Systemic and local human cytomegalovirus-specific T-cell response in lung transplant recipients

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INTRODUCTION

Human cytomegalovirus (HCMV) is a major cause of viral infection in lung transplant recipients (LTRs), often resulting in disseminated and/or local infection/disease. HCMV reactivation episodes may occur repeatedly, while the level of viral load may differ greatly between blood (systemic infection) and lungs (local infection) (Westall et al., 2004; Bauer et al., 2007; Gerna et al., 2007, 2009, 2012).

Recently, it has been documented that a T-cell-mediated immune response (both CD4⁺ and CD8⁺) is elicited during systemic HCMV infection in the post-transplant period and may confer protection against HCMV reactivation (Zeevi et al., 1998; Rowshani et al., 2005; Sester et al., 2005; Shlobin et al., 2006; Gerna et al., 2009, 2011). However, the differential role of the specific T-cell response in the two compartments (blood and lungs) has been scarcely investigated. Special attention has been paid to the differential HCMV-specific CD8⁺ T-cell response in lungs (bronchoalveolar lavage fluid - BAL) in comparison with blood (Zeevi et al., 1992; Shlobin et al., 2006; Westall et al., 2006; Pipeling et al., 2008), while few data on the clinical utility of the assessment of local T-cell responses have been reported.

This study divided 20 LTR patients into four groups according to viral load reached in blood and BAL at peak of infection. The T-cell response was determined in blood by cytokine flow cytometry (CFC) and in lungs (BAL) by the enzyme-linked immunospot (ELISpot) assay.

SUMMARY

It is debated whether human cytomegalovirus (HCMV) infection/disease of the pulmonary compartment in lung transplant recipients (LTRs) may be controlled by the HCMV-specific systemic T-cell response or requires a local (lung) T-cell response. Systemic and local HCMV loads were investigated in parallel by real-time PCR in 20 LTRs. T-cell responses were measured by intracellular cytokine staining of HCMV-specific IFN-γ⁺ CD4⁺ and CD8⁺ T-cells in PBMC, and by enzyme-linked immunospot (ELISpot) assay in lung (BAL) mononuclear cells. Patients were grouped at time of peak of infection based on viral load in blood and BAL. Immunological testing results showed that five patients with no HCMV infection (either local or systemic) had both local and systemic T-cell responses; four patients with systemic infection had no systemic T-cell response; five patients with both systemic and lung infection had neither local nor systemic T-cell responses; and six patients with lung infection had no local and a partial (only CD8⁺ in the absence of CD4⁺) systemic T-cell response. These results indicate that local immunity is associated with resolution of lung infection. Systemic T-cell response alone is not sufficient to provide lung protection from HCMV infection.

KEY WORDS: Lung transplant recipients, Systemic HCMV T-cell immunity, Local (lung) HCMV immunity, Intracellular cytoplasmic staining, Enzyme-linked immunospot (ELISpot) assay.

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linked immunospot (ELISpot) assay. Results seem to indicate that full protection (control) against systemic and local HCMV infection is achieved only when both compartments display a measurable T-cell response.

**MATERIALS AND METHODS**

**Patients**

Between 2008 and 2010, 20 HCMV-seropositive patients receiving lung (11 double-lung, and 9 single-lung) transplantation at the University Hospital, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Policlinico San Matteo, Pavia, Italy, were enrolled in this prospective observational study. Patients were monitored for HCMV infection both virologically and immunologically for at least one year. Inclusion criteria were male and female subjects >18 yrs of age with serological evidence of past HCMV infection in either the organ recipient or donor or both. All patients were submitted to a standard triple immunosuppressive regimen including tacrolimus, mycophenolate mofetil and low dose steroids, and were treated with steroid pulses when an episode of acute rejection was diagnosed. The study was approved by the Institutional Ethics Committee, and informed consent was obtained from each patient.

**Patient grouping**

The 20 patients enrolled between 2008 and 2010 were grouped on the basis of viral load determined in the blood and BAL samples collected closest to the peak of HCMV infection. Viral load values were referred to virological cut-offs previously established on a large series of patients for initiation of preemptive therapy in blood and BAL (Gerna et al., 2007, 2009). Group I, included five patients not reaching the cut-off for preemptive therapy either in blood or BAL; group II, four patients reaching the blood cut-off only; group III, five patients reaching both blood and BAL cut-offs; and group IV, six patients reaching the BAL cut-off only.

**Virological monitoring**

Serostatus determination, HCMV infection and disease definition, as well as guidelines for management of HCMV infection have been previously reported (Gerna et al., 2009). Systemic HCMV infection was diagnosed by determination of DNAemia by real-time PCR (Gerna et al., 2006; Furione et al., 2012) once a week in case of undetectable infection and twice a week in case of active HCMV infection until three months after transplantation, and monthly thereafter. Local HCMV infection was diagnosed by determination of viral DNA in BAL fluid (Gerna et al., 2006). BAL sampling was done at 1, 3, 6, and 12 months after transplant, and whenever clinically indicated. No patient received antiviral prophylaxis, whereas according to cut-offs previously established (Gerna et al., 2007, 2009), pre-emptive antiviral therapy for systemic HCMV infection was initiated when the cutoff of 1-3x10⁵ DNA copies [corresponding to 0.5-1.5x10⁶ International Units (Furione et al., 2012)]/ml blood was reached, or when the DNA level in BAL exceeded 1x10⁵ copies [corresponding to 0.5x10⁶ International Units (Furione et al., 2012)]/ml. Antiviral therapy consisted of i.v. 5 mg/kg/bid ganciclovir (GCV) or p.o. 900 mg/bid valganciclovir (VGCV); in case of GCV toxicity or GCV inefficacy, foscarnet (90 mg/kg bid) was administered i.v.

**Immunological monitoring**

**CFC assay**

HCMV-specific CD4⁺ and CD8⁺ T-cells were measured in PBMC with a previously in-house developed method based on the use of autologous, monocyte-derived, HCMV-infected, immature dendritic cells (DC), as a stimulus (Lozza et al., 2005). The frequency of HCMV-specific CD4⁺ and CD8⁺ T-cells producing interferon-γ (IFN-γ⁺) was determined by CFC (Waldrop et al., 1997; Lozza et al., 2005). Levels of HCMV-specific IFN-γ⁺ CD4⁺ and CD8⁺ T-cells greater than 0.4 cells/μl blood were considered protective (Gerna et al., 2006, 2011). Immunological monitoring was performed monthly for the first six months post-transplantation, then every three months until one year of follow-up.

**ELISpot assay**

The HCMV-specific T cell response in PBMC from 20 HCMV-seropositive and nine HCMV-seronegative healthy controls and five subjects with interstitial lung pathology as well as in lung mononuclear cells (LMC) from 20 HCMV-seropositive LTRs (and one HCMV-seronegative
control LTR) was determined by ELISpot after separation of LMC from BAL by depleting lung macrophages with a two-step plastic adhesion procedure. The ELISpot assay consisted of overnight coating in triplicate of 96-well microtiter plates with anti-human IFN-γ mAb (Thermo Scientific, Rockford, IL, USA), followed by addition of 2x10^5 PBMC/well or 1x10^5 LMC/well for 16h in the presence of HCMV-infected or non-infected (control) cell lysate (Microbix Biosystems, Mississauga, Ontario, Canada, 50 μg/ml) or PHA (15 μg/ml). Plates were then incubated with mouse biotinylated anti-IFN-γ mAb (Thermo Scientific) for 2h at 37°C, and HRP-streptavidin at RT for 30 min, prior to addition of aminoethylcarbazole substrate for 10-15 min. The final spot count was obtained by subtracting the number of spot forming cells (SFC) detected with the control antigen from the number of SFC observed with the HCMV-infected cell lysate. An HCMV-specific ELISpot response, in the presence of more than five SFC accounting for at least 7.5% of the maximal (PHA-stimulated) response, was considered a specific positive response.

**Statistical analysis**

ROC analysis was done to assess the performance of the T-cell response on the prediction of spontaneous control of HCMV infection. The Spearman correlation was calculated between the HCMV-specific T-cell response in PBMC as determined by CFC (after stimulation with infected-DC) and ELISpot (after stimulation with infected cell lysate), as well as between HCMV-specific T-cell responses in PBMC and LMC.

**RESULTS**

**Controls for the ELISpot assay**

All HCMV-seronegative subjects showed fewer than five HCMV-specific SFC/2x10^5 PBMC by the ELISpot assay (Figure 1A), whereas all HCMV-seropositive subjects showed values above this level.

In HCMV-seropositive subjects, the number of HCMV-specific SFC in response to infected cell lysate correlated weakly with the count/μl (P=0.03, r=0.54) and the percent (P<0.01, r=0.63) of HCMV-specific CD4+, but not CD8+ T-cells. The ELISpot assay was also performed on three HCMV-seropositive and two HCMV-seronegative immunocompetent subjects who underwent BAL as a helpful diagnostic procedure in the suspicion of an interstitial lung disease.

As reported in Figure 1B, no HCMV-specific T-cells were found in either blood or BAL of the two HCMV-seronegative subjects, or in BAL of the three HCMV-seropositive subjects, who showed varying levels of HCMV-specific SFC in PBMC.

![Figure 1](image-url) - (A, B) ELISpot assay on (A) peripheral blood mononuclear cells (PBMC) from 20 HCMV-seropositive and 9 HCMV-seronegative healthy control subjects, and (B) on PBMC or lung (LMC) from 3 HCMV-seropositive and 2 HCMV-seronegative immunocompetent subjects with suspected lung interstitial pathology. Dotted horizontal lines indicate the cut-offs for ELISpot HCMV-specific T-cell positivity in either blood (PBMC) or lung (LMC).
Groups of LTRs examined
The 20 HCMV-seropositive LTRs examined were divided into the following four groups, according to the magnitude of HCMV load in blood and lung (BAL) (Table 1).

Group I included patients #1 to #5, who had viral load levels lower than either blood or BAL cut-off and resolved the infection spontaneously, without requiring antiviral intervention. All these five patients showed both a systemic and local T-cell response.

Group II included patients #6 to #9, who had viral load levels in blood greater than the established cut-off and received pre-emptive antiviral treatment to control HCMV infection, whereas levels in BAL were below the cut-off. In three of these four patients, the lack of HCMV-specific CD4+ T-cells was associated with a lack of control of systemic infection (in patient #9, CD4+ and CD8+ could not be measured). In patients #8 and #9 a high number (119 and 484) of HCMV-specific SFC in lung appeared to be associated with control of local infection, while in patients #6 and #7 lung infection did not reach high viral load levels even in the absence of a detectable local T-cell response (which, however, was detected slightly later).

In group III (LTRs #10 to #14), both blood and lung DNA levels exceeded the relevant cut-offs, thus requiring antiviral treatment. Of these patients, one (#13) lacked both HCMV-specific CD4+ and CD8+ T-cells in blood, three (#10, #12 and #14) showed only HCMV-specific CD8+ T-cells in blood (in the absence of specific CD4+ T-cells), whereas patient #11 developed infection with a high HCMV load in blood and BAL (associated

TABLE 1 - Viral and immunologic findings in 20 LTRs at the time of peak of HCMV infection.

<table>
<thead>
<tr>
<th>Patient no. (group no.)</th>
<th>Peak HCMV DNA copies/ml*</th>
<th>HCMV-spec. T-cells/µl blood**</th>
<th>Outcome of HCMV infection</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Days after transplant</td>
<td>BAL</td>
<td>Blood</td>
</tr>
<tr>
<td>1 (gr. I)</td>
<td>82</td>
<td>200</td>
<td>neg</td>
</tr>
<tr>
<td>2</td>
<td>205</td>
<td>700</td>
<td>neg</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
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<td>2,000</td>
</tr>
<tr>
<td>4</td>
<td>94</td>
<td>11,250</td>
<td>neg</td>
</tr>
<tr>
<td>5</td>
<td>245</td>
<td>16,850</td>
<td>600</td>
</tr>
<tr>
<td>6 (gr. II)</td>
<td>28</td>
<td>37,700</td>
<td>305,600</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>19,300</td>
<td>285,000</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>2,000</td>
<td>275,000</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>5,150</td>
<td>346,200</td>
</tr>
<tr>
<td>10 I (gr. III)</td>
<td>30</td>
<td>199,700</td>
<td>636,100</td>
</tr>
<tr>
<td>II</td>
<td>86</td>
<td>97,300</td>
<td>259,000</td>
</tr>
<tr>
<td>11***</td>
<td>89</td>
<td>1,489,000</td>
<td>684,000</td>
</tr>
<tr>
<td>12</td>
<td>62</td>
<td>119,000</td>
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<td>13</td>
<td>41</td>
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</tr>
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<td>14</td>
<td>50</td>
<td>1,740,600</td>
<td>232,000</td>
</tr>
<tr>
<td>15 (gr. IV)</td>
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<tr>
<td>18 I</td>
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</tr>
<tr>
<td>20</td>
<td>62</td>
<td>1,073,100</td>
<td>14,000</td>
</tr>
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</table>

SFC, spot forming cells; LMC lung mononuclear cells. *Cut-offs were 1-3x10⁵ DNA copies/ml blood, and 1x10⁵ copies/ml BAL. **In bold: values exceeding immunological cutoffs (HCMV-specific T cells: 5 SFC in BAL by ELISpot, and 0.4 CD4⁺ and CD8⁺ T-cells/µl blood by cytokine flow cytometry). ***Acute rejection.
with radiologic findings of interstitial pneumo-
nia), notwithstanding the presence of both
HCMV-specific CD4+ and CD8+ T-cells (however,
this patient was receiving steroid treatment for
acute rejection). No SFC cut-off was reached in
LMC from any of these patients.
Finally, group IV included six patients (#15 to
#20), who developed high levels of HCMV infec-
tion (requiring treatment) in lungs only. A local
ELISpot-positive response was not detected in
LMC from any of these patients. A specific CD4+
T-cell response was detected in PBMC from one
patient (#19), whereas specific CD8+ T-cells were
detected in PBMC from five out of six patients, ex-
cept for patient #17.

Peripheral blood T-cell response and control
of systemic HCMV infection
The possibility of predicting spontaneous control
of systemic and lung HCMV infection by HCMV-
specific T-cell response determination was eval-
uated. For this purpose, patients examined prior
to (pre-inf) or during (inf) the development of
HCMV infection and reaching the cut-off for pre-
emptive therapy were considered “non-protect-
ed”, whereas patients examined after antiviral
treatment concomitantly with the appearance of
the T-cell response, in the absence of relapse
episodes (post-inf) or developing self-resolving
HCMV infection (no inf) were considered “pro-
tected”.
As shown in Figure 2A, the number of HCMV-
specific CD4+ T-cells above the cut-off was strong-
ly associated with the capacity to control systemic
HCMV infection (specificity 82%, PPV 95%). The
only samples showing HCMV-specific CD4+ T-
cells in “non-protected” patients were relevant to
patient #11 who developed HCMV infection,
while receiving high dosage steroid treatment for
acute rejection. On the other hand, the number of
specific CD8+ T-cells was less predictive of spon-
taneous control of systemic HCMV infection
(specificity 40%, PPV 90%) (Figure 2B).

Lung T-cell response and control of local
HCMV infection
The determination of HCMV-specific immune re-
sponse in lungs by ELISpot displayed a slightly
higher discriminatory capacity (AUC 0.78) as
compared to systemic immunity, and more ac-
curately predicted spontaneous control of local
HCMV infection (specificity 81%, PPV 94%, Figure 2E). However, NPV was lower (44%). This
means that the probability that a patient with a
positive local response is able to spontaneously
control the infection is greater than the risk of
developing a high level of infection in the absence
of an immune response.
Among the parameters of specific immune re-
sponse evaluated in peripheral blood, detection
of HCMV-specific CD4+ T-cells was slightly more
discriminatory in its capacity to control HCMV
infection in lungs than detection of CD8+ T-cells
(AUC 0.74 vs 0.66) (Figure 2C,D).

Correlation of lung and peripheral blood
T-cell responses to HCMV infection
The HCMV-specific T-cell response in lungs as
determined by ELISpot showed a fairly good cor-
relation with the number of HCMV-specific CD4+
T-cells in peripheral blood (P<0.001, r=0.70,
Figure 3A). On the other hand, the correlation of
local specific T-cell response with HCMV-specific
CD8+ T-cells was negligible (P=0.05, r=0.32,
Figure 3B).

Follow-up of LTRs with different patterns
of systemic and local T-cell response
The presence of both systemic T-cell immunity
in PBMC, and local T-cell immunity in LMC of
patient #2 (group I) was associated with a lack of
HCMV infection in both compartments (Figure
4A).
In patient #7 (group II), at onset of infection, the
level of HCMV-specific CD4+ T-cells in peri-
pheral blood below the established cut-off was asso-
ciated with a peak of infection at 30 days after
transplantation (in the presence of a high level of
HCMV-specific CD8+ T-cells), which was treated
with GCV (Figure 4B). Subsequently, concomi-
tantly with the appearance of local T-cell immu-
nity lung infection regressed (abortive infection),
while the development of specific CD4+ T cells in
blood was associated with a lack of systemic
HCMV reactivation.
In patient #14 (group III), the lack of HCMV-spe-
cific CD4+ T-cells in peripheral blood (following
steroid bolus administration) was associated with
systemic infection which was treated with GCV
and then VGCV (Figure 4C). In parallel, the same
antiviral treatment was required by the very high
level of viral load in BAL. Following initial treat-
A. DC-CD4-blood vs protection against systemic infection
- HCMV-spec. CD4+ T cells/μl blood
- non-protected vs. protected
- spec: 82%; PPV: 95%
- sens: 64%; NPV: 30%
- AUC: 0.80

B. DC-CD8-blood vs protection against systemic infection
- HCMV-spec. CD8+ T cells/μl blood
- non-protected vs. protected
- spec: 40%; PPV: 90%
- sens: 93%; NPV: 83%
- AUC: 0.80

C. DC-CD4-blood vs protection against lung infection
- HCMV-spec. CD4+ T cells/μl blood
- non-protected vs. protected
- spec: 85%; PPV: 85%
- sens: 78%; NPV: 22%
- AUC: 0.74

D. DC-CD8-blood vs protection against lung infection
- HCMV-spec. CD8+ T cells/μl blood
- non-protected vs. protected
- spec: 19%; PPV: 95%
- sens: 84%; NPV: 67%
- AUC: 0.66

E. CL-ELISpot-BAL vs protection against lung infection
- HCMV-spec. SFC/1x10^5 LMC
- non-protected vs. protected
- spec: 81%; PPV: 94%
- sens: 71%; NPV: 44%
- AUC: 0.76
ment, the subsequent appearance of HCMV-specific CD4+ T-cells in peripheral blood and T-cell immunity in LMC was not associated with new episodes (either systemic or local) of HCMV reactivation.

In patient #16 (group IV), the lack of local T-cell immunity induced a very high viral load in BAL prior to reaching the viral load cut-off in blood (Figure 4D).

The antiviral treatment controlled viral infection at both sites. Subsequently, while local immunity developed, presence of HCMV-specific CD4+ T-cells (along with CD8+ T-cells) was associated with the absence of new reactivation episodes.

**DISCUSSION**

The results of the present study indicate that:

1) group I patients did not reach either systemic or local viral load cut-off in the presence of both systemic and local T-cell responses;

2) group II patients exceeded viral load in blood (and were treated) in the absence of HCMV-specific CD4+ T-cells; (along with CD8+ T-cells) was associated with the absence of new reactivation episodes.

**FIGURE 2** - (A, B) Peripheral blood T-cell response measured by cytokine flow cytometry (CFC) [using autologous HCMV-infected dendritic cell (DC) stimulation] and control of HCMV systemic infection. The probability of predicting spontaneous control of systemic HCMV infection was evaluated by the Receiver-Operator characteristics (ROC) analysis of non-protected patients (those reaching the cutoff for pre-emptive therapy) vs protected patients (those with no infection or examined post-infection after development of T-cell immunity). The cut-off for the HCMV-specific CD4+ and CD8+ T-cells determined by CFC was 0.4 cells/μl blood. Both the CD4+ (A) and CD8+ (B) CFC assay showed a high discriminatory capacity (AUC 0.90 for both), but the CD4+ CFC assay showed higher specificity than the CD8+ assay in predicting spontaneous control of the infection. (C, D, E) Peripheral blood and lung (BAL) T-cell responses and control of lung HCMV infection. (C and D) As for CD4+ and CD8+ CFC assays on PBMC using infected dendritic cells (DC) as a stimulus, HCMV-specific CD4+ T-cells better correlated with control of HCMV infection in lung than CD8+ T-cells (AUC 0.74 vs 0.66). (E) The determination of HCMV-specific immune responses in lungs by ELISpot using infected cell lysate (CL) as a stimulus showed the highest discriminatory capacity (AUC 0.78) and the best prediction of spontaneous control of local HCMV infection (specificity 81%, PPV 94%). The low NPV (44%) means that the probability for a patient with a positive response to spontaneously control infection is higher than the risk to develop severe infection in the absence of a specific immune response.

**FIGURE 3** - Correlation of lung (BAL) and peripheral blood T-cell response to HCMV. (A) HCMV cell lysate (CL)-ELISpot-BAL: the HCMV-specific T-cell response in lung showed a fair correlation with the number of HCMV-specific dendritic cells (DC)-stimulated-CD4+-blood T-cells (r=0.70, P<0.001), whereas (B) the correlation was weak with the number of HCMV-specific DC-stimulated-CD8+-blood T-cells (r=0.32).
3) group III patients reached both systemic and local viral load cut-offs in the absence of both systemic CD4+ and CD8+ T-cells in peripheral blood prevented systemic infection. In the late post-transplant period, the appearance of HCMV-specific T-cells in BAL prevented lung infection. (B) Systemic HCMV infection. In this single-lung transplanted patient (pt #7), a peak of HCMV infection in blood was associated with lack of HCMV-specific CD4+ T-cells in blood and lack of immune response in BAL. Subsequently, the appearance of specific T-cells in both compartments controlled HCMV infection at both sites. (C) HCMV systemic and lung infection. In this single-lung transplanted patient (pt. #14), a peak of local infection was associated with lack of HCMV-specific T-cells in BAL and CD4+ T-cells in blood. Antiviral treatment controlled the infection, while appearance of HCMV-specific CD4+ T-cells in blood and T-cells in BAL prevented further episodes of HCMV reactivation. (D) HCMV lung infection (pt. #16). Initial lack of specific T-cells in BAL was associated with a peak of BAL infection, while in peripheral blood HCMV-specific CD4+ (although oscillating around the cut-off value) and CD8+ T-cells appeared to be able to control the systemic infection. In BAL, the late appearance of specific T-cells controlled further episodes of HCMV reactivation.

3) group III patients reached both systemic and local viral load cut-offs in the absence of both systemic CD4+ and local T-cell immunity;
4) group IV patients lacked a local T-cell response, thus reaching a very high viral load in BAL prior to developing local immunity.

Taken together, these findings indicate that both HCMV-specific CD4+ and CD8+ T-cells in blood are required to control systemic HCMV infection, whereas in the presence of an HCMV-specific T-cell response in LMC, patients seem to be able to control lung infection. Instead, HCMV-specific CD8+ T cells alone in PBMC do not confer long-term control of HCMV infection.

The documented presence of HCMV-specific CD4+ and CD8+ T-cells in peripheral blood prior to onset or during the development of a systemic HCMV infection indicates protection against progression of the infection towards HCMV disease (Gerna et al., 2006, 2011; Benmarzouk-Hidalgo et al., 2011). This has been shown to occur in LTRs and in other solid-organ transplant recipi-
ents, unless steroid treatment for transplanted organ rejection was administered or only a CD8+ (in the absence of CD4+) specific T-cell response was detected (Gerna et al., 2006, 2011). The sole presence of HCMV-specific CD8+ T cells in PBMC does not seem to confer protection against either systemic or local HCMV infection, as already reported (Gamadia et al., 2003; Gerna et al., 2011). Accordingly, in LTR the predictivity of subsequent HCMV DNAemia episodes by the quantiferon assay (which measures the IFN-γ CD8+ T cell response) was found to be low (AUC of ROC analysis= 0.639) (Weseslindner et al., 2012). With respect to this issue, it should also be considered that the ELISpot assay we adopted for the study of lung T cell responses mainly detects CD4+ T cells (due to LMC stimulation with soluble viral antigen), thus justifying the fair degree of correlation between HCMV-specific CD4+ T cells in the blood and control of local infection. As for local (lung) protection, we observed that detection of a specific T-cell response in LMC correlates with spontaneous control of HCMV lung infection and that, in the presence of HCMV reactivation, specific T cells can expand in the lung for at least one year.

In addition, we found that systemic HCMV-specific CD4+ T-cell immunity was more discriminatory in its capacity to control HCMV infection in lungs than CD8+ T-cells, and that systemic HCMV-specific CD4+ T-cells, correlated with local specific T-cell response, suggesting a possible role for peripheral blood CD4+ T-cells in the development of local immunity. One point of weakness of our study is the use of two different methods for investigating local and systemic T-cell immunity. This was due to the small number of cells often recovered from BAL. However, the association of systemic HCMV-specific T-cell (and namely CD4+) response and lack of HCMV reactivation, and the association of local T-cell response and lack of episodes of local HCMV reactivation seem to indicate that the T-cell response is needed to prevent HCMV reactivation episodes at both sites. A second issue deserving discussion is the relatively small number of patients studied. However, the distribution of 20 patients into four groups according to the relevant viral and immunological findings prompted us to draw the preliminary conclusions reported herein. This will represent the basis for a new prospective study which is starting in collaboration with other transplantation centers. As for timing of blood and BAL sampling, data reported in Table 1 are relevant to both samples collected simultaneously or very close to the peak of HCMV infection. Although different studies have reported an association between high viral load in BAL and parenchymal infection (Westall et al., 2004; Chemaly et al., 2005; Gerna et al., 2009), how the presence of virus in BAL correlates with lung pathology is still not clearly defined. In a recent study, we found that levels >1x10^5 HCMV DNA copies/ml BAL were detected in 100% of samples from LTRs with HCMV pneumonia (lung infection + inflammatory infiltrates), in 25% of samples from patients with HCMV infection in lungs and no inflammatory infiltrates, and only 3% of samples from LTR with no signs of lung infection (Gerna et al., 2009). Whether the T-cell subpopulations responsible for protection against systemic and local HCMV infection are the same or different remains to be defined. However, there are some preliminary indications that they may differ both in phenotype and function (Shlobin et al., 2006; Pipeling et al., 2008). The immune response to HCMV observed in the lung can be due either to the expansion of resident tissue T cells (de Bree et al., 2005; Schaerli and Moser, 2005) or to migration and subsequent expansion of peripheral blood HCMV-specific T-cells expressing homing receptors for the lung as a response to tissue inflammation after viral reactivation (D’Ambrosio et al., 2001).

Future clinical trials should be designed to verify whether episodes of HCMV infection in lungs, irrespective of viral load, should be left untreated, when a specific local T-cell response is present.

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REFERENCES


