Cytomegalovirus-specific T-cell dynamics in HIV infection

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Chapter 1

General Introduction

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T cells are well known to play a key role in the adaptive immune response to pathogens. Some pathogens, such as viruses, (myco-) bacteria, and parasites infect the host intracellularly, whereas worms, fungi, other parasites and bacteria do not enter cells and remain extracellularly. Both scenarios require a different response. Defence against extracellular pathogens depends upon T helper 2 cells and a strong B-cell response, where antibodies coat the pathogen, allowing for recognition and killing by phagocytosis, or activation of the complement pathway. Antibodies also play a role in intracellular infections by neutralisation of viruses and blocking viral entry. In addition, a T helper 1 cell response is required to control intracellular pathogens by inducing $CD8^+$ T cells that recognise the infected cells, and either kill by CTL-mediated cell death or inhibit for instance viral replication by production of cytokines, such as TNFα and IFNγ.

Cytomegalovirus infection

Human cytomegalovirus (CMV) is a double stranded DNA virus of the β-herpes virus family [1]. Depending on socio-economic circumstances, 50 to 100% of the human population is infected. Primary infection is established most likely in mucosal epithelial cells through saliva, but can also occur through blood contact. Since the virus uses many immune evasion strategies, it gets eliminated rarely, if ever [2]. The virus disseminates throughout the body, and remains latently present in many tissues and cell types of the host, most importantly in bone marrow precursors of monocytic peripheral blood cells [3]. Although asymptomatic in immunocompetent hosts, CMV (re-) infection or reactivation can cause serious clinical complications in case of congenital infection, and in immunocompromised individuals such as human immunodeficiency virus (HIV)-infected subjects and transplant recipients. The possible clinical presentation of CMV end-organ disease is diverse, ranging from retinitis, gastrointestinal disease, pneumonitis, meningo-encephalitis, radiculomyelitis, to a severe life-threatening illness with multi-organ failure (reviewed by Pass et al [3]).

T-cell immunity to CMV

Cellular immunity is thought to play a crucial role in controlling cytomegalovirus replication and prevention of disease [3]. With the development of class I HLA-peptide tetrameric complexes [4-6] and cytokine flow cytometry-based methods [7,8] to quantitate virus-specific $CD8^+$ and $CD4^+$ T-cell frequencies, together with the identification of highly immunodominant proteins and peptides [9,10], it became possible to study CMV-specific T-cell responses, and their role in protection from disease in detail. Stimulation with CMV lysate *in vitro* is widely used to measure IFNγ-producing CMV-specific $CD4^+$ T cells [11]. Recent developments in class II HLA-peptide tetrameric complexes will further enable detailed analysis of the $CD4^+$ T-cell response to CMV [12-15]. To further characterise T cells, various cell-surface

molecules have been proposed as phenotypic markers with a link to differentiation state and functional properties. CD45RA and CD45RO (isoforms of leukocyte common antigen CD45) are being used in most models, in combination with a number of receptors involved in co-stimulation, activation or lymphocyte homing [16-20]. A commonly used combination is CD45RA or RO in combination with co-stimulatory receptor CD27, a member of the TNFα super family. T-cell activation induces a shift from CD45RA to CD45RO expression that can be reversed under certain conditions. Since research described in this thesis focused on chronic CMV infection, we chose to use CD45RO. CD27 expression becomes down regulated irreversibly after interaction with its ligand CD70. Based on these two markers, $CD8^+$ T cells can be divided into naive $CD45RO^-CD27^+$, memory $CD45RO^+CD27^+$, memory/effector $CD45RO^+CD27^-$ and effector $CD45RO^-CD27^-$ $CD8^+$ T cells [16,21]. $CD4^+$ T-cell nomenclature differs from $CD8^+$ T-cell subsets in that the $CD4^+CD45RO^+CD27^-$ subset is defined as the effector population [22], and the fully differentiated $CD4^+CD45RO^-CD27^-$ is a rarely observed subset in healthy individuals. Although pathways of T-cell differentiation have been studied extensively, their lineage relationship is still not fully understood. Several models have been proposed based on linear or divergent pathways, and pathways with formation of different memory (and effector) T-cell subsets (reviewed in [23]). During chronic viral infections in human, based on the CD45RO/CD27 nomenclature, T cells have been reported to differentiate from memory towards effector phenotype [21].

CMV in healthy individuals

The immune response to CMV appears to be dominated by the pp65 lower matrix protein, and in HLA-A2 individuals by the NLVPMVATV epitope of the pp65 lower matrix protein specifically [9,10,24]. In combination with CMV lysate, which is known to express high levels of pp65, the $CD8^+$ and $CD4^+$ T cell-response against CMV can be analysed. In healthy CMV-seropositive individuals, numbers of CMV-specific $CD8^+$ T cells can be readily detected. They have been shown to be heterogeneous for the capability to express IFNγ and perforin [25]. In addition, it has been shown that there is a considerable variability over time in both CMV-specific $IFN\gamma^+CD8^+$ and $IFN\gamma^+CD4^+$ T-cell responses [26]. Interestingly, a correlation has been shown between IFNγ-producing CMV-specific $CD4^+$ versus $CD8^+$ T-cell frequencies in healthy donors, but not in asymptomatic CMV-seropositive renal transplant recipients who are on standard immunosuppressive drug therapy [27]. In addition, CMV-specific $tetramer^+CD8^+$ T cells are mainly of $CD45RO^+CD27^+$ memory phenotype in the majority of healthy donors and patients before renal transplantation, although some individuals express predominantly a $CD45RO^-CD27^-$ effector phenotype [27]. Another study has shown that CMV-specific IFNγ-producing $CD8^+$ T cells however, are found mainly in $CD45RO^+CD27^-$ memory/effector or $CD45RO^-CD27^-$ effector subsets [28].

CMV in transplantation

CMV-specific immune responses are studied in transplantation settings and during HIV infection, because it is in these particular conditions that CMV retinitis or other end-organ disease occurs mostly. In CMV-seronegative renal transplant recipients, specific $CD8^+$ cytotoxic T cells do develop upon primary CMV infection (through a CMV-seropositive donor organ), regardless the presence or absence of clinical signs and symptoms. However, unlike asymptomatic patients, the CMV-specific IFNγ-producing $CD4^+$ T cell response does not precede the specific $CD8^+$ T-cell response in symptomatic patients, but is delayed until after commencing antiviral therapy [29]. During CMV reactivation in immunosuppressed transplant recipients, percentages of CMV-specific $CD8^+$ T cells are higher and increased over time, shifting to the $CD45RO^-CD27^-$ effector subset [27]. In CMV-seropositive stem cell transplantation patients, it has been described that the presence of a larger fraction of dysfunctional CMV-specific $CD8^+$ T cells, and not lower absolute numbers nor frequencies of $CD8^+$ or $CD4^+$ T cells, is associated with the risk of CMV-related complications [30].

CMV in HIV-infected patients

In the natural course of HIV infection, CMV retinitis and/or other end-organ disease as an AIDS-defining illness [31,32] occurs particularly when $CD4^+$ T-cell counts fall below 50 cells/μl. CMV-specific $CD4^+$ T cells can be detected at higher numbers in HIV^+ compared to HIV^- subjects, and show a Th1-type response [8]. Higher T-cell proliferative responses to CMV antigen are associated with decreased risk of CMV retinitis [33,34]. CMV-specific $CD8^+$ T cells are also present in high numbers, a large proportion expresses effector cytokines [35,36], and they are mainly $CD45RO^{-(+)}$ $CD27^-$ $CD28^-$ $CCR7^-$ $perforin^+$ [37-39]. It is still a point of discussion, whether in humans $CD4^+$ T-cell help is associated with the activity of cytotoxic T lymphocytes (CTLs), as has been shown in animal models and suggested in human studies [37-39]. Although CMV-related morbidity and mortality was a major problem in individuals progressing to AIDS before the development of highly active anti-retroviral therapy (HAART), at the time the experimental work of this thesis started, little was known about the CMV-specific immune response. In particular progression to CMV end-organ disease in HIV-infected individuals who are not treated with HAART, and receive no CMV medication, was poorly understood. This thesis provides new insights in CMV-specific T-cell responses in these clinical settings.

Scope of the thesis

In this thesis we aimed to determine immunological factors that may contribute to progression to AIDS with CMV end-organ disease. To this end, we analysed dynamics of CMV-specific $CD8^+$ and $CD4^+$ T cells in terms of number, function and phenotype in the natural course of HIV-1 infection. In chapter 2, dynamics of CMV-specific $CD8^+$ T cells, studied longitudinally in terms of numbers, and the expression of functional molecules IFNγ, as well as perforin and granzyme B, are presented. In parallel, analyses of CMV-specific $CD4^+$ T-cell response, with IFNγ production as a

read-out, and the measurements of intracellular CMV load in PBMC are described. HIV-infected individuals, who progressed to AIDS with CMV end-organ disease, were compared to progressors to AIDS without CMV end-organ disease and long-term asymptomatics in order to determine which cells may play important roles in protection from disease progression. Chapter 3 describes the results of continued studies of the $CD8^+$ CMV-specific T-cell response in more detail by analysis of a panel of frequently used phenotypical markers in order to test if impaired maturation of CMV-specific $CD8^+$ T cells contributed to disease development. In addition, we aimed to define the phenotype of the total CD4 T-cell population in these HIV-infected patients to determine what happens to CMV-driven shaping of the immune response, as described in HIV-negative CMV-seropositive individuals without CMV end-organ disease. Chapter 4 describes the synthesis of MHC class II tetramers in the context of HLA-DR3. Two epitopes from different pathogens were covalently linked to the HLA-DRB1*0301 β-chain and transfected in Drosophila Schneider cells for expression and subsequent protein purification. Since CMV-specific $CD4^+$ T cells may play an important role in progression to CMV end-organ disease, the CMV-specific $CD4^+$ T-cell response was analysed in detail as described in chapter 5. For the first time CMV-specific HLA-DR3 tetrameric molecules were used in parallel with proliferative capacity, cytokine production (i.e. IFNγ and IL-2) and phenotypic analyses. In chapter 6, we present the CMV-specific IFNγ-producing T-cell response phenotypically in HIV-infected children with or without continuous CMV shedding. In these children, expansions of the total $CD45RA^+CD27^-$ effector $CD8^+$ T cell population correlates with CMV seropositivity as well as CMV shedding. We aimed to define if this expansion was due to CMV-specific $CD8^+$ effector T-cell expansions. Chapter 7 presents a longitudinal analysis of HIV-, Epstein-Barr virus (EBV)- and CMV-specific $CD8^+$ T cells in rapid (i.e. progressors to AIDS) and slow progressors to AIDS (i.e. long-term asymptomatics), in order to compare virus-specific $CD8^+$ T-cell differentiation. Expression of the phenotypic molecule CD27 as well as the effector molecules perforin and granzyme B were measured in relation to viral load. Finally, in chapter 8, the results from the different chapters are discussed in the context of current literature, culminating in some final concluding remarks.

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