Cytomegalovirus-specific T-cell dynamics in HIV infection
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Chapter 2

Dynamics of CMV-specific T cells in HIV-1-infected individuals progressing to AIDS with CMV end-organ disease

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asymptomatics with more than 7 years of asymptomatic follow-up and CD4 counts $>400/\mu l$ (classification of the Centres for Disease Control 1993). None of the individuals received anti-(retro)viral therapy during the study period. For the AIDS-CMV group, time points were studied from just after HIV seroconversion or study entry up to CMV diagnosis. The earliest time point studied in the AIDS-OI and the long-term asymptomatics groups was between 1 and 2 years after HIV seroconversion. The latest time point studied for AIDS-OI was on average 5 years (around the time of AIDS diagnosis) and for long-term asymptomatics 10 years after HIV seroconversion. Characteristics of the study participants are summarised in Table 1.

**HLA-class I tetramer staining in parallel with intracellular IFNγ staining after antigen-specific stimulation**

It has been well characterised that the immune response to CMV in HLA-A2 individuals is dominated by the NLVPMVATV (NLV) epitope of the pp65 lower matrix protein [11,12]. HLA-A2 NLVPMVATV peptide-tetrameric complexes were produced, and four-colour fluorescence analysis was performed as described previously [13]. Briefly, PBMC were stained with HLA-A2-NLV tetramers. After fixation and permeabilisation (Becton Dickinson reagents, hereafter BD, San José, California, USA), cells were stained (intracellularly) with fluorochrome-conjugated monoclonal antibodies (mAb) against CD8, perforin (BD) and granzyme B (Sanquin Reagents, Amsterdam, the Netherlands). In parallel, PBMC were stimulated either with 10 μg/ml of NLVPMVATV peptide, or with CMV lysate (BioWhittaker, Walkersville, USA; 60 μl/ml or Microbix Biosystems, Toronto, Canada; 10 μl/ml) in the presence of 2 μg/ml anti-CD28 (Sanquin Reagents) and CD49d (BD) mAb at 37°C for 6 hours. After 1.5 hours, 5% (w/v) monensin was added [13]. As a positive control for the capacity of PBMC to produce IFNγ, phorbol myristate acetate (PMA) and ionomycin (Sigma-Aldrich, Zwijndrecht, the Netherlands; 5 ng/ml and 1 μg/ml respectively) were added. Unstimulated cells were used as a negative control. Cells were placed at 4°C overnight, after which they were stained as described above with mAb against CD8, and IFNγ (BD), or CD4, CD3 (BD), and IFNγ. Cells were fixed in Cellfix (BD), and up to 400,000 events acquired using a FACS Calibur flow cytometer (BD). Lymphocytes were gated by forward-sideward scatter, and data were analysed using the software programme CELL Quest 3.3 (BD). In case of IFNγ measurements, the number of responding T cells was calculated after subtracting negative control values. Data are expressed in absolute numbers per μl blood, to take into account that during HIV infection and progression to AIDS, total CD4$^+$ T-cell numbers drop dramatically.

**Viral load determination**

CMV viral load was determined in PBMC with the COBAS AMPLICOR™ CMV MONITOR KIT (Roche Diagnostics, Almere, the Netherlands) as described previously [14]. In short, PBMC in lysis buffer were added to the lysis reagent that
Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Subject</th>
<th>Classification</th>
<th>AIDS defining illness</th>
<th>HIV status</th>
<th>Months AIDsfree</th>
<th>CD4 count AIDSf</th>
<th>Months from AIDS to CMV</th>
<th>CD4 count CMVo</th>
<th>Months from AIDS to Death</th>
<th>Age at AIDS diagnosis</th>
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<tr>
<td>8001</td>
<td>CMV disease</td>
<td>CMV disease, Candidiasis, Kaposi sarcoma</td>
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<td>20</td>
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<td>CMV disease</td>
<td>SC</td>
<td>84</td>
<td>50</td>
<td>-</td>
<td>50</td>
<td>9</td>
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<td>Toxoplasmosis</td>
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<td>SC</td>
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<td>-</td>
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<td>SC</td>
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<td>29.5</td>
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<td>SC</td>
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<td>390</td>
<td>-</td>
<td>-</td>
<td>15.5</td>
<td>36</td>
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<tr>
<td>1215(^7)</td>
<td>Progressor</td>
<td>Candidiasis</td>
<td>SC</td>
<td>71</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>23</td>
<td>28</td>
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</tbody>
</table>

1 CMV = cytomegalovirus; CMV disease = CMV other end-organ disease than retinitis; LTA = long-term asymptomatic.
2 PCP = Pneumocystis carinii pneumonia.
3 SC = seroconversion; SP = seroprevalence; these individuals have seroconverted approximately between 0.5 to 1.5 years before study entry.
4 CD4 counts at time of AIDS diagnosis.
5 CD4 counts at time of CMV diagnosis.
6 n.a. = not available.
7 HLA-B7-expressing individual.
Chapter 2

contained a quantitation standard. DNA was precipitated with isopropanol, and pelleted by centrifugation. The pellet was washed, and resuspended in specimen diluent. The extracted sample was added to PCR Master Mix and placed in the COBAS AMPLICOR instrument for automated amplification, detection and quantification of CMV DNA. The detection limit is 100 copies. Copy numbers between 1 and 100 are considered to be positive, but cannot be quantitated.

Statistical analysis
Wilcoxon signed rank tests were performed to compare values at early and late time points, within patient groups. Differences between the patient groups were analysed using the Mann-Whitney test. Correlations were tested using the Spearman's non-parametric correlation test. All statistical analyses were performed using the software programme SPSS 10.00 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Numbers of CMV-specific CD8+ T cells increase over time prior to onset of CMV-EOD
HLA-A2-NLV tetramers were used to determine CMV-specific CD8+ T-cell numbers. Figure 1 shows the results of all time points in the AIDS-CMV group. To be able to analyse the data, early time points as defined as the earliest time point after HIV seroconversion or study entry, and late time points, on average 10 years after HIV seroconversion for long-term asymptomatics, 5 years after HIV seroconversion for progressors to AIDS-OI, and time points between 5 and 11 months before CMV end-organ disease for progressors to AIDS-CMV were analysed (Figure 2).

In long-term asymptomatics (Figure 2a), numbers of A2-NLV-tetramer+CD8+ T cells were readily detected, which increased significantly during follow-up (median 8.51 to 49.33/\mu l; p=0.043*, Wilcoxon). Progressors to AIDS-OI (Figure 2a) had relatively low and stable numbers of A2-NLV-tetramer+CD8+ T cells (median 0.73 to 1.39/\mu l; p=1.000, Wilcoxon). Interestingly, in progressors towards AIDS-CMV (Figures 1a and 2a), like in long-term asymptomatics, A2-NLV-tetramer+CD8+ T cells were detected at high and increasing numbers (median 8.77 to 21.58/\mu l; p=0.093, Wilcoxon). Both long-term asymptomatics and progressors to AIDS-CMV contained more A2-NLV-tetramer+CD8+ T cells than the progressors to AIDS-OI early (p=0.050* and p=0.024* respectively, Mann-Whitney) and late (p=0.014* and p=0.005* respectively, Mann-Whitney) in HIV infection.

Lack of IFNy-producing CMV-specific CD8+ T cells within progressors to AIDS-CMV
To investigate responsiveness of the CMV-specific CD8+ T cells, the number of IFNy-producing CD8+ T cells was measured using intracellular IFNy staining, after stimulation with NLV peptide. In long-term asymptomatics (Figure 2b), IFNy+CD8+
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Figure 1. Dynamics of CMV-specific CD8\(^+\) T cells, CD4\(^+\) T cells and CMV load in the course of HIV-1 infection in progressors to AIDS-CMV. T-cell responses were analysed in progressors to AIDS-CMV. Patient samples were analysed for a) numbers of A2-NLV-tetramer\(^+\)CD8\(^+\) T cells/\(\mu l\) blood; b) numbers of IFN\(\gamma\)+CD8\(^+\) T cells/\(\mu l\) blood after stimulation with NLV peptide; c) percentage of perforin\(^+\)granzyme B\(^+\) cells within A2-NLV-tetramer\(^+\)CD8\(^+\) T cells; d) numbers of IFN\(\gamma\)+CD4\(^+\) T cells/\(\mu l\) blood after stimulation with CMV lysate; and e) CMV load in copies/\(10^6\) PBMC (dotted line represents detection limit). X-axis indicates time in months. Due to the variation with respect to the time to CMV end-organ disease (e.g. light blue versus green line in Figure 1a), the data is plotted retrospectively from the time of onset of CMV end-organ disease. Each colour represents the same patient in different analyses.
Figure 2. Comparison of early and late time points of CMV-specific T-cell responses between groups. Early and late time points from the longitudinal data of long-term asymptomatics (left column), progressors to AIDS-OI (middle column) and progressors to AIDS-CMV (right column) were compared using the Wilcoxon test. The two-tailed p-values are depicted in the upper right or left corner of the graphs. Depicted are a) numbers of A2-NLV-tetramer$^+$CD8$^+$ T cells/μl blood; b) numbers of IFN$\gamma^+$CD8$^+$ T cells/μl blood after stimulation with NLV peptide; c) percentage of IFN$\gamma^+$ cells within A2-NLV-tetramer$^+$CD8$^+$ T cells.
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cells; d) percentage of perforin/granzyme B⁺ cells within A2-NLV-tetramer⁺CD8⁺ T cells; and e) numbers of IFNγ⁺CD4⁺ T cells/μl blood after stimulation with CMV lysate. In long-term asymptomatics, the upper graph is depicted with a scale from 0 to 80 cells/μl blood and the lower graph with a scale from 0 to 6 cells/μl, like the other graphs of AIDS-OI and AIDS-CMV, for better comparison. Dots represent individual patients and the median is shown as a bar.

T cells could be detected (median 2.82 to 17.22/μl; p=0.138, Wilcoxon), whereas IFNγ⁺CD8⁺ T cells in progressors to AIDS-OI (Figure 2b) were present at low numbers (median 0 to 0.68/μl; p=0.109, Wilcoxon). In progressors to AIDS-CMV (Figures 1b and 2b), IFNγ⁺CD8⁺ T-cell numbers increased towards, but dropped before the time of CMV event (median 0 to 2.25/μl; p=0.327, Wilcoxon). In the cumulative data from all individuals, correlations were found between the number of A2-NLV-tetramer⁺CD8⁺ T cells and IFNγ⁺CD8⁺ T cells both early (p=0.826, p<0.01*, Spearman's) and late (p=0.787, p<0.01*, Spearman’s; data not shown).

As depicted in Figure 2c, percentages of IFNγ-producing cells within A2-NLV-tetramer⁺CD8⁺ T cells were lowest at the late time point in progressors to AIDS-CMV (median 6%) compared to long-term asymptomatics (median 28%) or progressors to AIDS-OI (median 58%), and differed at the late time point between long-term asymptomatics and progressors to AIDS-CMV (p=0.019*, Mann-Whitney). Thus, despite high numbers of CMV-specific A2-NLV-tetramer⁺CD8⁺ T cells before onset of CMV-EOD, a relatively low proportion of these cells was able to produce IFNγ after stimulation with peptide.

Percentage of perforin and granzyme B expressing CMV-specific CD8⁺ T cells increases to high levels in progressors to AIDS-CMV

To investigate potential cytotoxic capacity of the CMV-specific A2-NLV-tetramer⁺CD8⁺ T cells, perforin and granzyme B expression within these cells was analysed. In all patient groups, percentages of single perforin⁺ T cells were low, whereas single granzyme B⁺ T cells were detected abundantly in the CMV-specific CD8⁺ T cells (data not shown). In addition, perforin granzyme B⁺ T cells could be detected in all groups. In long-term asymptomatics (Figure 2d), high percentages of T cells expressing both perforin and granzyme B were detected (median 24.40 to 43.75%; p=0.138, Wilcoxon). In progressors to AIDS-OI (Figure 2d) these percentages were lower and stable (median 19.45 to 19.89%; p=1.000, Wilcoxon). For progressors to AIDS-CMV (Figures 1c and 2d), the fraction of these cells started at similar percentages as the progressors to AIDS-OI, and increased towards onset of CMV-EOD (median 22.75 to 54.73%; p=0.080, Wilcoxon). Within all individuals, the number of A2-NLV-tetramer⁺CD8⁺ T cells and perforin/granzyme B⁺ A2-NLV-tetramer⁺ T cells correlated significantly (early, p=0.990, p<0.01*, Spearman’s; late, ρ=0.884, p<0.01; data not shown). Interestingly, at the late time point, progressors to AIDS-OI had significantly lower percentages of perforin⁺ granzyme B⁺ CMV-specific CD8⁺ T cells compared to progressors to AIDS-CMV (p=0.050*, Mann-Whitney).

Thus, even though in progressors to CMV-EOD a relatively low fraction of CMV-
specific CD8\(^+\) T cells was capable of producing IFN\(\gamma\), expression of the cytotoxic molecules perforin and granzyme B was not impaired.

**IFN\(\gamma\)-producing CMV-specific CD4\(^+\) T cells are lost in the year before the first CMV-associated clinical event**

CMV-specific CD4\(^+\) T cells were studied using intracellular IFN\(\gamma\) expression after stimulation with CMV lysate. In long-term asymptomatics (Figure 2e), IFN\(\gamma\)^+CD4\(^+\) T cells were readily detected (range 0 to 75.58/\(\mu\)l), and stable (median 0.88 to 0.11/\(\mu\)l, \(p=0.893\), Wilcoxon). In progressors to AIDS-OI (Figure 2e), IFN\(\gamma\)^+CD4\(^+\) T cells could be detected though at lower numbers (median 0.08 to 0.51/\(\mu\)l, \(p=0.285\), Wilcoxon). Remarkably, in progressors to AIDS-CMV (Figures 1d and 2e), even though IFN\(\gamma\)^+CD4\(^+\) T-cell numbers could be detected early in infection (range 0 to 2.71/\(\mu\)l), they dropped significantly over time from a median of 0.30 to 0/\(\mu\)l (\(p=0.028\), Wilcoxon). However, no correlation could be found in the HIV\(^+\) data between numbers of IFN\(\gamma\)^+CD8\(^+\) T cells and IFN\(\gamma\)^+CD4\(^+\) T cells. Also, absolute CD4\(^+\) T-cell number and number of IFN\(\gamma\)^+CD8\(^+\) T cells did not correlate.

The disappearance of IFN\(\gamma\)^+CD4\(^+\) T cells in progressors to AIDS-CMV could not be explained by an absolute lack of responsiveness of CD4\(^+\) T cells, since stimulation with PMA and ionomycin readily induced IFN\(\gamma\) expression by CD4\(^+\) T cells both in absolute numbers and percentages (data not shown). Thus, the disappearance of IFN\(\gamma\)-producing CMV-specific CD4\(^+\) T cells in progressors to AIDS-CMV was associated specifically with the development of CMV disease.

**CMV load can be detected a few months prior to the onset of CMV-EOD**

To investigate the role of CMV replication, CMV load was measured in a large number of PBMC samples. No CMV load was detectable in long-term asymptomatics. In progressors to AIDS-OI, CMV load was detected in a few samples at the later time points, but too low to be quantified (data not shown). In all progressors to AIDS-CMV, CMV load was detectable. In 8 out of 10 individuals, CMV load could be quantified in samples obtained in the year before the onset of CMV-EOD, and ranged from 148 to 18184 copies per 10\(^6\) PBMC (median 439 copies per 10\(^6\) PBMC; Figure 1e). Although CMV load did not correlate with the CMV-specific CD8 or CD4 response, it did relate to disease progression, since only in progressors to AIDS-CMV, load could be detected in quantifiable amounts.

**DISCUSSION**

In this study, longitudinal samples from long-term asymptomatics, progressors to AIDS-OI, and progressors to AIDS-CMV were analysed in terms of CMV-specific CD8\(^+\) and CD4\(^+\) T-cell responses in parallel to CMV load in PBMC in order to investigate which factors might contribute to CMV-associated disease progression.
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Staining with HLA-A2-NLV tetramers clearly showed that CMV-specific A2-NLV-tetramer<sup>+</sup>CD8<sup>+</sup> T cells were present in all individuals, and increased over time both in long-term asymptomatics and progressors to AIDS-CMV. These increasing numbers of CMV-specific T cells may be induced by episodes of CMV reactivation. CMV load was indeed detectable and much increased in the AIDS-CMV group prior to CMV diagnosis. This may suggest that long-term asymptomatics are successful at keeping the CMV load below detection limits during follow-up, whereas progressors to AIDS-CMV fail to control CMV replication. Progressors to AIDS-OI were shown to have low numbers of CMV-specific T cells, which fits the low numbers of virus-specific T cells in general [15,16]. This may be explained by a lower capacity to proliferate in response to viral antigens as has been shown for HIV-specific CD8<sup>+</sup> T cells [17]. As very low levels of CMV DNA were detectable at some of the later time points, and no clinical CMV-related symptoms were observed, this suggests that CMV reactivation may simply be less severe despite the low numbers of CMV-specific CD8<sup>+</sup> T cells.

We used IFNγ as a functional read-out, and showed in progressors to AIDS-CMV much lower percentages of IFNγ<sup>+</sup>CD8<sup>+</sup> T cells within A2-NLV-tetramer<sup>+</sup>CD8<sup>+</sup> T cells compared to progressors to AIDS-OI and long-term asymptomatics, suggesting CMV-specific CD8<sup>+</sup> T-cell dysfunction. Indeed, in three HIV-1-infected individuals with low or no CD4<sup>+</sup> T cells at all, also dysfunction of HIV and CMV-specific CD8<sup>+</sup> T cells was shown [18]. Likewise, this has been described for HIV-specific CD8<sup>+</sup> T-cells in individuals progressing to AIDS [19], and for EBV-specific CD8<sup>+</sup> T-cells in HIV-1-infected individuals progressing to AIDS-related non-Hodgkin lymphoma [15]. In contrast, the percentages of perforin and granzyme B double positive CMV-specific CD8<sup>+</sup> T cells were highest in progressors to AIDS-CMV, indicating that these individuals do have a substantial number of CTLs with cytotoxic capacity [5].

Low numbers of IFNγ-producing CMV-specific CD8<sup>+</sup> T cells in combination with high numbers of both perforin and granzyme B expression, indicates that in CMV-specific T cells, IFNγ release and perforin and granzyme B expression are not necessarily linked [20]. As CMV is a typical cytopathic virus [1], that has been shown to depend more on the antiviral effect of cytokines like IFNγ and TNFα, than on the perforin-dependent granule exocytosis pathway [21], the decreased IFNγ-producing capacity of the CMV-specific cells may well be crucial.

The most conspicuous observation in this study was that in the progressors to AIDS-CMV, CMV-specific IFNγ<sup>+</sup>CD4<sup>+</sup> T cells disappeared in the year before onset of CMV-EOD suggesting that CMV-specific IFNγ<sup>+</sup>CD4<sup>+</sup> T cells could play an important role in protection from CMV-EOD. Dysfunction of CTL (in terms of IFNγ) and memory B cells was shown to be associated with SIV-induced impairment of CMV-specific CD4<sup>+</sup> T helper cells in rhesus macaques [22], and CMV-specific IFNγ has been shown to act as an immunological predictor of CMV control [23]. However, no correlation was found between numbers of IFNγ-producing CD8<sup>+</sup> and CD4<sup>+</sup> T cells as has been shown in healthy donors, but not in asymptomatic CMV-seropositive
renal transplant recipients who are on basic immunosuppressive drug therapy [24]. Possibly, the IFNγ produced by the CMV-specific CD4⁺ T cells may well have a role in controlling CMV directly, like IFNγ produced by the CD8⁺ T cells.

In conclusion, our data show that progressors to AIDS-CMV lose CMV-specific IFNγ⁺CD4⁺ T cells in the year before onset of CMV-EOD, in parallel with a sharp increase in load. CMV-specific CD8⁺ T cells remain in high numbers, with a high proportion of these cells expressing perforin and granzyme B, and a low proportion producing IFNγ. The decrease in IFNγ production in the CD8 compartment may be due to insufficient CD4 Th1 cells, and may allow for CMV dissemination. High viral load leads to high numbers of virus-infected cells and induces high numbers of perforin⁺granzyme B⁺A2-NLV-tetramer⁺CD8⁺ T cells. These high numbers of cytotoxic T cells in combination with the large numbers of infected cells, may lead ultimately to disease in the organ(s) infected, irrespective of the type of CMV-EOD (data not shown). Our data suggests that the balance between CD4⁺ T cells, CD8⁺ T cells, IFNγ and perforin/granzyme B is very important to control CMV replication and prevent virus-associated immune pathology.

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REFERENCES


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