Mechanisms of decreasing HIV-1 specific CD8+ T cell activity during progression to AIDS
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Summary

HIV-1 infection is characterised by a gradual loss of CD4+ T cell counts and loss of immune functions. HIV-specific cytotoxic T lymphocyte (CTL) activity can be readily found in most infected individuals, however, after prolonged chronic HIV-infection, CTL activity is lost leading to increased viral load and opportunistic infections.

In this thesis the clonal composition of CD4+ and CD8+ T cell subsets is examined, and phenotypic and functional characteristics of HIV-specific CD8+ T cells were followed in order to investigate the mechanism of the declining antiviral CD8+ T cell activity during the course of HIV infection.

The prologue chapter 2 is an introduction into CD8+ T cell subsets and the technique of CDR3 spectratyping. Phenotypic separation was performed to isolate naive CD27+CD45RA+, memory CD27+CD45RA−, and effector CD27−CD45RA+/− T cells and these subsets were investigated for replicative history and clonal composition. Telomer lengths provided evidence that memory and effector T cells had significantly shorter telomer lengths than naive T cells indicating that repeated rounds of cell divisions had occurred in these subsets.

By CDR3 spectratyping, the RNA-transcripts of the gene encoding the T cell receptor (TCR) are analysed for size diversity. Each T cell has generated its clonotypic TCR gene in order to generate a large variety of TCR. The more different TCR sizes, the more different T cells are present. CDR3 spectratyping revealed that naive T cells were composed of many different CDR3 sizes, whereas the repeated divisions of memory and effector T cells were antigen-driven as CDR3 size patterns were perturbed by clonal expansions.

The CDR3 spectratyping in chapter 3 showed that both the memory and naive CD4+ T cell subsets were considerably skewed in HIV-1 infected individuals. At that time, missing CDR3 sizes were regarded as holes in the repertoire, which should be replenished after antiretroviral treatment. Following treatment, the diversity of both subsets showed an initial rise in the presence of clonal expansions in peripheral blood, together with the initial redistribution of CD4+ T cells. After one year of treatment, clonal expansions were reduced in size resulting in more diverse repertoires, however, not as diverse as healthy controls. The data reflected the repopulation kinetics of the CD4+ T cells which indicated that initial redistribution of expanded clones repopulated peripheral blood early during therapy, and that the regeneration of new CDR3 sizes occurred as slow as the replenishment of naive CD4+ T cells.

CD8+ T cell populations were composed of large clonal expansions that dramatically skewed TCR diversity in naive and memory CD8+ T cells (Chapter 4). In contrast to CD4+ T cell repertoires, therapy reduced the number and or sizes of the clonal expansions, presumably due to suppression of HIV-antigens and immune activation. However, while dominant CD8+ T cell clones disappeared, other clones emerged in peripheral blood during the first months of treatment. By comparison of CDR3 sizes in lymph-nodes before therapy, it appeared that several clones migrated from lymph-nodes into the blood during therapy. By temporally and spatially tracking CDR3 sizes evidence was obtained for recirculation of T cell clones after antiretroviral treatment.

Although regeneration of new CDR3 sizes in the naive T cell pool could be observed in several occasions in chapters 3 and 4, missing CDR3 sizes may not have reflected truly physical absence of T cells clones as became clear in chapter 5. In two individuals' CD4+ T cell populations, several CDR3 sizes were undetectable at one point in time, and became detectable again the next time-point. These individuals did not undergo interventions which may account for thymic rebound or other sort of regeneration of naive T cells, hence the reappearance of previously undetectable CDR3 sizes indicated that these CDR3 sizes were never depleted, but had been temporarily undetectable. In the discussion chapter 6, the possible interpretations from CDR3 spectratyping are discussed.

In part two, HLA-peptide tetrameric complexes were used to identify virus specific CD8+ T cells. This allowed for phenotypic and functional analysis of virus specific T cells on the single cell level. In some occasions, EBV- specific CD8+ T cells were included to compare these with HIV-specific T cells.

In chapter 7 HLA-A2 restricted HIV-specific T cells were followed during the natural course of HIV-infection in 16 individuals comprising mainly long-term asymptomatics. Early in infection, an inverse correlation was found between the number of HIV-specific CD8+ T cells and viral load. In addition, the
group with high CTL numbers early in infection had a delayed rate of disease progression compared to the group with lower numbers of HIV-specific CD8+ T cells. Strikingly, progression to AIDS was not necessarily correlated with loss of HIV-specific T cells. These HIV-specific T cells appeared to be mainly of the memory CD27+CD45RO+ phenotype.

In chapter 8 it was investigated whether the inverse correlation was evident in HIV-infected individuals expressing other HLA-types. In this cross-sectional study again an inverse correlation was found for HLA-A2 restricted HIV-specific CD8+ T cells and viral load, however, this was not true for HLA-B8 or -B57 restricted T cells. A weak inverse correlation was observed for individuals with CD4+ T cell counts higher than 400 cells per microliter blood, however, the group with lower CD4 counts contained several subjects with both high numbers of tetramer+ HIV-specific T cells and high viral load. This was not explained by mutations in the virus epitopes as only 4 of 14 investigated subjects contained minor virus populations with mutated epitopes. Alternatively, tetramer+ T cells may lack antiviral activity. This was investigated by intracellular IFNγ staining after stimulation with the same peptide as complexed in the tetramers. Indeed, many tetramer+ T cells appeared not to produce interferon gamma (IFNγ). Moreover, the percentage of IFNγ+ tetramer+ T cells correlated with delayed disease progression. This suggested that many HIV-specific T cells are impaired in antigenic responsiveness and these may not be able to eliminate virus infected cells. The lack of IFNγ production appeared to be related to the maturation status, as high percentages of CD27+ were correlated with IFNγ+ percentages of tetramer+ T cells.

In chapter 9 the role of CD27- virus specific T cells on disease progression was further investigated. Most HIV-specific T cells expressed CD27 and this remained relatively stable during the natural course of infection. In contrast, high percentages of EBV-specific T cells developed into CD27- effector T cells during prolonged HIV-infection. However, those individuals who developed EBV-related non-Hodgkin lymphomas did not accumulate EBV-specific CD27- T cells. In addition, early in HIV infection (1 to 3 years after seroconversion) CD27- tetramer+ T cells specific for HIV were inversely correlated with viral load, and positively correlated with AIDS free survival. Finally, EBV-specific T cells who matured into CD27- T cells also showed higher percentages of IFNγ production. These data suggest that CD27- T cells are important effector T cells for protection against viral disease.

Chapter 10 describes the longitudinal development of antigen induced IFNγ production of HIV-specific T cells. In order to distinguish between presence and function, HIV-specific CD8+ T cells were detected by tetramer staining in parallel with intracellular IFNγ staining. During the natural course of infection, tetramer+ T cells remained relatively stable, whereas the number of IFNγ producing T cells decreased in most individuals. Also the percentage of IFNγ+ T cells relative to tetramer+ T cells decreased in most individuals when peptide stimulation was performed, however, PMA and ionomycin stimulation did not change the percentage of IFNγ producing T cells. The number of functional IFNγ producing T cells decreased concordant with decreasing CD4+ T cell numbers, suggesting that IFNγ productive capacity is dependent on CD4+ T cell help. These data indicated that HIV-specific CTL activity decreases not due to physical depletion but due to progressive impairment of HIV-specific CD8+ T cell function.

In the longitudinal study, one HLA-B57 expressing individual was included, who showed above average IFNγ production. In chapter 11, the percentage of IFNγ producing tetramer+ T cells for Gag-specific T cells restricted to HLA-A2,-B8, and -B57 were investigated. Indeed, significantly higher percentages of IFNγ producing T cells were found among HLA-B57 restricted T cells. This difference was reproducible within individuals positive for HLA-B57 and -A2 or -B8. In addition, HLA-B57 restricted T cells needed lower concentrations of peptide to produce IFNγ than T cells restricted to HLA-A2 or -B8.

In chapter 12 it was investigated whether antiretroviral therapy was able to enhance T cell function as measured by antigen specific IFNγ production. HIV-specific T cells were compared with EBV specific T cells by tetramer staining and IFNγ Elispot assays. Most individuals decreased in number of HIV-specific T cells after start of therapy and had constant levels of EBV specific T cells as measured by tetramer staining. Elispot assays, however, showed that HIV and EBV specific IFNγ producing T cells showed somewhat different kinetics than tetramer+ T cells, resulting in an improvement of the ratio IFNγ+ T cells to tetramer+ T cells. Despite the relative improvement of EBV specific T cells, no clear effect was observed on...
EBV load. The enhanced IFNγ producing capacity indicated that therapy is capable of improving the antiviral immune response.

In chapter 13 the relation between virus-specific CD8+ T cells and virus load is discussed. CTL follow the kinetics of viral load because CTL numbers depend on antigenic stimulation, and CTL decline in the absence of viral antigens. Within individuals, the CTL and viral load are positively correlated, however, due to individual differences in CTL antiviral capacity, individuals may differ in the number of CTL needed to maintain viral replication at a given level. Therefore an inverse correlation can be observed in a cross-sectional analysis. Furthermore, changes in numbers of viral antigens (like antiretroviral therapy or escape mutations) may induce similar kinetics in CTL numbers. Vice versa, changes in either CTL numbers (infusion/depletion) or capacity (lack of CD4 help) may allow inverse kinetics of viral load in time. Chapters 8 through demonstrate that indeed the CTL capacity decreases during the natural course of infection allowing an uncontrolled rise in viraemia, ultimately leading to AIDS. Because of the observed impaired CTL function, it is argued that besides antiretroviral therapy, additional treatment is necessary to overcome lack of CD4+ T cell help and to boost T cell responses. Several recent studies have shown the beneficial effects of additional vaccination next to conventional treatments, which may help the immune system to clear or control HIV infection.