Cytomegalovirus-specific T-cell dynamics in HIV infection
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Chapter 5

Loss of CMV-specific CD4+ T-cell cytokine production and proliferative capacity precedes progression to HIV-related CMV end-organ disease

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Submitted for publication
ABSTRACT

**Objectives:** To define function and phenotype of CMV-specific CD4⁺ T cells in HIV-infected individuals with different clinical end-points to identify which factors might be related to progression to CMV end-organ disease.

**Design:** Longitudinal analysis of CMV-specific CD4⁺ T cells in progressors to AIDS with CMV end-organ disease (AIDS-CMV) compared to long-term asymptomatics (LTA) and progressors to AIDS with opportunistic infections (AIDS-OI).

**Methods:** Production of IFNγ and IL-2, phenotype, and proliferative capacity of CMV-specific CD4⁺ T cells were analysed after stimulation of PBMC with CMV lysate.

**Results:** Numbers of CMV-specific IFNγ-producing CD4⁺ T cells were higher than IL-2-producing CD4⁺ T cells. In LTA and progressors to AIDS-OI, numbers of IFNγ-producing CD4⁺ T cells remained detectable during follow-up, but decreased sharply in individuals progressing to AIDS-CMV a year before onset of CMV end-organ disease. In parallel, CMV-specific IL-2 production and proliferative capacity were lost in progressors to AIDS-CMV. Most CMV-specific cytokine-producing CD4⁺ T cells were of the CD27⁻ phenotype. Initially, the majority of the IFNγ⁺CD4⁺ T cells were of the CD45RO⁻CD27⁻ effector subset, but during progression to AIDS-CMV a shift in phenotype to the highly differentiated CD45RO⁺CD27⁻ subset was observed.

**Conclusions:** Our data indicate that loss of CMV-specific cytokine production and proliferative capacity is associated with progression to AIDS-CMV. Accumulation of CD4⁺ T cells with a CD45RO⁺CD27⁻ phenotype suggests that persistent antigen exposure drives differentiation of CMV-specific CD4⁺ T cells towards a non-IL-2-producing, poorly proliferating, and highly differentiated “effector” subset, which eventually also fails to produce IFNγ in patients developing AIDS-CMV.
INTRODUCTION

Even though cytomegalovirus (CMV) infection normally does not lead to symptomatic disease, it can cause serious clinical complications in immunocompromised individuals. Recent studies have shown that in CMV infection next to CD8⁺ T cells, CD4⁺ T cells play an important role in protection from clinical complications. CMV-seronegative transplant recipients receiving a CMV-seropositive organ are likely to undergo primary CMV infection. This does not lead to symptomatic disease in individuals who mount a normal immune response. However, in patients who showed a delayed IFNγ⁺CD4⁺ T-cell response, symptoms were apparent [1]. Similarly, CMV reactivation in CMV-seropositive transplant recipients was associated with the absence of IFNγ⁺CD4⁺ T cells in the blood [2]. In HIV-infected individuals, loss of IFNγ⁺CD4⁺ T cells was observed prior to onset of symptomatic disease only in individuals who progressed to AIDS-CMV [3], further suggesting an important role for these CD4⁺ T cells in preventing disease progression.

In addition to the apparent importance of CMV-specific IFNγ⁺CD4⁺ T cells, HIV-specific IL-2⁺CD4⁺ T cells that have proliferative properties have been associated with low viral load and long-term non-progressing HIV-1-infected individuals [4,5]. Furthermore, Zaph et al [6] recently showed that presence of such IL-2⁺CD4⁺ T cells were protective in *Leishmania major* infection in mice.

CD4⁺ T cells can be classified into different subsets based on the production of cytokines [7], or on the expression of differentiation markers [8-10]. Based on the molecules CD45RO and CD27, the CD4⁺ T-cell population can be divided in CD45RO⁺CD27⁺ naive; CD45RO⁺CD27⁺ memory; and CD45RO⁺CD27⁻ effector CD4⁺ T cells [8,11,12]. In another frequently used model CD4⁺ T cells are divided into CD45RO⁺CCR7⁺ central memory, CD45RO⁺CCR7⁻ effector memory and CD45RO⁻CCR7⁻ terminally differentiated T cells based on CCR7 expression [9]. IL-2 production and proliferative capacity are features of (central) memory CD4⁺ T cells, whereas IFNγ production at the site of infection and decreased proliferative capacity are characteristics of effector (memory) CD4⁺ T cells [13,14]. Highly differentiated CD45RO⁻CD27⁻ CD4⁺ T cells are rarely detected in healthy individuals [13] but can be observed in chronic viral disease settings such as CMV, EBV and HIV-1 infection [15,16].

To study the role of CMV-specific CD4⁺ T cells in progression to CMV end-organ disease, we selected a group of HIV-infected individuals, who were not receiving treatment for HIV nor CMV, and were either long-term asymptomatics, or progressors to AIDS with or without CMV end-organ disease. IFNγ and IL-2 production, proliferative capacity and phenotype of CMV-specific CD4⁺ T cells were determined. In addition, we used novel HLA-DR3 tetrameric molecules containing a DR3-restricted CMV-derived epitope in order to study epitope-specific CD4⁺ T cells.
Chapter 5

METHODS

Study population

This study was performed on longitudinal peripheral blood mononuclear cell (PBMC) samples from participants of the Amsterdam Cohort Studies on HIV-1 infection and AIDS among homosexual men. Eight (4 HLA-DR3+) of the 25 HIV-seropositive individuals were defined as progressors to AIDS with CMV retinitis and/or other end-organ disease (AIDS-CMV), 9 (4 HLA-DR3+) as progressors to AIDS with Opportunistic Infections, but without CMV end-organ disease (AIDS-OI), and 8 (3 HLA-DR3+) as long-term asymptomatics (LTA) with more than 7 years of asymptomatic follow-up, CD4 counts > 300/μl (classification of the Centres for Disease Control 1993), and no history of CMV end-organ disease. None of the individuals received anti-viral therapy during the study period, except HLA-DR3+ LTA 0164 who had started highly active anti-retroviral therapy (HAART) at the late time point and was therefore excluded from group analyses in this study.

For the AIDS-CMV group, an early time point around one year after HIV seroconversion or study entry was compared with a late time point between 9 to 12 months before CMV diagnosis. In case follow-up was longer than 5 years, also a middle time point around 5 years after HIV seroconversion was included. For the HLA-DR3-positive individuals in the AIDS-CMV group, a time point between 12 to 20 months before CMV diagnosis was chosen as the late time point. The early time point studied in the progressors to AIDS-OI and LTA groups was around 1 year after HIV seroconversion. The late time point studied was around 5 years (around the time of AIDS diagnosis) in progressors to AIDS-OI, and 10 years after HIV seroconversion for LTA. Group characteristics of the study participants are summarised in Table 1.

Intracellular cytokine staining after antigen-specific stimulation

Four-colour fluorescence analysis was performed as described previously [17,18]. Briefly, PBMC were stimulated with 10 μl/ml CMV lysate (MicrobiX Biosystems, Toronto, Canada) in the presence of 2 μg/ml anti-CD28 (Sanquin Reagents, Amsterdam, the Netherlands) and CD49d (Becton Dickinson (BD), San José, California, USA) monoclonal antibodies (mAb) at 37°C for 6 hours. After 1 hour, 1 μl/ml Brefeldin A (“GolgiPlug”, BD) was added. As a positive control for the capacity of PBMC to produce cytokines, 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. Subsequently, cells were placed at 4°C overnight, after which they were stained extracellularly with fluorochrome-conjugated mAb against CD45RO (BD) and CD49d (BD), CD4, CD3 (BD), CD27 (Sanquin Reagents). Cells were fixed in Cellfix (BD), and 100,000 up to 300,000 events were acquired using a FACSCalibur flow.
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cytometer (BD). Lymphocytes were gated by forward-sideward scatter, and data were analysed using the software program CELL Quest (BD). In case of intracellular IFNγ and IL-2 measurements, the number of responding T cells was calculated after subtracting negative control values. Percentages of CD45RO⁺CD27⁺, CD45RO⁺CD27⁻ and CD45RO⁻CD27⁻ CD4⁺ T cells in Figure 3c were expressed as percentages within these three T-cell subsets. Data are expressed in absolute numbers per µl blood, to take into account that during progression to AIDS, total CD4⁺ T-cell numbers drop dramatically.

Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Subject</th>
<th>Classification¹</th>
<th>AIDS defining illness²</th>
<th>Months</th>
<th>CD4 count</th>
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¹ CMV = cytomegalovirus; CMV disease = CMV other end-organ disease than retinitis; LTA = long-term asymptomatic.
² PCP = Pneumocystis carinii pneumonia; KS = Kaposi sarcoma; C. meningitis = Cryptococcal meningitis.
³ CD4 counts are in absolute numbers/µl.
⁴ = HLA-DR3-positive individual.
⁵ At this time point individual 0164 had started HAART, and was therefore excluded from data analyses.
Antigen-specific proliferation assay

In vitro T-cell proliferation to CMV lysate was measured using CFSE (5,6-carboxyfluorescein diacetate succinimidyl ester) (Molecular Probes, Leiden, The Netherlands) according to the manufacturer's protocol. Briefly, PBMC were labelled with CFSE and incubated with 10 µl/ml CMV lysate at 37°C for 6 days. As a positive control, 0.4 µg/µl anti-CD3 and 4 µg/µl anti-CD28 (Sanquin Reagents) were added and medium alone was used as a negative control. At day 6, cells were stained extracellularly with fluorochrome-conjugated mAb against CD4 and CD3 (BD). Cells were fixed in Cellfix (BD), and 60,000 up to 300,000 events were acquired using a FACSCalibur flow cytometer (BD). Stimulation indices (SI) were calculated by dividing the percentage proliferation of CD3⁺CD4⁺ T cells after stimulation by the percentage proliferation of unstimulated cells.

HLA-class II tetramer staining directly ex vivo and after expansion

In the context of HLA-DR3 (HLA-DRB1*0301), peptide p510-522 from CMV lower matrix protein pp65 was defined as a CD4-dependent epitope and used to produce HLA-DR3 tetrameric molecules (Bronke et al, submitted). Briefly, Drosophila Schneider cells were used to produce HLA-DR3 monomeric molecules, which consist of the extracellular domain of the α-chain of HLA-DRB1*0101, an acid leucine zipper, and His-tag, and the extracellular domain of the β-chain of HLA-DRB1*0301 (plus p511-522 epitope covalently linked), a complementary basic leucine zipper and biotinylation domain. His-tag allowed for the purification of the monomeric molecules expressed and excreted in culture supernatant, and the biotinylation domain to form tetramers through streptavidin binding.

PBMC from HLA-DR3⁺ HIV-infected individuals were incubated with CMV-specific HLA-DR3 tetramer at 37°C for 1 h. In parallel, PBMC were also labelled with CFSE as described above, and incubated with 5 µg/ml p510-522 peptide in the presence of 2 µg/ml anti-CD28 and CD49d mAb at 37°C for 6 days. At day 6, cells were incubated with tetramer at 37°C for 1 h. Subsequently, cells were stained at RT for 20 minutes with fluorochrome-conjugated mAb against CD4 and/or CD3 (BD). Finally, cells were fixed in Cellfix (BD), and 60,000 up to 300,000 events were acquired using a FACSCalibur flow cytometer (BD). Propidium iodide (PI) was added to distinguish between dead and life cells.

Statistical analysis

Wilcoxon 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. All statistical analyses were performed using the software programme SPPS 11.5 (SPSS Inc., Chicago, Illinois, USA).
RESULTS

CMV-specific CD4+ T cells mainly produce IFNγ and some IL-2, and loose both during progression to AIDS-CMV

Stimulation with CMV lysate and intracellular cytokine staining was used to analyse CMV-specific T cells. As shown in Figure 1, IFNγ-producing as well as IL-2-producing CMV-specific CD4+ T cells could be detected in all individuals.

Figure 1. Early and late CMV-specific cytokine-producing CD4+ T-cell responses. Early and late time points from the longitudinal data of LTA (left column), progressors to AIDS-OI (middle column) and progressors to AIDS-CMV (right column) were compared. Depicted are a) numbers of CMV-specific single IFNγ+CD4+ T cells/μl blood; b) numbers of IFNγ-IL-2 double positive CD4+ T cells/μl blood; c) numbers of single IL-2+CD4+ T cells/μl blood after stimulation of PBMC with CMV lysate. Dots represent individual patients and the median is shown as a bar. The two-tailed p-values from Wilcoxon tests comparing early and late time points are depicted in the upper right corner of the graphs.

In LTA, single IFNγ+CD4+ T cells were readily detected and their number increased in 3 out of 5 individuals (median 1.67 to 5.81/μl; p=0.144, Wilcoxon). Progressors to AIDS-OI (Figure 1a) initially had similar numbers of single IFNγ+CD4+ T cells but they decreased in 5 out of 7 individuals (median 6.26 to 1.32/μl; p=0.176, Wilcoxon). Interestingly, in progressors towards AIDS-CMV...
(Figure 1a), single IFNγ⁺CD4⁺ T cells were detected in 4 out of 5 individuals initially, but they decreased to hardly detectable levels in all individuals a year before onset of CMV end-organ disease (median 6.05 to 0.63/μl; p=0.080, Wilcoxon). In all groups, the number of IL-2-producing CD4⁺ T cells was lower than the number of IFNγ⁺producing CD4⁺ T cells (Figures 1b and c; note different y-axis scale).

In LTA and progressors to AIDS-OI, single IL-2⁺CD4⁺ T cells (median 0.01 to 0.05/μl, LTA; and median 0.315 to 0.036/μl, AIDS-OI; Figure 1b) as well as IFNγ⁺IL-2⁺ CD4⁺ T cells (median 0.14 to 0/μl, LTA; and median 0.644 to 0.114/μl, AIDS-OI; Figure 1c) could be detected at both time points. In progressors to AIDS-CMV, similar to single IFNγ⁺CD4⁺ T cells, both single IL-2⁺ as well as IFNγ⁺IL-2⁺ CD4⁺ T cells decreased significantly to hardly detectable levels in all individuals a year before onset of CMV end-organ disease (median 0.144 to 0.01/μl IL-2⁺; p=0.043, Wilcoxon, Figure 1b; median 0.828 to 0/μl IFNγ⁺IL-2⁺; p=0.043, Wilcoxon; Figure 1c). Thus, in progressors to AIDS-CMV, CMV-specific CD4⁺ T cells produced mainly IFNγ and both IFNγ⁺-producing and IL-2⁺-producing CD4⁺ T cells were lost during progression to CMV end-organ disease.

**CMV-specific CD4⁺ T cells loose proliferative capacity a year before onset of CMV end-organ disease**

To determine proliferative capacity of the CMV-specific CD4⁺ T cells, PBMC were labelled with CFSE dye and stimulated for 6 days with CMV lysate (Figures 2a, b and c). Cell division results in dilution of the CFSE dye. Stimulation indices (SI) were determined by dividing the percentage proliferation of CD3⁺CD4⁺ T cells after stimulation by the percentage proliferation of unstimulated cells. Proliferation remained relatively stable in LTA (median stimulation index (SI) 3 to 5.4; p=0.917, Wilcoxon; Figures 2a and c) as well as in progressors to AIDS-OI (median SI 13.4 to 8.4; p=0.893, Wilcoxon; Figure 2c). In progressors to AIDS-CMV, however, CMV-specific CD4⁺ T-cell proliferation decreased significantly in all individuals towards progression to CMV end-organ disease (median SI 2.8 to 1.4; p=0.043, Wilcoxon; Figures 2b and c). One of the progressors to AIDS-CMV showed a peak in proliferation around 5 years after HIV seroconversion, but well before onset of CMV end-organ disease. Interestingly, this same individual showed a peak in the number of IFNγ and IL-2 co-producing CD4⁺ T cells at this time point as shown in Figure 1b, illustrating that IL-2 production and proliferation may be linked. Thus, in progressors to AIDS-CMV loss of proliferative capacity was observed, which paralleled the loss of cytokine-producing CMV-specific CD4⁺ T cells.

**CMV-specific IFNγ⁺-producing CD4⁺ T cells are preferentially of the CD45RO⁺CD27⁻ phenotype a year before onset of CMV end-organ disease**

IFNγ⁺-producing CD4⁺ T cells were stained with the cell surface markers CD45RO and CD27 to determine the phenotype of these CMV-specific CD4⁺ T cells. Figure 3a shows FACS analysis of a representative progressor to AIDS-CMV at an early and late time point. IFNγ⁺CD4⁺ T cells were readily detected in most individuals and the
Loss of CMV-specific CD4⁺ T cells and onset of disease

Figure 2. Proliferative capacity of CMV-specific CD4⁺ T cells. In a) FACS analysis after 6 days of stimulation with CMV lysate of CFSE-labelled PBMC of a representative LTA at the early (left) and late (right) time point; and in b) a representative progressor to AIDS-CMV at the early and late time point. Numbers indicate percentages of CFSE⁺CD4⁺ T cells that were used to calculate stimulation indices. In c) early and late time points from the longitudinal data of LTA (left column), progressors to AIDS-OI (middle column) and progressors to AIDS-CMV (right column) were compared. Proliferative capacity as indicated by stimulation index is shown on the y-axis. Dots represent individual patients and the median is shown as a bar. The two-tailed p-values from Wilcoxon tests early and late time points are depicted in the upper left corner of the graphs.

three major subsets (CD45RO⁺CD27⁻, CD45RO⁺CD27⁺, and CD45RO⁻CD27⁻) are depicted in Figure 3c. In LTA, most IFNγ⁺CD4⁺ T cells resided in the CD45RO⁺CD27⁻ effector subset both early and late in HIV infection (median 65.2 to 73.4%; p=0.465, Wilcoxon). In progressors to AIDS-OI, also most of the cells were found in the CD45RO⁺CD27⁻ effector subset, which remained stable during HIV infection (median 77.2 to 81%; p=0.600, Wilcoxon). In progressors to AIDS-CMV, initially the majority of the IFNγ⁺CD4⁺ T cells were of the CD45RO⁺CD27⁻ effector subset, but during progression to AIDS-CMV a shift in phenotype to the
CD45RO⁺CD27⁻ subset was observed (median 67.6 to 23% CD45RO⁺CD27⁻ versus median 18.5 to 73% CD45RO⁺CD27⁻; p=0.068 for both subsets, Wilcoxon).

**Figure 3. Phenotype of CMV-specific total IFNγ- or IL-2-producing CD4⁺ T cells.** In a) IFNγ-producing CD4⁺ T cells and phenotype of a representative progressor to AIDS-CMV at the early (upper) and late (lower) time point; and in b) IL-2-producing CD4⁺ T cells and phenotype of the same individual. Numbers in the upper right-hand corner indicate percentages of IFNγ⁺ within CD4⁺ T cells and percentages of the 4 quadrants, respectively. In c), early and late time points from the longitudinal data of LTA (left column), progressors to AIDS-OI (middle column) and progressors to AIDS-CMV (right column) were compared. Percentages of CD4⁺ T cells in a particular subset are shown on the y-axis. Memory CD45RO⁺CD27⁺ T cells are depicted by black (●), effector CD45RO⁺CD27⁻ T cells as grey (○), and fully differentiated CD45RO⁺CD27⁻ T cells as white (□) circles. Dots represent individual patients and the median is shown as a bar.

IL-2-producing CD4⁺ T cells were also characterised phenotypically in cases where enough IL-2-producing T cells were present. We were able to analyse all progressors to AIDS-OI, 1 out of 4 progressors to AIDS-CMV (Figure 3b) and 2 out of 6 LTA. Interestingly, the largest subset of IL-2-producing CD4⁺ T cells was of the CD45RO⁺CD27⁺ memory phenotype (median 54.2 to 34.4%; p=0.500, Wilcoxon), followed by CD45RO⁺CD27⁻ effector (median 42 to 29.3%) and CD45RO⁺CD27⁻ (median 5.6 to 0%) CD4⁺ T cells (data not shown). Overall, CD45RO⁺CD27⁺ memory cells were significantly more abundant in IL-2-producing compared to IFNγ-
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producing CD4⁺ T cells, early (median 51.38 versus 4.3%; p=0.008, Wilcoxon) and late (median 33.3 versus 5.5%; p=0.093, Wilcoxon) in HIV infection.

Thus, CMV-specific IFNγ-producing CD4⁺ T cells had differentiated towards the highly differentiated CD45RO⁺CD27⁺ phenotype during progression towards AIDS-CMV. In addition, the majority of IFNγ⁺CD4⁺ T cells were found in the CD45RO⁺CD27⁺ subset, whereas the majority of IL-2⁺CD4⁺ T cells were found in the memory subset.

![Diagram](image)

**Figure 4.** MHC class II tetramer staining of an epitope of CMV pp65 in the context of HLA-DR3. In a), *ex vivo* tetramer staining on PBMC of an LTA at the early (upper) and late (lower) time point, and tetramer staining on CFSE-labelled PBMC after stimulation with peptide for 6 days of the same individual (middle left), a progressor to AIDS-OI (middle right) and a progressor to AIDS-CMV (right). In b), *ex vivo* tetramer staining on PBMC of an LTA at the early (upper) and late (lower) time point, and tetramer staining on CFSE-labelled PBMC after stimulation with peptide for 6 days of the same individual. This individual had started HAART at the late time point. Numbers in the upper right-hand corner indicate percentages of tetramer⁺ within CD4⁺ T cells and in the upper left-hand corner percentages of tetramer⁺CFSE⁻ cells within CD4⁺ T cells.
Detection of CMV-specific CD4+ T cells using HLA-DR3 tetrameric molecules specific for an epitope from CMV pp65

Epitope-specific CD4+ T cells were analysed using HLA-DR3 tetrameric molecules containing an epitope (peptide 511-522) from pp65. We identified 11 HLA-DR3+ individuals, who could be classified in either group of LTA (n=3), progressors to AIDS-OI (n=4) or AIDS-CMV (n=4). In one LTA patient, reasonable numbers of tetramer+CD4+ T cells could be detected directly ex vivo (0.47 to 0.49/μl (i.e. 0.10 to 0.13%) tetramer+ in CD4+ T-cell population; Figure 4a LTA). In addition, these tetramer+CD4+ T cells could be expanded by in vitro stimulation with the specific peptide. After 6 days of culture of CFSE-labelled PBMC, 2.4% to 0.13% tetramer+CFSElow cells were detected in the CD4+ T-cell population (Figure 4a), confirming the observed direct tetramer staining in this individual. This individual clearly showed a decline in tetramer+CFSElow cells. In another LTA, tetramer+CD4+ T cells could be detected only after stimulation. Furthermore, we were able to detect tetramer+CD4+ T cells after stimulation in one progressor to AIDS-CMV at an early time point, but not at the late time point (2.88 to 0.05% tetramer+CFSElow in the CD4+ T-cell population; Figure 4a AIDS-CMV). In progressors to AIDS-OI, however, no CMV-specific CD4+ T cells could be detected even after stimulation (median 0.03 to 0.04% tetramer+CFSElow in the CD4+ T-cell population; Figure 4a, a representative AIDS-OI patient). Thus, CMV-specific HLA-DR3 tetramer+CD4+ T cells can be detected in HIV-1-infected individuals during the course of HIV infection both ex vivo and after 6-day expansion by in vitro stimulation with the peptide of interest.

Interestingly, in one LTA (who had been excluded from this study, since he had already started HAART at the late time point), tetramer+CFSElow cells could be detected clearly late in infection (Figure 4b), suggestive of restoration of CMV-specific proliferative capacity after HAART.

DISCUSSION

The aim of this study was to define function and phenotype of CMV-specific CD4+ T cells in HIV-infected individuals with different clinical end-points to identify which factors might be related to progression to CMV end-organ disease. Therefore we studied long-term asymptomatic HIV+ individuals (LTA), progressors to AIDS with opportunistic infections (AIDS-OI), and progressors to AIDS with CMV disease and/or retinitis (AIDS-CMV) in the natural course of HIV infection, longitudinally.

In all individuals, numbers of CMV-specific IFNγ-producing were found to be substantially higher compared to IL-2-producing CD4+ T cells, and especially the number of single IL-2-producing CMV-specific CD4+ T cell was very low. These observations are compatible with another study in which HIV-1-infected individuals with various patient characteristics were analysed [15]. In primary CMV infection and during CMV latency in transplantation patients, CMV-specific CD4+ T cells have been reported to produce IFNγ and TNFα, but no IL-2 or IL-4 [2]. Even though
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Harari et al [5] did report a relatively equal distribution of IFNγ and/or IL-2-producing CMV-specific CD4+ T-cell populations, single IFNγ+ numbers were still shown to be higher than IFNγ+IL-2+ numbers, which were in turn higher than single IL-2+CD4+ T-cell numbers.

In progressors to AIDS-CMV, a sharp decline was observed in IFNγ-producing CD4+ T cells a year before onset of CMV end-organ disease. This finding is in line with our previous work [3], where we showed that IFNγ+CD4+ T cells were lost during progression to CMV end-organ disease. In addition to the decline in IFNγ-producing cells, CMV-specific IFNγ+IL-2+ as well as the already scarce single IL-2+CD4+ T cells decreased to non-detectable levels. These data suggest that during progression to AIDS-CMV, CMV-specific cytokine-producing CD4+ T-cell responses are lost. However, not only the cytokine production of CD4+ T cells were lost, but also their proliferative capacity was impaired. This impaired proliferation may be linked directly to the loss in IL-2 production. IL-2 is a strong inducer of T-cell proliferation, and other studies have suggested that lack of proliferation of HIV-specific CD4+ T cells is indeed caused by their inability to produce IL-2 [4,5].

Several studies have implied that infection with CMV could have a dominant effect on shaping the T-cell immune response [19-21]. In progressors to AIDS-CMV, CD45RO+CD27-CD4+ and CD28+CD27+CD4+ T cells have been shown to significantly increase over time within the CD4+ T-cell population as a whole [16]. Collectively these data implied that there is a CMV-driven maturation in the polyclonal CD4+ T-cell population over time in HIV infection that is strongest in progressors to AIDS-CMV. Here, we extended our study of CMV-specific IFNγ-producing CD4+ T cells by analysing these cells phenotypically. Remarkably, within the IFNγ+CD4+ T cells in progressors to AIDS-CMV we observed a striking shift from CD45RO+CD27- effector to the CD45RO+CD27+ subset during progression to AIDS-CMV. In LTA and progressors to AIDS-OI, IFNγ+CD4+ T cells were mainly found within the CD45RO+CD27- effector subset during the course of HIV infection. Antigenic load has been associated with antigen-specific CD4+ (and CD8+) T-cell differentiation [4,22-24] and loss of IL-2- CD45RO+CCR7+ central memory cells. CMV replication may well play a role in the shift in differentiation status of CMV-specific T cells, not only because of its high antigenic properties but also since high CMV load (indicative of active CMV replication) could be detected in PBMC just before onset of symptomatic disease [3].

Harari et al recently reported that memory (i.e. the ability to mount an accentuated response to antigen re-encounter) of an immune response can be found in different phenotypic subsets depending on antigen persistence and antigen load, which is different for different viral infections [23]. For HIV-specific CD4+ T cells, IL-2-production has been identified to be associated with protective immunity, i.e. low viral load and a long-term asymptomatic clinical course, and is now considered a better marker for disease progression, whereas HIV-specific single IFNγ+CD4+ T cells were shown to increase in progressors to AIDS [5]. CMV-specific immunity may not resemble HIV-specific immunity. Memory CMV-specific CD4+ T cells may also
produce IFNγ and express a slightly more differentiated phenotype, possibly due to repetitive antigen exposure [23]. IFNγ-producing CMV-specific CD4+ T cells remain associated with progression to AIDS-CMV and seem important in preventing CMV end-organ disease. Indeed, IFNγ-producing CMV-specific CD4+ T-cell numbers are more abundant than IL-2-producing cells. Moreover, both populations were lost in progression to AIDS-CMV.

A number of HLA-DR3+ HIV-1-infected individuals were identified, who could be analysed with our novel CMV-specific HLA-DR3 tetrameric molecules in order to determine whether the observed loss of cytokine-producing CD4+ T cells could be due to physical depletion. Despite focussing only on T cells specific for a single epitope, we were able to detect CMV-specific CD4+ T cells in several of the HLA-DR3+ patients. Interestingly, tetramer+CD4+ T-cell numbers tended to decline over time in LTA. Only in one LTA, tetramer+CD4+ T cells could be detected late in infection, which was in fact a time point after this individual had started HAART. Unfortunately, in patients who progressed to AIDS-CMV we were not able to detect DR3-restricted CD4+ T cells late in infection. This could indicate that fewer of these cells were present in progressors to CMV end-organ disease. Also after 6-day stimulation, no DR3-restricted, p511-522-specific CD4+ T cells were detected. Since in progressors to AIDS-CMV, proliferative capacity of the CMV-specific CD4+ T cells was impaired, detection of these cells after expansion seems therefore unlikely. Furthermore, we used a CMV pp65-derived epitope, p511-522, which we have defined in the context of HLA-DR3. Although our previous work (Bronk et al, submitted) did clearly identify this HLA-DR3 epitope, there may be non-DR3 restricted epitopes that are more immunodominant. Moreover, not in all individuals is pp65 the immunodominant protein for the CMV-specific CD4+ T-cell response [25]. Therefore we cannot exclude the possibility that we might have missed a response.

In conclusion, IFNγ- and IL-2-producing CMV-specific CD4+ T cells as well as CMV-specific proliferative capacity were lost in progressors to AIDS-CMV before onset of CMV end-organ disease. CMV-specific IFNγ-producing CD4+ T cells shifted towards highly differentiated CD45RO−CD27− phenotype during progression towards AIDS-CMV, and eventually disappeared. Antigenic presence could play an important role in driving the differentiation of the CMV-specific CD4+ T cells towards the CD45RO−CD27− phenotype. Probably, loss of these CMV-specific IFNγ+CD4+ T cells (and IFNγ+CD8+ T cells) allows uncontrolled dissemination of the virus and subsequent immunopathology. Especially in HIV infection, where chronic immune activation seems to exhaust immune responses in general, this could play an important role.

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Chapter 5


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