Mechanisms of decreasing HIV-1 specific CD8+ T cell activity during progression to AIDS
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Evolution of HIV specific T cells during the course of HIV infection

The relation between viral load and antiviral CD8+ T cells

The current view on a CD8+ T cell immune response to viral infection has been simply shown in a recent study on LCMV infection in the mouse model (127). Acute viral infection is characterised by an initial burst of viraemia followed by a rapid and massive expansion of virus-CD8+ T cells. This massive antiviral response results in the suppression of viral replication, and ultimately to virus elimination. Due to decreased levels of antigen, subsequently the bulk of the virus-specific CD8 T cells lack antigenic stimulation and dies of apoptosis, however, a subpopulation remains and establishes a virus-specific memory pool (Figure 1A). In this model, the virus has been cleared and memory cells presumably reside in a resting state, until reactivated by recurrent infection. Similar kinetics have been found for T cell expansions in acute EBV and HIV infection (14,125,126).

Virus infections that are not cleared but controlled at low viral load levels establish a chronic infection, in which similar kinetics can be observed. Latent EBV infection for instance, is characterised by substantial fluctuations (185). CTL levels may be relatively low when EBV load is low. Occasional viral burst are accompanied by re-emerging EBV-CTL, which suppress viraemia and re-establish the resting state of infection (185,239). In this system, virus load and CTL follow the same longitudinal kinetics by reciprocal stimulation and suppression. The changes in viral burden and induction of CTL are characterised by interdependent kinetics; viral load induces the expansion of virus specific CTL, sufficient levels of CTL suppress viral load and lack of viral-antigen reduces CTL numbers. These kinetics of virus burden and CTL numbers show a positive correlation in time (Figure 1A).

HIV load and HIV-specific CD8+ T cells

Likewise, primary HIV-1 infection is accompanied by major CD8+ T cell expansions of selective Vb usage and high levels of HIV-specific cytotoxic T cells (86). This acute CTL mediated immune response is correlated with initial suppression of viraemia (13-15). By the use of CTLp LDA assays and tetramer staining a temporal positive correlation was observed indicating that CTL responses follow the viral dynamics until CTL reach sufficient levels to suppress viral load, analogous to the LCMV model.

HIV infection differs from many other viral infection models in that viral clearance is not accomplished. On the contrary, chronic HIV infection is characterised by continuous high virus production (240,241). Early in the chronic phase, equilibrium is established between viral load and immune response. The level of viral load at the equilibrium (the viral setpoint) is a determinant...
for disease progression (242). It is argued that the height of the viral setpoint in turn is determined by the initial CTL response: a strong and rapid CTL response during acute infection suppresses the acute viral burst and establishes a low viral setpoint, and vice versa (15,16). The stronger an individual's CTL response, the lower his viral burden at steady state (Figure 1B).

The relation between viral load and CTL numbers depends on the proliferative responsiveness and the lytic capacity of the CTL (243). The presence of viral antigen induces proliferation of antigen-specific CTLs, and CTLs that encounter infected cells kill target cells or secrete antiviral cytokines. The proliferative responsiveness of the antigen-specific T cell determines the number of CTL in relation to a given antigen load; the efficacy of the CTL to eliminate infected cells determines the viral load. Therefore, among individuals the equilibrium between viral load and CTL can differ because of individual differences in CTL properties (or viral properties which are not discussed here). If individual differences are restricted to variation in proliferative responsiveness, the relation between CTL and viral load can be relatively simple and can show an inverse correlation (243)(Figure 1B). However, individuals can also differ widely in CTL efficacy, as suggested by differential CD27 expression and IFNγ production (chapters 8-12). Therefore, for each infected subject, distinct levels of CTL and viral load may determine the steady state, and individuals may vary in the number of CTL necessary to maintain low viral load. Variance in effectiveness of CTL to suppress viral replication can explain why progressors with high viral load can have equal levels of CTL as non-progressors have, and why long-term asymptomatics can have low viral load despite low numbers of CTL (222,243) (Figure 1C). Only in homogenous study populations in which similar T cell responses are measured, inverse correlations may be evident.

In this thesis it is shown that in a homogenous group of mainly long-term asymptomatics, the number of HIV-specific, HLA-A2+ tetramer positive T cells inversely correlates with viral load, and that the number of HIV-specific T cells correlates with disease free follow up (26)(Chapter 7). In a more heterologous group, only a moderate correlation was found only in the subpopulation with high CD4+ T cell counts (Chapter 8, ref.(189)). This is likely due to the fact that not all tetramer+ T cells are equally efficient in virus suppression, as antigen induced IFNγ production differed substantially among individuals. Therefore, tetramer staining is not representative of the true antiviral capacity. Conversely, the percentage of CD27 negative HIV-specific T cells, inversely correlated with viral load and again positively correlated with AIDS free survival (Chapter 9). The percentage CD27− effector cells represents a functional characteristic of CTL, as CD27− T cells contain more perforin, and can kill target cells without prior restimulation. Therefore, the dichotomy between tetramer staining and antiviral activity is corrected for and CD27− tetramer+ T cells may better approach the number of antiviral CTL.

**Perturbation of the viral load-CTL equilibrium**

As mentioned above, the relation between HIV-load and HIV-specific CTL in chronic HIV-infection can be described as a longitudinal positive correlation when an individual subject is investigated. Within one individual, the CTL response will follow most of the fluctuations of antigen load as expansion and survival of CTL depends on the presence of antigenic stimulation. Nonetheless, both positive and negative correlations can be observed in time after perturbation of the equilibrium through suppression or enhancement of either viral load or CTL numbers, either artificial or natural.

Antiretroviral therapy acts directly on viral replication and artificially reduces the antigenic burden. Therefore the equilibrium between virus load and CTL is perturbed in a person in time. Expanded T cells will receive lower antigenic activation signals resulting in shrinking of a subset, and the remaining of a smaller population (Figure 2) (89) (90)(Chapter 4). This observation shows that HIV-specific T cell expansions are antigen driven, and the HIV-specific T cell pool is maintained through the presence of antigen.

CTL do not detect viral load, but detect viral epitope-peptides on the surface of infected cells. For individual CD8+ T cells, disappearance of the epitope has the same effect as disappearance of virus infection. Epitopes that mutate to unprocessed or unrecognized amino acid sequences result in the persisting of CTL recognizing the original peptide sequence (19,22)(Figure 2). However, many HIV-infected subjects are described with dominant CTL responses against epitopes that are not mutated and remain recognized (174,244)(Chapter 8, ref.(189)).
Opposed to reduction of antigenic load, vaccination increases antigen burden. Harmless virus-like particles, virus –proteins, -DNA, or -peptides do not enhance viral pathogenesis but may stimulate T cells specific for the administered particles to ideally higher levels than natural infection accomplishes (Figure 2). Previously, various vaccines have been tested, mostly without inducing protective immunity, or just inducing moderate changes in CTL activity such as Gag protein vaccination (212,213). In chapter 11, two of these patients are described and are shown to have temporal increases in CD8+ T cell numbers, reduction in viral load, and moderate increases in Gag-specific tetramer+ CD8+ T cells during or after vaccination. More recently, promising results have been obtained with prime boost DNA / recombinant virus vaccines, cytokine augmented vaccines, and vaccination administered to patients treated with antiviral drugs has led to increased CTL responses (202,245-247).

Infusion of HIV-specific CTL can lead to a temporal reduction in viral load (27). The infused CTL migrated to the site of infection and colocalised with infected CD4+T cells, however, a few weeks to months later, the infused CTL disappeared and viral load rebounded (27). In this study the elevation of CTL numbers had a reciprocal effect on the virus load. In other studies, infused CTL selected for escape mutants or massively died of apoptosis, leading to rapid progression to AIDS (145,248). Opposite to CTL infusion, depletion of CTL leads to severe increase in viraemia, as observed in SIV infected macaques. Antibody mediated depletion of CD8+ T cells resulted in loss of control of primary infection, and increased viraemia during chronic infection, whereas after gradual replenishment of CD8+ T cells, viral load was suppressed again (28,29).

When the apparent antigenic burden is changed, CTL and apparent antigen load follow similar kinetics (therapy, vaccination, escape). Conversely, when CTL numbers are changed, viral load shows inverse kinetics (depletion, infusion). An additional potential perturbation of the virus-CTL equilibrium is the change in CTL efficacy to destroy infected cells. The experiments described in Figure 2 demonstrate that CTL and viral load directly respond to changes in the equilibrium, suggesting a dynamic and functional interaction. This means that HIV-specific T cells are not completely unresponsive. CTL are readily induced in vivo and after strong in vitro stimulation, cytolitic T cell clones can be cultured from each tetramer+ T cell (88,195). Still, HIV-infection eventually ends in the loss of the CTL response in favour of HIV load, leading to AIDS.

The steady state may also be influenced by gradually changing factors. Progressive loss of CD4+ T cells or polyclonal T cell function can be taken as parameters that reflect gradual changes in the equilibrium between virus load and CTL response. It has been shown that HIV-specific CD8+ T cells are impaired in antigen-specific IFNγ production and in cytolytic activity (192,198,199), and that decreased IFNγ production of EBV-specific T cells in response to peptide stimulation has been shown to be a correlate for progression to AIDS-related non-Hodgkin lymphoma (185). In this thesis, the gradual loss of IFNγ production in response to antigenic stimulation is described. The decrease in IFNγ producing T cells is similar to the decrease in IFNγ producing T cells is similar to the decrease in CTLp frequencies in individuals progressing to AIDS (30). It is argued that lack of antigen induced IFNγ production is a reflection of loss of antiviral activity. Although absolute HIV-specific T cells remain stable during the course of infection, fewer functional CTL are left to kill virus infected cells, and this may cause the balance between CTL and viraemia to gradually shift in favour of the virus.
Impaired function of HIV-specific CD8+ T cells

This thesis describes the loss of peptide-specific T-cell responses in HIV-infected individuals. T cells also showed progressively reduced responsiveness to polyclonal stimulation like PMA+ionomycin, CD3 antibodies or PHA (42,44,45), which could be restored by CD28 costimulation (44). When stimulated in the presence of costimulatory molecules such as CD28 and CD49d monoclonal antibodies, most HIV-specific T cells do produce IFNγ in response to peptide-antigen (177,195), suggesting that excessive stimulation is capable to induce HIV-specific T cell responses. Similarly, differences in antigen responsiveness of EBV-specific T cells in healthy donors have been attributed to different stimulation requirements (191). These different requirements may represent central- and effector-memory T cells (191,197), and in healthy donors, these subsets are balanced with respect to control of viraemia. HIV infection is characterized by high viraemia, which would suggest the need of high frequencies of effector cells. However, most HIV-specific T cells lacked phenotypic markers for effector functions and in contrast resembled memory T cells with regard to expression of CD27, CD45RA, CCR7 (177,249)(Van Baarle, submitted Chapter 9). In addition, large frequencies of HIV-specific T cells showed impaired production of antiviral proteins such as IFNγ or perforin (88,162,177,192,193,199)((189)Chapter 8), suggesting impaired antiviral effector function. Apparently, HIV-specific T cells are impaired in maturation to fully differentiated effector CTL. We argue that lack of IFNγ production in short-term incubation assays reflects impaired effector function and that high IFNγ production reflects strong effector function and correlates with delayed disease progression. Although lack of IFNγ production does not necessarily exclude other effector functions, several conditions are found in which IFNγ production was correlated with the clinical status. First decreased IFNγ production correlates with progressive infection and CD4+ T cell depletion (Chapters 8,10)(178). The protective HLA-B57 allele is associated with higher IFNγ production (Chapter 11). Finally, therapy improves the clinical condition of patients coinciding with their IFNγ production (Chapter 12).

Since IFNγ production decreased during progression to AIDS, we favour the hypothesis that antiviral CTL activity is failing because of dysfunctional CD8+ T cells, not because of depletion or because of proliferative exhaustion of CD8+ T cells. Whether this is due to lack of CD4+ T cell help remains to be investigated, but alternative help for CD8+ T cell may be provided by immuno therapeutic interventions. Promising results have been obtained for individuals treated early in infection who undergo structured treatment interruptions (STI). During untreated periods, autologous virus acts as a booster for immune responses while because of early treatment adequate numbers of HIV-specific CD4+ T cells were preserved (184). However, this is not an option for many other individuals who have already lost crucial immune functions (250) Alternatively, chronically treated individuals may benefit from vaccine like adjuvants that can boost immuneresponses in the absence of viral replication (201,202). In addition, IL2 is currently evaluated as an immunostimulatory adjuvant (251). It has become evident that neither antiretroviral therapy, nor the natural immune response is capable of eliminating HIV infection. Additional immunotherapies should be developed to improve CD4 and or CD8 T cell responses, to clear residual HIV replication.