t-test.

**Results**

We performed 104 lactulose mannitol (LM) tests on POD 10 (baseline values), 18 during active CMV infection, and 9 on POD 40 without CMV infection. Twenty-