CHAPTER 4: CD8⁺LYMPHOCYTE DYNAMICS IN PRIMARY CMV INFECTION

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Transplantation Proceedings 2000, in press
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Introduction
Cytomegalovirus (CMV) infection is a frequent infectious complication after renal transplantation. During active and even latent CMV infection, viral DNA can be detected in specimens from many tissues of the human body (1;2). Primary CMV infection leads to considerable immune activation. In particular, expansions of CD8<sup>pos</sup> T cells and γδ T cells from human peripheral blood shortly after primary CMV infection have been described (3;4). CD8<sup>pos</sup> T cells are presumed to deliver "the lethal hit" to CMV infected cells. In accordance, shortly after primary CMV infection, the majority of the expanded CD8<sup>pos</sup> T cells express granzyme B, to execute cytotoxic functions(5). Rendering CMV infected cells susceptible to CD8<sup>pos</sup> T cell mediated killing requires surface expression of MHC class I molecules, loaded with CMV derived peptides. The CMV genome, however, contains many genes that impair MHC class I mediated recognition by CTL's (6). Therefore, the immune system positions redundant mechanisms to eliminate CMV infected cells. Notably, natural killer cells (NK cells) add to control of CMV infection in vivo (7).

In human peripheral blood, two populations of CD8<sup>pos</sup> lymphocytes can be visualized. One population consists of CD8<sup>pos</sup> T-cells. The majority of these cells express the CD8αβ heterodimer, with high cell surface levels of the CD8α chain. Immunocytochemical stainings of human peripheral blood mononuclear cells (PBMC) with CD8α directed antibodies reveal these cells as CD8<sup>bright</sup> lymphocytes in flow cytometric analyses. The second population consists of CD3<sup>neg</sup> NK cells, expressing CD8αα homodimers in low concentrations on the cell surface, appearing as CD8<sup>dull</sup> lymphocytes in flow cytometry.

In many human and murine viral infections, the majority of the expanded CD8<sup>pos</sup> T cell populations from peripheral blood and spleen during lymphocytosis were shown to be constituted of cells specific for few dominant epitopes of the infecting virus, rather than being caused by bystander T cell activation (8-11). In mice, viral infections can be timed and dosed. Moreover, murine and viral strains with specific properties are used to infect the animals. These circumstances are infrequently encountered in humans. However, primary CMV infection can relatively reliably be foreseen in some groups of organ transplant recipients. Specifically, renal transplant recipients, who are seronegative before transplantation (R-) and receive organs from CMV seropositive organ donors (D+) are highly at risk for acquiring CMV, for the donated kidney may contain the CMV genome or even CMV particles. We previously demonstrated that CMV-DNAemia developed with a median of 25 days after transplantation in 5 out of 7 of these D+R- combinations (12). These patients allowed, by frequent sampling, careful observation of the development of human CD8<sup>pos</sup> lymphocytes from before infection through the complete acute phase of the infection.

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Method

Patients
Seven 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.

Determination of T cell subsets
At each time point, total leukocyte-and differential counts were determined using standard techniques on EDTA-anticoagulated whole blood. CD8\textsuperscript{pos} T cell frequency was determined by flow cytometry as described (12). NK cell frequencies were determined by flow cytometry using CD56-PE and CD16-PE and CD3-FITC (NK simultest, BectonDickinson (BD), San José, CA, USA). Frequencies of cells within a lymphocyte gate, negative for CD3 expression and positive for CD16/CD56 were designated NK cell frequencies. Lymphocyte counts were calculated by multiplying leukocyte counts and percentages of lymphocytes. CD8\textsuperscript{pos} lymphocyte counts and NK cell counts were determined by multiplying lymphocyte counts and the frequency of CD8\textsuperscript{pos} cells or NK cells respectively within a lymphocyte gate.

CMV PCR
Quantitative PCR for the detection of CMV-DNA was performed in EDTA whole blood samples as described for plasma or serum (13).

Results
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. Five out of seven patients who were seronegative before transplantation and received organs from seropositive organ donors were diagnosed as having a primary CMV infection during follow up of at least 100 days after transplantation. Of these five patients, only one received ganciclovir treatment initiated after persistent fever, and radiological evidence of interstitial pneumonia. The other four patients did not receive ganciclovir treatment and maintained basic immune suppressive therapy throughout the study.
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Shortly after transplantation, few CD8 \textsuperscript{dull} cells were observed in peripheral blood from the here studied renal transplant recipients (figure 1A, left panel).

A.

<table>
<thead>
<tr>
<th>Day</th>
<th>Relative Cell Number</th>
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<tr>
<td>10</td>
<td>![Histogram Image]</td>
</tr>
<tr>
<td>50</td>
<td>![Histogram Image]</td>
</tr>
<tr>
<td>103</td>
<td>![Histogram Image]</td>
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Figure 1 CD8\textsuperscript{pos} lymphocyte dynamics in primary CMV infection.

A. Histograms of CD8-PE fluorescence (X-axis, arbitrary units, log scale) versus relative cell number (Y-axis) of electronically gated lymphocytes. The histograms show results from one representative patient sampled before (left panel, 10 days after transplantation), shortly after (middle panel, 50 days after transplantation) and long after first detection of CMV-DNA (right panel, 103 days after transplantation).

B. CD8 lymphocyte numbers (left panel, Y-axis, cells/liter peripheral blood) and NK cell numbers (right panel, Y-axis, cells/liter peripheral blood) versus time (X-axis, days after transplantation) of samples from one representative patient (same patient as in figure 1A.). The vertical dotted line at day 29 after transplantation designates the first detection of CMV-DNA. $\bigcirc$=CD8\textsuperscript{dull} lymphocytes, $\bullet$=CD8\textsuperscript{bright} lymphocytes, $\blacklozenge$=natural killer cells.

In the 5 seroconverting patients, but not in the non-converters, a CD8\textsuperscript{dull} population appeared shortly after first detection of viral DNA (figure 1A, middle panel). This population gradually decreased in peripheral blood in the course of 25 to 50 days after the first detection of CMV-DNA (figure 1A left right panel and figure 1B left panel). Likely, the population of CD8\textsuperscript{dull} cells consists of NK cells. To exclude that CD8\textsuperscript{pos} T cells downregulate CD8\textalpha from the cell surface and thereby appear as CD8\textsuperscript{dull} cells, we performed flow cytometric analysis to determine CD3\textsuperscript{neg} NK cell numbers. Concomitant with the rise and fall of CD8\textsuperscript{dull} cell numbers, a rise and fall in NK cell numbers was observed (figure 1B right panel). The CD8\textsuperscript{bright} T-lymphocyte numbers similarly increased, shortly after detection of CMV-DNA.
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(mean change (peak number before-peak number after first CMV-DNA detection) = 0.42 x 10<sup>9</sup> cells/liter blood p=0.0018). In contrast to the CD8<sup>dull</sup> population, these cells either continued to rise when the CD8<sup>dull</sup> numbers decreased from blood or remained at an elevated number during the study period (figure 1B left panel).

Discussion

Since CD8 and NK cell mediated responses are important in the control of CMV infection, we addressed the issue of CD8 dynamics in primary CMV infection by frequent sampling of peripheral blood and detailed subset analysis in patients, highly at risk of developing primary CMV infection. Indeed, five out of seven of D+R- patients did experience CMV-DNAemia, commencing within the first month after transplantation. In accordance with others, CD8 lymphocytosis rapidly followed the CMV-DNAemia (3). Several groups noted that CD8<sup>pos</sup> T cells progressively acquire NK cell markers like CD57 in the course of CMV infection (3). We found that NK-cells significantly add to the CD8-lymphocytosis during the first 50 days after the infection, but not thereafter. CD3<sup>neg</sup> NK cell- and CD8<sup>bright</sup> T cell dynamics then segregate into an expanding CD8<sup>pos</sup> T cell compartment and a shrinking NK cell compartment. It remains to be established how these mechanisms contribute to reversal of the CMV infection into latency.

Reference List


