Immune responsiveness in immunosuppressed patients

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CHAPTER 3: CD4<sup>POS</sup> T CELL DYNAMICS IN PRIMARY CMV INFECTION

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**Introduction**

Cytomegalovirus (CMV) infection is an important cause of morbidity following renal transplantation. Many cell types interact in the specific immune defense against CMV infection (1). Starting with initially infected host cells, activation, migration, proliferation and/or differentiation occurs of professional antigen presenting cells, CD4^{pos} and CD8^{pos} T cells, γδ T cells, B cells and NK cells. In mice, B cell responses against murine CMV are strictly dependent on CD4^{pos} cells (2). Moreover, in a well-studied model of murine lymphocytic choriomeningitis virus (LCMV) infection, maintenance and diversity of LCMV specific CD8^{pos} T cells were shown also to depend on CD4^{pos} T cells (3). Thus, CD4^{pos} T cells appear to function as crucial regulatory cells in specific immune response against CMV. We initiated an observational study into CD4^{pos} lymphocyte dynamics in human primary CMV infection. CMV-seronegative recipients of kidneys from seropositive donors were studied longitudinally. CD4^{pos} T lymphocyte counts were determined by weekly intervals and related to the amount of CMV-DNA present in the peripheral blood compartment.

**Methods**

**Patients**

Six patients, who were CMV-seronegative before transplantation and received a kidney transplant from a CMV-seropositive donor, were studied. Immunosuppressive drug therapy consisted of 10 mg prednisolone per day and cyclosporin (Neoral) aimed at blood trough levels of about 150 ng/ml. No prophylactic ganciclovir was given; ganciclovir treatment of primary CMV infection was initiated only in case of CMV-disease. Peripheral blood was collected weekly during about 20 weeks. On sampling days, venous puncture was performed before immunosuppressive medication was taken. The study was approved by the local medical ethical committee.

**CMV PCR**

Quantitative PCR for the detection of CMV-DNA was performed in EDTA whole blood samples as described for plasma or serum (4).

**Determination of T cell subsets**

At each time point, total leukocyte-and differential counts were determined using standard techniques on EDTA-anticoagulated whole blood. CD4^{pos} T cell frequency was determined by flowcytometry as described in (5). Lymphocyte counts were calculated by multiplying leukocyte counts and percentages of lymphocytes. CD4^{pos} lymphocyte counts were determined by multiplying lymphocyte counts and the frequency of CD4^{pos} cells within a lymphocyte gate.
Results and Discussion

CMV seronegative renal transplant recipients were chosen for this study for the apparent reason that the exact time point of infection was known, viz. the time of transplantation with an organ from a CMV seropositive donor. Four out of six patients who were CMV-seronegative before transplantation and received an organ from a CMV-seropositive donor were diagnosed as having a primary CMV infection during follow up. Of these four patients, only one received ganciclovir treatment initiated after persistent fever and radiological evidence of interstitial pneumonia. The other three patients did not receive ganciclovir treatment and maintained basic immune suppressive therapy throughout the study. Around the time of first detection of CMV-DNA, lymphocyte counts declined (not shown). This decrease was mainly due to a dip in the peripheral blood CD4<sup>pos</sup> lymphocyte counts (figure 1, left and upper right panels). In the CMV-seronegative renal transplant recipients who did not acquire primary CMV infection (i.e. remained negative in CMV-PCR), this decrease in CD4<sup>pos</sup> lymphocyte counts was not observed (figure 1, lower right panel). Moreover, CD8<sup>pos</sup> T cells did not decline in peripheral blood. With a median of 7 days after first detection of CMV-DNA, CMV specific CD4<sup>pos</sup> T cells emerged in the peripheral blood compartment (5). To our knowledge, the decline in CD4<sup>pos</sup> lymphocyte counts in the absence of a serious decline of CD8<sup>pos</sup> lymphocyte numbers just before detectable primary CMV infection was not previously documented. Decrease of the CD4<sup>pos</sup> lymphocyte number from the peripheral blood may be due to either physical deletion or redistribution of these cells to e.g. secondary lymphoid organs. We favor the second hypothesis. First, CMV does not preferentially infect CD4<sup>pos</sup> lymphocytes, if it infects T-lymphocytes at all. Therefore, these cells presumably do not suffer from direct cytopathic effects due to infection. Second, several lines of evidence support the notion that viral infection leads to retention of lymphocytes in the secondary lymphoid organs. After initiation of highly active anti-retroviral therapy in HIV infected patients, peripheral blood CD4<sup>pos</sup> T cells rapidly rise, accompanied by a decline of lymphocytes in lymphnodes (6;7). Moreover, even in localized infections, virus specific T cells mainly reside in secondary lymphoid organs (8). In addition, IFNγ, which is elicited by viral infections, was shown to induce redistribution of human memory/effecto r T cells (9). We therefore hypothesize that our observation of temporal disappearance of CD4<sup>pos</sup> lymphocytes from the peripheral blood reflects an early mechanism in CMV infection of retaining lymphocytes in the appropriate localization to encounter antigen presenting cells.
Figure 1 Peripheral CD4<sup>pos</sup> T lymphocyte numbers decline at the time of appearance of CMV-DNA in primary CMV infection in renal transplant recipients, but not in uninfected patients.

Time after transplantation (X-axis) versus CD4<sup>pos</sup> lymphocyte count (left Y-axis, closed circles) and CMV-DNA (right Y-axis, open circles) of representative CMV-seronegative renal transplant recipients of kidneys from CMV-seropositive donors. Left two panels and upper right panel: patients who acquired primary CMV infection. Lower right panel: patient, who did not acquire primary CMV infection.

Reference List


