Symptomatic and Asymptomatic Viral Recrudescence in Solid
Organ Transplant Recipients and its Relationship with Antigen-
Specific CD8+ T-cell Response

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Running title: T-cell dynamics and viral recrudescence

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Abstract

Using ex vivo antigen-specific T-cell analysis, we found that symptomatic cytomegalovirus recrudescence in transplant recipients was co-incident with reduced expression of IFN-γ by virus-specific CD8+ T-cells and an up-regulation of CD38 expression on these T-cells, although there was no significant change in the absolute number of virus-specific cells (as assessed by MHC-peptide multimers). In contrast, HLA class I-matched transplant patients with asymptomatic viral recrudescence showed increased expansion of antigen-specific T-cells and highly stable IFN-γ expression by epitope-specific T-cells. These studies suggest that a strong functional T-cell response plays a crucial role in defining the clinical outcome of acute viral recrudescence.
Clinical presentation of acute latent viral infections and its interaction with host T-cell responses has recently been investigated in order to understand the dynamics of immune regulation and to develop better therapeutic strategies (5, 9, 10, 20). Previous studies have proposed a role for a number of potential factors, such as viral load and cytokine dysregulation, in controlling the symptoms of acute viral infection (1, 17). It is entirely feasible that the dynamics of emergence of the virus-specific T-cell response during the early stages of viral recrudescence may delimitate the patterns of clinical symptoms in different individuals (11, 13, 16). Indeed, massive expansion of CD8$^+$ T-cells specific for Epstein-Barr virus latent and lytic antigens, which is often a feature of acute EBV infection, suggests that these T-cell responses are recruited to control the active viral infection (2). However, understanding the biological significance and the longitudinal dynamics of these T-cells during acute viral infections in humans is often difficult, and is complicated by the nature of immune responses in naturally out-bred individual patients. We have addressed some of these limitations by analyzing the dynamics of T-cell responses to a panel of CD8$^+$ T-cell epitopes in a group of HLA class I-matched unrelated human subjects undergoing acute human cytomegalovirus (HCMV) infection with contrasting clinical symptoms. We studies three broad groups of transplant patients - (a) individuals with asymptomatic viral recrudescence, (b) individuals with symptomatic viral recrudescence and (c) individuals with no evidence of viral recrudescence. In each of these groups of patients we longitudinally analyzed CD8$^+$ T-cell responses using ex vivo ELISPOT assays and MHC-peptide multimer analysis. In addition, we also assessed the viral load in these individuals to determine whether there was any correlation with either T-
cell dynamics and/or clinical symptoms.

Peripheral blood samples from a cohort of 15 HLA class I-matched solid organ transplant patients (renal or heart and/or lung; SOT) were collected into EDTA collection tubes. These blood samples were collected at multiple time points (see figure 1), cryopreserved and used for T-cell assays and viral load analysis. All blood samples were collected following informed consent, and the study was approved by the relevant human ethics committees. Clinical diagnosis of symptomatic viral recrudescence was based on laboratory diagnosis (pp65 antigenemia; ≥10 positive cells/10^6 PBMC) and previously published clinical criteria outlined by the American Society of Transplantation (8). Patients with symptomatic HCMV disease were treated with oral and intravenous ganciclovir (the exact period of treatment is indicated in figure 1 as shaded area) with the exception of patient N, who received cidofovir. Patient L also received Foscarnate and Valganciclovir. The transplant immunosuppressive regimes have been outlined elsewhere (14). Briefly, these patients received cyclosporin A, mycophenolate mofetil and Prednisolone.

In the first set of studies, we longitudinally analyzed the HCMV-specific T-cell responses using ELISPOT assays and MHC-peptide pentamer/tetramer staining in these transplant patients as described previously (3, 4). For these assays, HCMV epitopes restricted through various HLA class I alleles (HLA-A1, HLA-A2, HLA-B7 and HLA-B8) were used in this study (Table 1). Data from each of these SOT recipients is presented in Fig. 1 (panel A-O). Longitudinal analysis of immune responses clearly illustrated that those SOT patients who either showed no evidence of viral
recrudescence (Fig. 1, panel A-E) or asymptomatic viral recrudescence (Fig. 1, panel F-J) maintained a stable virus-specific IFN-γ expression by CD8+ T-cells throughout the follow-up period. These responses were towards epitopes derived from both structural and/or IE-1 antigens. In contrast, HLA matched SOT recipients who were diagnosed with symptomatic viral recrudescence (Fig. 1, panel K-O and Table 2) showed significant fluctuations of their virus-specific IFN-γ expression by CD8+ T-cells. These fluctuations ranged from a 3-120,000 fold reduction in the virus-specific CD8+ T-cell responses. One of the interesting aspects of this longitudinal analysis was that patients with symptomatic viral recrudescence showed reduced IFN-γ expression (>5 fold reduction) by virus-specific CD8+ T-cells in >70% of their blood samples, while <10% of the blood samples from SOT recipients with no viral recrudescence or asymptomatic viral recrudescence showed reduced IFN-γ expression by virus-specific CD8+ T-cells. Furthermore, T-cell responses towards multiple epitopes showed a contemporaneous pattern of fluctuation which is consistent with our previous finding in healthy virus carriers(3). Most importantly, the reduction in the IFN-γ expression by antigen-specific T-cells in four of the five symptomatic recipients preceded the clinical diagnosis of active disease. It is important to mention here that the viral load in asymptomatic or symptomatic recipients showed no correlation with either IFN-γ expression by HCMV-specific T-cells or clinical symptoms. Taken together, these analyses strongly suggest that a stable antigen-specific CD8+ T-cell response is crucial to preventing symptomatic clinical syndrome following latent viral recrudescence.
To determine whether the reduced IFN-γ expression by virus-specific T-cells in individuals with symptomatic viral recrudescence was due the loss of HCMV-specific T-cells, PBMC from all SOT recipients were stained with MHC-peptide tetramers/pentamers and anti-CD8 antibody (Fig. 2, panel A). Data presented in figure 2, panel B shows that although there was very little difference in the overall numbers of HCMV-specific T-cells in SOT recipients who either showed no viral recrudescence or symptomatic recrudescence, a significant increase in the number of HCMV-specific T-cells was observed in individuals with asymptomatic recrudescence. We hypothesize that this increase in the HCMV-specific T cells in these individuals is not unexpected as the viral recrudescence would provide increased stimulation to these cells resulting in their expansion and thus provide protection from clinical disease. It is highly likely that in addition to the reduced IFN-γ expression, the lack of expansion of HCMV-specific T-cells in individuals with symptomatic recrudescence may also contribute towards the development of clinical disease. Furthermore, we also observed a significant increase in the expression of CD38 on the HCMV-specific CD8⁺ T-cells in SOT recipients with symptomatic viral recrudescence when compared to recipients who either showed no viral recrudescence or asymptomatic recrudescence (Fig. 2, panel C). No significant difference in the expression of CD62L or CD27 on HCMV-specific CD8⁺ T-cells was observed during or after anti-viral prophylaxis (data not shown). Previous studies on HIV-infected individuals have shown that an increased expression of CD38 on CD8⁺ T-cells is co-incident with chronic HIV disease progression to AIDS(12, 15).
In conclusion, this study is the first to demonstrate directly that a broadly directed CD8\(^+\) T-cell response with strong functional activity was coincident with the protection from symptomatic viral recrudescence. In addition, we have also shown that terminal differentiation/exhaustion of antigen-specific T-cells (as indicated by the increased CD38 expression) may contribute towards the development of clinical symptoms following viral recrudescence. These conclusions are strongly supported by previous studies on other viral infections which has shown that the breadth of the CTL response may be important in preventing viral pathogenesis\((1, 18)\). Another important implication of this study relates to the potential use of T-cell functional analysis as a diagnostic tool for identifying the levels of virus-specific T-cell responses which would predict the increased or decreased risk of symptomatic HCMV recrudescence \((6, 7, 19)\). The diagnostic application of this technology will require an extended analysis on a larger cohort of transplant patients in various clinical settings to determine if the protection from HCMV disease in transplant patients is dependent on T-cell responses directed against a broad range of antigens rather than on any one single antigen.
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References


occurs in the absence of blood T-cell repertoire perturbations despite high levels of systemic viral load. Blood 98:3739-3744.


Figure Legends

Figure 1: Longitudinal functional analysis of HCMV-specific T-cells in HLA class I-matched SOT recipients using IFN-γ ELISPOT assays and peptide epitopes from HCMV antigens (see Table 1). Data from individual recipient is presented in each panel. Data from individuals with no evidence of viral recrudescence are presented in panels A-E, with asymptomatic recrudescence are presented in panels F-J and with symptomatic recrudescence are presented in panels K-O. Data presented in panels A-E, K, L and N is based on SOT patients who received heart and/or lung transplant, while data in panels F-J, M and O is based on renal transplant recipients. Grey shaded areas in panels K-O indicates the time period when clinically active HCMV disease was diagnosed and the patient was being treated with anti-viral medication (see Table 2). Patients B, D and E received anti-viral prophylaxis based on oral ganciclovir. The results are expressed as spot forming cells (SFC) per 10^6 CD3^+CD8^+ T-cells. Also shown in panels F-O is the HCMV load in peripheral blood of these patients as HCMV copies/10^6 PBMC. Blood Sample collection time points (weeks) for each of the donors are indicated on X-axis.

Figure 2: Ex vivo enumeration of HCMV-specific CD8^+ T-cells using MHC-peptide multimers. PBMC from SOT recipients were co-stained with anti-human
CD8-tricolor labeled antibody and PE-labeled MHC-peptide tetramers/pentamers. The fluorescence intensity was assessed with a FACSCalibur and data analyzed using Cellquest software. Representative data plots for three different HCMV epitopes; NLV (HLA A2-restricted), VTE (HLA A1-restricted) and TPR (HLA B7-restricted) are shown in Panel A. Panel B shows percentage of MHC-peptide multimer positive CD8$^+$ T-cells in samples from individuals with no evidence of viral recrudescence, with asymptomatic recrudescence and with symptomatic recrudescence. Panel C shows the phenotypic analysis of HCMV-specific T-cells in these individuals. HCMV-specific T-cells were co-stained with CD38 antibody. Data presented in panels B & C is based on all the blood samples collected at different time points as indicated in Fig. 1. P values were calculated using the non-parametric Mann-Whitney test.
Table 1: List HLA class I-restricted HCMV epitopes used in this study

<table>
<thead>
<tr>
<th>Epitope Sequence (code)</th>
<th>HLA restriction</th>
<th>Antigen</th>
<th>Antigen Expression kinetics</th>
<th>ELISPOT</th>
<th>MHC-peptide Tetramer</th>
</tr>
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<tbody>
<tr>
<td>YSEHPTFTSQL (YSE)</td>
<td>HLA A1</td>
<td>pp65</td>
<td>Late/structural</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>RPERNGFTVL (RPH)</td>
<td>HLA B7</td>
<td>pp65</td>
<td>Late/structural</td>
<td></td>
<td>√</td>
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<tr>
<td>NLVPMATV (NLV)</td>
<td>HLA A2</td>
<td>pp65</td>
<td>Late/structural</td>
<td></td>
<td>√</td>
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<tr>
<td>VTEHDTLVL (VTE)</td>
<td>HLA A1</td>
<td>pp50</td>
<td>Late/structural</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>TPRVTGGAM (TPR)</td>
<td>HLA B7</td>
<td>pp65</td>
<td>Late/structural</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>VLEETSVML (VLE)</td>
<td>HLA A2</td>
<td>IE-1</td>
<td>Immediate-early</td>
<td></td>
<td>√</td>
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<tr>
<td>ELRKKMMY (ELR)</td>
<td>HLA B8</td>
<td>IE-1</td>
<td>Immediate-early</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>QIKVRVDMV (QIK)</td>
<td>HLA B8</td>
<td>IE-1</td>
<td>Immediate-early</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>Patient Code*</td>
<td>Clinical Syndrome</td>
<td>Anti-viral therapy</td>
<td></td>
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<td>--------------</td>
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<tr>
<td>Patient K (D-/R-)</td>
<td>Systemic HCMV infection without defined end organ involvement</td>
<td>Treated with ganciclovir (IV, 400 mg bd) followed by oral ganciclovir (1000 mg tid)</td>
<td></td>
<td></td>
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<tr>
<td>Patient L (D+/R-)</td>
<td>Clinical disease with very high viral load and antigenemia</td>
<td>Treated with ganciclovir (IV, 400 mg bd, followed by valganciclovir 450 mg bd). Also treated with a combination of foscarnet (4 g), IV ganciclovir (460 mg) and CMV hyperimmunoglobulin</td>
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<tr>
<td>Patient M (D+/R-)</td>
<td>Systemic HCMV infection with Gastric involvement</td>
<td>Treated with ganciclovir (IV, 160 mg bd) followed by oral ganciclovir (1000 mg tid)</td>
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<td></td>
<td></td>
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<tr>
<td>Patient N (D+/R-)</td>
<td>HCMV syndrome with end organ disease (liver, pancreas, brain and gut)</td>
<td>Treated with Cidofovir (200 mg weekly)</td>
<td></td>
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<tr>
<td>Patient O (D+/R+)</td>
<td>HCMV syndrome without defined end organ involvement</td>
<td>Treated with ganciclovir (IV, 325 mg bd) followed by oral ganciclovir (1000 mg tid)</td>
<td></td>
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*Patient code refers to the panels in Figure 1
Fig. 1
Asymptomatic recrudescence

Symptomatic recrudescence

NLV (HLA A2, pp65)

VTE (HLA A1, pp50)

TPR (HLA B7, pp65)

CD8-Tricolor

MHC-peptide multimer-PE

Fig. 2
No viral recrudescence
Asymptomatic recrudescence
Symptomatic recrudescence

% CD3+CD8+ MHC-peptide multimer +ve cells

B

p = ns
p = 0.0274
p = 0.0443

% CD3+CD8+ CD38+ MHC-peptide multimer +ve cells

C

p = 0.005
p = ns
p = 0.0004

Fig. 2