Differential Usage of Cellular Niches by Cytomegalovirus versus EBV- and Influenza Virus-Specific CD8+ T Cells

Ester M. M. van Leeuwen, Jasper J. Koning, Ester B. M. Remmerswaal, Debbie van Baarle, René A. W. van Lier and Ineke J. M. ten Berge

*J Immunol* 2006; 177:4998-5005; doi: 10.4049/jimmunol.177.8.4998

http://www.jimmunol.org/content/177/8/4998
Differential Usage of Cellular Niches by Cytomegalovirus versus EBV- and Influenza Virus-Specific CD8+ T Cells

Ester M. M. van Leeuwen,‡ Jasper J. Koning,* Ester B. M. Remmerswaal,‡
Debbie van Baarle,§§ René A. W. van Lier,* and Ineke J. M. ten Berge††

Immunological memory provides long-term protection against reinfection or reactivation of pathogens. Murine memory T cell populations may be compressed following infections with new pathogens. Humans have to retain memory T cells directed against a variety of microbes for many decades. Under these circumstances, the effect of pathogens that mount robust T cell reactivity on the pre-existing memory directed against unrelated microbes is unknown. In this study, we studied peripheral blood memory CD8+ T cells directed against different viruses following primary CMV infection in renal transplant recipients. The entrance of CMV-specific CD8+ T cells expanded the Ag-primed CD8+ T cell compartment rather than competing for space with pre-existing memory T cells specific for persistent or cleared viruses. Neither numbers nor phenotype of EBV- or influenza-specific CD8+ T cells was altered by primary CMV infection. CMV-specific CD8+ T cells accumulated over time, resulting in increased total CD8+ T cell numbers. Additionally, they acquired a highly differentiated cytolytic phenotype that was clearly distinct from EBV- or influenza-reactive T cells. Thus, the human immune system appears to be flexible and is able to expand when encountering CMV. In view of the phenotypic differences between virus-specific T cells, this expansion may take place in cellular niches different from those occupied by EBV- or influenza-specific T cells, thereby preserving immunity to these pathogens. The Journal of Immunology, 2006, 177: 4998–5005.

The development and maintenance of immunological memory are essential for long-term protection against pathogens in case of reinfections. Memory T cells specific for pathogens that are cleared from the host are maintained without further contact with Ag, but do require extrinsic factors for their survival and homeostatic proliferation (1–3). The cytokines IL-7 and IL-15, both members from the common γ-chain family of cytokines, have in this regard been identified as essential factors (4–8). Concerning memory T cells specific for persistent viruses, the requirements for their maintenance appear to be different. A minor role is fulfilled by IL-7, whereas regular contact with the cognate Ag seems to be essential to maintain these memory T cells (9, 10). During life, humans are exposed to a wide variety of pathogens, and consequently a large population of memory T cells with different specificities is formed. With respect to viral infections, particularly virus-specific CD8+ T cells have been studied. Whether CD8+ memory T cells with different viral specificities are maintained as separate populations or, alternatively, influence each other’s presence is still a matter of debate. Several studies in mice have shown that infection with a new virus, and consequent emergence of an additional population of memory cells, results in permanent loss of pre-existing memory T cells against unrelated pathogens (11–13). In this way, the overall size of the memory T cell pool remains relatively constant, and memory T cells have to compete for physical space and/or homeostatic cytokines (14). There appears to be a difference between T cells specific for viruses that are cleared by the host and viruses that persist. The loss of memory T cells against previously encountered pathogens was more profound after persistent infection than after infection with a virus that was cleared from the host, and was a continuously ongoing process (15).

The question whether the development of memory cells to a new infection indeed impairs the pre-existing memory T cell population is of special interest in humans, because during their life span many different Ags are being encountered. However, probably because of the limitations in tools and especially the difficulty in tracking primary infections in humans, no data are available yet on this subject. We had the opportunity to study human primary CMV infection in renal transplant recipients. This allowed us to analyze possible changes in peripheral blood memory CD8+ T cells specific for other viruses upon entrance of CMV-specific T cells in the system. Moreover, in individuals that were CMV seropositive, we analyzed the impact of renal transplantation and start of immunosuppressive therapy on virus-specific memory CD8+ T cells. Besides numbers of virus-specific T cells, we also tested the phenotype of the memory T cells. In this way, we were able to examine the flexibility and adaptations of the human immune system to a new situation.

Materials and Methods

Subjects

Renal transplant recipients were divided in three groups based on CMV serostatus, i.e., CMV seropositive (n = 18; median age, 53; range, 31–64), CMV seronegative (n = 11; median age, 45; range, 17–60), and patients experiencing a primary CMV infection (n = 9; median age, 33; range, 24–67). All patients included in this study were EBV seropositive. From the moment of transplantation, patients were treated with different combinations of immunosuppressive drugs as listed in Table I. Dosages were as
follows: prednisolone (P) (10 mg daily), cyclosporin A (CsA) (at dosages guided by trough levels aimed at 100 ng/ml), mycophenolate mofetil (MMF) (1000 mg, twice daily), tacrolimus (Tac) (at dosages guided by trough levels aimed at 12.5 ng/ml), anti-CD25 treatment (basiliximab) (20 mg on days 0 and 4), and azathioprine (Aza) (2 mg/kg, once daily). No differences were observed between patients on different medication. Because no differences were observed between patients with CMV reactivation (n = 12 as defined by a positive PCR for CMV-viral load in a CMV-seropositive patient) compared with CMV-seropositive patients without CMV reactivation (n = 6), data were pooled. Measurement of EBV viral load was not a standard procedure in our protocol and was only performed on PBMCs of five patients at the same time points used for the analysis, and no EBV was detected in those samples. All patients gave written informed consent, and the study was approved by the local medical ethical committee.

**PBMCs**

Heparinized peripheral blood samples were collected, and PBMCs were isolated using standard density gradient centrifugation techniques. Subsequently, these cells were cryopreserved until the day of analysis. From all patients, PBMCs were used from just before transplantation and 1 year after transplantation. From patients with a primary CMV infection, cells were also studied at a time point just after the peak of the CMV viral load.

**CMV-PCR, EBV-PCR, anti-CMV IgG, and anti-EBV IgG**

Quantitative PCR for CMV was performed in EDTA whole-blood samples. This analysis was done every week for at least 18 wk after transplantation, or as long as the PCR remained positive. Thereafter, the viral load was measured 6, 9, and 12 mo after transplantation. To determine CMV serostatus, anti-CMV IgG was measured in serum using the AxSYM microparticle enzyme immunoassay (Abbott Laboratories) according to the manufacturer’s instructions. Measurements were calibrated relative to a standard serum. Quantitative PCR for EBV was performed on 500,000 PBMCs. The EBV serostatus was investigated by determination of IgG specific for EBV-VCA and EBNA by ELISA (Biotest AG).

**Tetrameric complexes**

The following HLA-peptide tetrameric complexes were obtained from Sanquin: HLA-A2 tetramer loaded with the CMV pp65-derived NLVPMATV peptide, HLA-B7 tetramer loaded with the CMV pp65-derived TPRVTGGGAM peptide, HLA-A2 tetramer loaded with EBV BMLF1-derived GLCTLVAML peptide, HLA-B7 tetramer loaded with EBV

### Table 1. Numbers of patients analyzed and their respective immunosuppressive drugs

<table>
<thead>
<tr>
<th>CMV Seropositive</th>
<th>CMV Seronegative</th>
<th>Primary CMV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/CsA/MMF</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>P/MMF/Tac/CD25</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>P/CsA/MMF/CD25</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>P/Aza</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>P/CsA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>P/MMF/Tac</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations used in this paper: P, prednisolone; CsA, cyclosporin A; MMF, mycophenolate mofetil; Tac, tacrolimus; Aza, azathioprine; FLU, influenza; RSV, respiratory syncytial virus.*
EBNA3A-derived RPPIFIRRL peptide, HLA-B8 tetramer loaded with EBV EBNA3A-derived FLRGRAYGL peptide, HLA-B8 tetramer loaded with EBV BZLF1-derived RAKFKQLL peptide, HLA-A2 tetramer loaded with FLU M1-derived GILGFVFTL peptide, HLA-A1 tetramer loaded with RSV M-derived YLEKESIYY peptide, and HLA-B7 tetramer loaded with RSV NL-derived peptide NPKASLLSL. HLA-A1 tetramer loaded with FLU NP-derived CTELKLSDY peptide was obtained from Proimmune. All tetramers used were allophycocyanin conjugated. In the text, the different tetramers will be named after the virus, the HLA type, and the first three amino acids of the peptide sequence, e.g., CMV A2 NLV tetramer. EBV-specific tetramers are directed against latent epitopes (EBV B7 RPP and EBV B8 FLR, referred to as EBV-latent) or against lytic epitopes (EBV B8 RAK, referred to as EBV-lytic).

Immunofluorescent staining and flow cytometry

PBMCs were washed in PBS containing 0.01% (w/v) NaN₃ and 0.5% (w/v) BSA. A total of 500,000 PBMCs was incubated with an appropriate concentration of tetrameric complexes in a small volume for 30 min at 4°C, protected from light. Fluorescent-labeled mAbs (concentrations according to manufacturer’s instructions) were then added and incubated for 30 min at 4°C, protected from light. For analysis of expression of surface markers, the following Abs were used in different combinations: CD127 (IL-7Rα)-PE, CD27-PE, and CD8-PerCP-Cy5.5 (all BD Biosciences). Cells were washed and analyzed using a FACSCalibur flow cytometer and CellQuest software (BD Biosciences).

Statistical analysis

The two-tailed Mann-Whitney U test was used for analysis of differences between groups. The Wilcoxon matched pairs test was used for analysis of differences within groups. Values of $p < 0.05$ were considered statistically significant.

Results

Transient drop in memory $T$ cell numbers during primary CMV infection

We aimed to study the effect of the appearance of CMV-specific CD8⁺ T cells after primary CMV infection on pre-existing memory CD8⁺ T cells. Therefore, we analyzed peripheral blood frequencies and absolute numbers of EBV-, influenza (FLU)-, and respiratory syncytial virus (RSV)-specific CD8⁺ T cells before infection with CMV, at the peak of the CMV viral load, and after 1 year. We studied nine renal transplant recipients experiencing a primary CMV infection caused by transplantation of a kidney derived from a CMV-seropositive donor in a CMV-seronegative recipient. The first positive CMV PCR was usually detected a few weeks after transplantation (median, 4; range, 4–9) and remained positive for a period of 15 wk (median) (range, 7–48). The maximum height of the CMV viral load PCR ranged from 3,400 to 430,000 copies/ml with a median value of 73,000; no correlation was present between the height of the viral load and the number of weeks that the PCR remained positive.

CMV-specific CD8⁺ T cells could obviously not be found before infection, but were present in high frequencies within CD8⁺ T cells at the peak of infection and the percentage declined thereafter (Fig. 1, A and B, upper panels). Interestingly, the absolute number of CMV-specific CD8⁺ T cells in peripheral blood did not diminish after the viral load became undetectable and the virus had reached its latency stage (Fig. 1C,
upper panel). CD8⁺ T cells specific for latent and lytic epitopes of EBV and FLU decreased in percentage as soon as CMV-specific CD8⁺ T cells appeared, and this percentage remained unchanged after 1 year (Fig. 1, A and B). In contrast, absolute numbers of EBV- and FLU-specific CD8⁺ T cells showed a temporary drop during the acute phase of infection, but recovered thereafter, because 1 year later similar numbers could be found as before CMV infection (Fig. 1C). Because working in humans limits analysis to the peripheral blood compartment, we have to take into consideration that the effect of distribution of virus-specific cells between blood, the secondary lymphoid organs, and other tissues is unknown.

In patients that remained CMV seronegative during the course of transplantation, the percentage of CD8⁺ T cells specific for the persistent EBV increased in most of the patients (5 of 6 and 7 of 11 for EBV-latent and -lytic epitopes respectively; NS), whereas absolute numbers remained unchanged (Fig. 2). In these patients, percentages and absolute numbers of FLU-specific CD8⁺ T cells did not alter (Fig. 2, lower panels).

**FIGURE 3.** Numbers of CMV-specific cells increase 1 year after transplantation. A, Dot plots of two representative CMV-seropositive patients showing the increase of CMV-specific CD8⁺ T cells and numbers of FLU- and RSV-specific CD8⁺ T cells 1 year after transplantation in one patient and the EBV-specific CD8⁺ T cells in another patient. Numbers indicate the percentage of virus-specific cells within CD8⁺ T cells. B, Graphs show the changes in frequencies of CMV-, EBV-latent, EBV-lytic, FLU- and RSV-specific CD8⁺ T cells from CMV-seropositive renal transplant patients before and 1 year after transplantation. Data of individual patients are shown with distinct symbols, and the same symbol is used throughout for the same patient. C, Similar in absolute numbers. *, Significant difference, p < 0.05.
We also studied virus-specific cells in patients that were already CMV seropositive before transplantation. Twelve of the 18 patients studied experienced a CMV reactivation, indicated by a positive PCR for CMV viral load during the first year after transplantation. The maximum PCR value varied between 400 and 600,000 copies/ml with a median value of 7,750 copies/ml. The first positive PCR was usually detected early after transplantation (median, 7 wk; range, 4–27) and CMV reactivation lasted for a few weeks (median, 5 wk; range, 1–38). Because we did not find differences in the data we collected between patients that did or did not have a CMV reactivation, we pooled all data from the CMV-seropositive patients.

In CMV-seropositive patients, CMV-specific CD8+ T cells increased both in percentages and in absolute numbers during the course of transplantation (Fig. 3, upper panels). The percentages of EBV- and FLU-specific cells within CD8+ T cells decreased (Fig. 3, A and B). This was not significant for EBV-latent CD8+ T cells, but only 2 of 12 patients did not show this decrease in percentage. Absolute numbers of EBV- and FLU-specific CD8+ T cells showed some changes in individuals, but overall no alterations were observed (Fig. 3C).

**Total CD8+ T cell percentages and absolute numbers increase as a result of CMV infection**

The inconsistency between the changes in percentages and absolute numbers of virus-specific cells prompted us to analyze the total CD8+ T cell population in peripheral blood. Fig. 4 shows that, in patients experiencing a primary CMV infection, both percentages and absolute numbers of CD8+ T cells increased significantly. The same accumulation of CD8+ T cells was seen during the course of transplantation in CMV-seropositive, but not in CMV-seronegative individuals. Before and 1 year after transplantation, CMV-seropositive individuals had more CD8+ T cells than CMV-seronegative patients, whereas patients with a primary CMV infection resembled CMV-seronegative patients before, and CMV-seropositive patients after transplantation. Together with the data in Figs. 1 and 3, this showed that primary infection with CMV and the consequent increase in CMV-specific CD8+ T cells induced an expansion of the memory CD8+ T cell pool during the latency phase.

**FIGURE 4.** Frequencies and absolute numbers of total CD8+ T cell population are affected by CMV seropositivity. A and B, Frequencies (A) and absolute numbers (B) of total CD8+ T cells are shown in the three groups of patients before and 1 year after transplantation. *, Significant difference, p < 0.05.

**Only CMV-specific CD8+ T cells acquire a more differentiated phenotype, which is reflected in the total CD8+ T cell population**

Phenotypic markers can be used to characterize CD8+ T cells and determine their differentiation status. It has been described that human memory CD8+ T cells have a preferential phenotype depending on the virus they recognize (16, 17). Loss of expression of the costimulatory molecule CD27 or the chemokine receptor CCR7 is regarded as a differentiation event. Re-expression of CD45RA is only seen in cells that have progressed very far along the differentiation pathway. Expression of the cytokine receptor IL-7Ra has been described to be different between CD8+ T cells that recognize viruses that are cleared from the host compared with cells specific for persistent viruses (9). This suggests that expression remains low when cells regularly encounter their Ag, but, like loss of expression of CD27 and CCR7, it can also be regarded as a differentiation event.

We analyzed the phenotype of the various virus-specific CD8+ T cells before and after transplantation in patients experiencing a primary CMV infection (Fig. 5A) and in patients that were already CMV seropositive before transplantation (Fig. 5, B and C). When CMV-specific CD8+ T cells are first detectable during primary CMV infection, they exhibit a CD27−IL-7Ra−CD45RA− phenotype (Fig. 5A, upper panels; data not shown and Ref. 9). After the peak of the CMV infection, a low percentage of CMV-specific CD8+ T cells re-expressed IL-7Ra, whereas a considerable percentage lost expression of CD27, indicating differentiation of the cells (Fig. 5A). Consistent with the differentiation of these cells, in all patients CD45RA was re-expressed on the large majority of CMV-specific CD8+ T cells (data not shown). EBV-specific CD8+ T cells recognizing latent or lytic epitopes did not change their phenotype as a consequence of the primary CMV infection (Fig. 5A, lower two panels). Most cells remained CD27−, and expression of IL-7Ra was heterogeneous and showed some variations within individuals but was overall not altered after primary CMV infection. In patients that were already CMV seropositive, the most apparent change in phenotype was seen in the CMV-specific CD8+ T cells. Before transplantation, most of these cells already had a CD27−IL-7Ra− phenotype as has been described before (16, 18). One year later, expression of both CD27 and IL-7Ra was even significantly lower, and more cells re-expressed CD45RA, whereas they all remained CCR7− (Fig. 5, B and C, upper panels, and data not shown).
shown). This indicates that the CMV-specific CD8⁺ T cells have progressed further along the differentiation pathway (16, 17). In general, EBV-specific CD8⁺ T cells did not clearly change their phenotype and remained mostly CD27⁺IL-7Rα⁺CCR7⁻CD45RA⁻ (Fig. 5, B and C, lower panels, and data not shown). The expression of CD27 slightly, but significantly, decreased on EBV-specific T cells recognizing latent epitopes, although most cells still were CD27⁺. In addition, the expression of IL-7Rα on CD8⁺ T cells specific for lytic EBV epitopes increased in 8 of 11 patients, but this did not reach statistical significance.

Like the increase in CMV-specific CD8⁺ T cells was reflected in the total CD8⁺ T cell population, a similar observation was made for the phenotypic changes (Fig. 6). In CMV-seropositive individuals, the expression of CD27 and IL-7Rα
decreased, whereas expression of CD45RA increased on total CD8<sup>+</sup> T cells in peripheral blood. That this was an effect depending on CMV-infection was confirmed by the absence of phenotypic changes of CD8<sup>+</sup> T cells in CMV-seronegative individuals, whereas primary CMV infection dramatically changed the composition of the CD8<sup>+</sup> T cell pool. The expression of CD27 and IL-7Rα on the CD8<sup>+</sup> T cells decreased significantly after primary CMV infection, whereby these cells showed differentiation toward effector-type cells. The difference between CMV-seropositive and CMV-seronegative patients concerning the phenotype of CD8<sup>+</sup> T cells that was already present before transplantation became even more pronounced after 1 year (Fig. 6).

**Discussion**

In this study, we had the opportunity to investigate whether in humans infection with a new virus would have an impact on the virus-specific memory T cells already present. We determined that absolute numbers of these pre-existing memory T cells in peripheral blood were not affected by the appearance of CD8<sup>+</sup> T cells specific for the new CMV infection. The fact that percentages decreased is explained by the increase in total CD8<sup>+</sup> T cells after infection with CMV. We thus confirm the finding by others that CMV infection leads to decreased percentages of EBV-specific cells in elderly individuals (19). However, the conclusion cannot be that EBV-specific CD8<sup>+</sup> T cells diminished in numbers. Therefore, it is unlikely that infection with CMV would impair EBV-specific T cell responses. Our findings do not corroborate the data retrieved from mice studies where competition between CMV- and EBV-specific CD8<sup>+</sup> T cells was shown (11–14), especially because CMV will reside in endothelial cells and monocytes, the peripheral blood is not so strictly regulated that the entrance of new cells leads to the disappearance of others. Instead, the human immune system is flexible and adapts to a subsequent infection by allowing an increase in the CD8<sup>+</sup> T cell pool to make space for the newly generated virus-specific memory cells.

It would be interesting to know whether expansion of the CD8<sup>+</sup> T cell pool also occurs upon infection with other viruses, especially viruses that are cleared from the host such as influenza, but this is difficult to assess in humans. On one hand, it is feasible that the immune system would respond in a similar way to different viral infections as the clonal expansion of virus-specific CD8<sup>+</sup> T cells is common in all antiviral immune responses. In contrast, it has been described that, after clearance of a virus, 90–95% of the effectors die and only a very small population survives to become memory T cells (20). However, the data in Fig. 1C show that absolute numbers of CMV-specific CD8<sup>+</sup> T cells in peripheral blood do not decline after the acute phase of infection. It is known from previous studies that CMV-specific CD8<sup>+</sup> T cells are highly activated during the acute phase of primary infection and become resting during latency. Moreover, they gradually change toward a more differentiated phenotype (21). Nevertheless, the number of CMV-specific CD8<sup>+</sup> T cells apparently remains very similar. We have to note here that our analysis was limited to the peripheral blood compartment, which means that we have no data on numbers or phenotype of virus-specific CD8<sup>+</sup> T cells in secondary lymphoid organs and tissues of these patients. However, because during latency CMV resides in endothelial cells and monocytes, the peripheral blood is probably an appropriate place to investigate CMV-specific T cells. Moreover, we recently published that CMV- and EBV-specific CD8<sup>+</sup> T cells did not differ in frequency (or phenotype) between paired samples of peripheral blood and lung tissue, suggesting a relatively equal distribution in at least tissues and blood (22).

In this study, we analyzed samples from renal transplant recipients that immunologically differ from healthy individuals in that they take immunosuppressive medication. How could this affect our results? It has been described before that immunosuppressed
individuals have higher percentages of CMV-specific cells and that these cells have a more differentiated phenotype (9, 18, 23). We believe that this can be explained by more frequent reactivations of CMV because of insufficient control by the immune system during the latency stage. Besides the reactivations where we can detect the viral load, this might also occur at a low level below the detection threshold of the PCR. In the patients with a primary CMV infection, the effect might be more clear or faster than in healthy individuals because of the higher viral load in patients. But in general we predict that the immune system will behave in a similar way in healthy individuals it might just be more pronounced in the individuals we studied. The fact that we studied transplant recipients even emphasizes that the immune system is flexible and can adapt to new situations, even when it is suppressed by medication.

The increase in total CD8+ T cells and the concurrent change in the phenotype of the CD8+ T cell population are only seen in CMV-seropositive individuals. The large impact of CMV infection on the composition of the total CD8+ T cell pool has been described before, as CMV-seropositive individuals have more differentiated CD8+ T cells (24). The question remained whether all these CMV-specific cells or whether CMV infection by itself would also have an effect on CD8+ T cells with other specificities. Based on several findings from our current study and in literature, we favor the notion that the changes observed are due to an increase in CMV-specific cells. First, the changes seen in CMV-seropositive individuals during transplantation are similar to the alterations after primary CMV infection when CMV-specific CD8+ T cells enter the system. Second, in peripheral blood, only CMV-specific cells have this far differentiated CD27- IL-7Rα-CD45RA+ phenotype (9, 16, 25, 26). Third, the increase in number and the changes in phenotype of CMV-specific CD8+ T cells were already substantial in our analysis even though we only measured T cells recognizing a single epitope from one protein of CMV. This implies that the total response to CMV is much larger, also considering the broad immune response to CMV that has recently been described (27). Fourth, we observed no changes in CD8+ T cells recognizing other viruses upon primary CMV-infection or in CMV-seropositive individuals during the first year after transplantation. Lastly, it has been shown before that the higher the percentage of CMV-specific CD8+ T cells, the more these cells have a CD27- phenotype (23).

Why especially CMV infection has such a large impact on the total T cell pool remains unknown, but factors that are likely to contribute are the large reservoir of CMV during latency, the persistent nature of the virus, and its capacity to evade the immune system in several ways (28, 29). These are all reasons that could explain why the immune system has evolved in such a prominent way to control CMV infection.

Acknowledgments

We thank Gijs van Schijndel (Department of Viro-Immunology, Sanquin Research at Central Laboratory for Bloodtransfusion, Amsterdam, The Netherlands) for the generation of tetramer complexes, and the technicians from the Department of Clinical Virology (Academic Medical Center, Amsterdam, The Netherlands) for performing CMV PCRs and CMV and EBV serology.

Disclosures

The authors have no financial conflict of interest.

References


The Journal of Immunology

5005

infection, the effect might be more clear or faster than in healthy indi-

viduals because of the higher viral load in patients. But in general we predict that the immune system will behave in a similar way in healthy individuals it might just be more pronounced in the individuals we studied. The fact that we studied transplant recipients even emphasizes that the immune system is flexible and can adapt to new situations, even when it is suppressed by medication.

The increase in total CD8+ T cells and the concurrent change in the phenotype of the CD8+ T cell population are only seen in CMV-seropositive individuals. The large impact of CMV infection on the composition of the total CD8+ T cell pool has been described before, as CMV-seropositive individuals have more differentiated CD8+ T cells (24). The question remained whether all these CMV-specific cells or whether CMV infection by itself would also have an effect on CD8+ T cells with other specificities. Based on several findings from our current study and in literature, we favor the notion that the changes observed are due to an increase in CMV-specific cells. First, the changes seen in CMV-seropositive individuals during transplantation are similar to the alterations after primary CMV infection when CMV-specific CD8+ T cells enter the system. Second, in peripheral blood, only CMV-specific cells have this far differentiated CD27- IL-7Rα-CD45RA+ phenotype (9, 16, 25, 26). Third, the increase in number and the changes in phenotype of CMV-specific CD8+ T cells were already substantial in our analysis even though we only measured T cells recognizing a single epitope from one protein of CMV. This implies that the total response to CMV is much larger, also considering the broad immune response to CMV that has recently been described (27). Fourth, we observed no changes in CD8+ T cells recognizing other viruses upon primary CMV-infection or in CMV-seropositive individuals during the first year after transplantation. Lastly, it has been shown before that the higher the percentage of CMV-specific CD8+ T cells, the more these cells have a CD27- phenotype (23).

Why especially CMV infection has such a large impact on the total T cell pool remains unknown, but factors that are likely to contribute are the large reservoir of CMV during latency, the persistent nature of the virus, and its capacity to evade the immune system in several ways (28, 29). These are all reasons that could explain why the immune system has evolved in such a prominent way to control CMV infection.

Acknowledgments

We thank Gijs van Schijndel (Department of Viro-Immunology, Sanquin Research at Central Laboratory for Bloodtransfusion, Amsterdam, The Netherlands) for the generation of tetramer complexes, and the technicians from the Department of Clinical Virology (Academic Medical Center, Amsterdam, The Netherlands) for performing CMV PCRs and CMV and EBV serology.

Disclosures

The authors have no financial conflict of interest.

References


The Journal of Immunology

5005