T cell development in human cytomegalovirus infection
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Summary
This thesis focuses on the generation and maintenance of cellular immunity against CMV in renal transplant recipients under immunosuppressive therapy. The development and maintenance of CMV-specific T cell responses in primary infection, latency and reactivation episodes is described in the first chapters of this thesis. Next, the impact of CMV-specific CD8\(^+\) T cells on alloreactive immunity is studied in the last chapter.

In chapter 1, the CMV-specific CD4\(^+\) T cell response is analysed in asymptomatic primary CMV-infection. Longitudinal analyses shows that CMV-replication becomes apparent between 18 and 25 days in these patients. CMV-specific CD4\(^+\) T cells become emerge into peripheral blood with a minimum of 7 days and a maximum of 14 days after the first appearance of CMV-DNA. Shortly after the detection of CMV-specific CD4\(^+\) T cells CMV-specific IgM followed by IgG antibody titres can be measured. Whereas CMV-specific CD4\(^+\) T cells during primary infection expressed the activation marker CD38 and the cell cycle marker Ki-67 and had not lost CD27 as compared to CMV-specific CD4\(^+\) T cells analysed in latently infected individuals, in both groups these cells expressed VLA-4 and CD11a. Also in both primary and latently infected individuals CMV-specific CD4\(^+\) T cells displayed a Th1 cytokine profile, i.e. upon stimulation these cells secreted IFN\(\gamma\) and TNF\(\alpha\) but not IL-2 or IL-4. Remarkably, in patients experiencing primary CMV-infection, CMV-specific CD4\(^+\) T cells could only be detected for a short period in peripheral blood, inferring redistribution of these cells, most probably to peripheral target sites.

In Chapter 2, both CMV-specific CD4\(^+\) and CD8\(^+\) T cell responses were analysed in healthy individuals and renal transplant recipients during latency. In healthy individuals, the CD4\(^+\) T cell response measured by antigen-induced IFN\(\gamma\) production correlated with the CMV-specific CD8\(^+\) T cell antigen induced IFN\(\gamma\) response, whereas no correlation was found between the percentage IFN\(\gamma\) producing CD4\(^+\) T cells and the percentage CMV-specific CD8\(^+\) T cells as measured by specific tetramer staining. In renal transplant recipients, also no correlation was found between IFN\(\gamma\) producing CMV-specific CD4\(^+\) and CD8\(^+\) T cells. When the percentage of CMV-specific CD8\(^+\) T cells, enumerated by tetramer staining was compared in healthy individuals and renal transplant recipients, renal transplant recipients showed an increase in CMV-specific CD8\(^+\) T cells and this cell population was enriched for cytotoxic
CD27⁺ CD8⁺ T cells. However, in both healthy individuals and renal transplant recipients, CMV-specific T cell populations with either a predominantly CD27⁺ non-cytotoxic or CD27⁻ cytotoxic memory cell phenotype could be discerned. Analysis of renal transplant patients prior to transplantation and 1 year after transplantation showed that the transition of CMV-specific non-cytotoxic CD27⁺ memory cell to CD27⁻ cytotoxic memory cells was concurrent with viral replication as measured by viral shedding, thereby inferring that viral replication upon start of immunosuppressive drug therapy induces qualitative and quantitative changes in the CD8⁺ T cell compartment.

Chapter 3 analyses the kinetics and dynamics of CMV-specific antibody and CD4⁺ and CD8⁺ T cell responses in asymptomatic and symptomatic primary CMV-infection. In asymptomatic infection, CMV-specific CD4⁺ T cells are the first to appear in peripheral blood, followed by CMV-specific CD8⁺ T cells and antibody responses. Here it is shown that the absence of a CMV-specific CD4⁺ IFNγ⁺ T cell response is concomitant with the development of CMV-disease, whereas neither the emergence of CMV-specific antibody responses nor the emergence of the CMV-specific CD8⁺ T cells is different in time. Also with respect to the differentiation pattern of the CMV-specific CD8⁺ T cell population, as well as their capacity for direct ex-vivo lysis of CMV-peptide loaded target cells, no difference was found compared to primary infection in asymptomatic individuals. Furthermore, it is shown that in the acute phase of the antiviral response, at viral load peak value, CMV-specific CD8⁺ T cells are CD27⁺ and CD45R0⁺, a phenotype formerly designated as not cytotoxic, yet contain perforin and Granzyme B and are capable of target cell killing.

Chapter 4 summarises the findings of chapter 2 and 3 and their implications for the interpretation of virus-specific subset analyses in health and disease.

Chapter 5 describes the phenotypical changes of CMV-specific CD8⁺ T cell populations in vivo in situations of viral reactivation and relates the size and phenotype of virus-specific CD8⁺ T cell populations to the amount of antigenic activation, most notably the induction of CD70. Upon reactivation both CMV-specific CD27⁻CD45R0⁻ and CD27⁻CD45RA⁺ CD8⁺ T cell populations proliferate and increase in number of cells, whereas phenotypically CD27⁺CD45R0 CMV-specific CD8⁺ T cells differentiate to CD27⁻ cytotoxic cells. In vitro stimulation of CD27⁺ CMV-specific CD8⁺ T cells with specific peptide in combination with IL-2 or IL-15 induced CD70 expression and consequent differentiation to
CD27+ CD8+ cytotoxic CMV-specific T cells whereas stimulation with CMV-peptide and IL-21 propagated outgrowth of CMV-specific CD8+ T cells but not differentiation.

Chapter 6 analyses the proliferation requirements of cytotoxic CCR7CD28CD27CD45RA+, antigen specific cells in vitro. Antigen specific stimulation could induce proliferation of these cells when stimulated with peptide in the presence of CMV-antigen, IL-2, IL-15 and IL-21. Upon stimulation and subsequent proliferation, CD45 changed its isoform from CD45RA to CD45R0, as was CCR7 reexpressed on activated cells. These results show that CD27CD45RA+ CD8+ T cells are not end-stage differentiated effector cells without proliferative potential and can be expanded provided with specific stimulation signals.

Chapter 7 investigates possible cross-reactivity of CMV-specific CD8+ T cells to alloantigens. CMV-specific CD8+ T cells could be induced to proliferate by alloactivation and this proliferation could be blocked by MHC class I antibodies but not class II antibodies, inferring this proliferation was TCR induced. Also EBV specific CD8+ T cells proliferated on alloantigen stimulation, and, when assessed in the same responder these two distinct virus-specific populations showed a different responsiveness upon different allo-MHC class I molecules.

In chapter 8 a new model is introduced regarding the generation of virus-specific CD8+ T cells and its consequences regarding the interpretation of subset analyses in different viral infections are discussed.