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

The immune system protects the human body against various potential pathogens by recognizing and eliminating non-self structures. The acquired immune response largely depends on lymphocytes such as CD4 and CD8 T-cells. These cells possess receptors to specifically recognize invading pathogens and are able to suppress or eliminate the infection. The human immunodeficiency virus (HIV), however, is able to establish a chronic infection without being completely eradicated by T cells. Initially, HIV-specific T cells do suppress the replication of HIV, but eventually lose control of the infection. In addition, after prolonged incubation, HIV-infected individuals lose their protective immunity against other pathogens than HIV. The basic mechanism of this immunodeficiency, defined as acquired immunodeficiency syndrome (AIDS), is not completely clear yet.

Figure 1. Antigen presenting cells (APC) present antigens to CD4+ T cells leading to reciprocal activation of APC and CD4 helper cell (A). APC and CD4+ T cell can now provide direct or indirect help to CD8+ T cells (Arrow). CD8+ T cells recognize target cells (B) by binding of the CD8 T cell receptor to the peptide antigen presented by MHC class I proteins on the surface of infected cells (C). The CDR3 region of the TCR determines which peptide is recognized by the T cell.

The cellular immune response

The T cell mediated immune response generally starts with the uptake of foreign antigen by dendritic cells, at the site of initial infection. These cells are specialised in collecting material and presenting it to CD4+ T cells, for which they are called antigen presenting cells (APC) (Figure 1A). A CD4+ T cells that recognizes its cognate antigen presented by an APC is activated and in turn activates the APC by CD40 CD40L interactions (1,2). This starts of the immune response and leads to the direct or indirect activation of CD8+ T cells. The CD8+ T cells are cells specialised in recognizing and killing infected cells (Figure 1B), and thus are the mediators of viral clearance or control of virus infection (3).

This scenario includes two checkpoints at which the presence of a foreign pathogen is verified. Recognition of the pathogen by CD4+ T cells at the initiation of the cellular immune response, and recognition of infected cells by CD8+ T cells. This recognition is mediated by binding of the T cell receptor (TCR) with major histocompatibility (MHC) complexes (Figure 1C).

Each cell is required to show the immune system what sorts of proteins are synthesised within the cell. When a cell is infected, non-self proteins are synthesised in the cell. All nucleated cells express antigen-presenting proteins, the MHC complexes, in order to show their intracellular contents. MHC class I presents small peptides of ~9 amino-acids that are generated by proteolytic cleavage of intracellular proteins. MHC alleles differ in binding pockets for peptide binding. Peptides with appropriate amino acids at specific locations can bind in the peptide binding groove. The MHC-peptide complex is transported to the cell surface and exposes the peptide to surveying CD8+ T cells. Specialised APC have an additional antigen presentation mechanism by which extracellular antigens can be presented to CD4+ T cells in the context of MHC class II complexes.

CD4+ and CD8+ T cells recognize foreign peptides presented by MHC complexes via their T cell receptor (TCR) (4,5). This multimeric complex is composed of 2 TCR chains α and β, and accessory CD3 chains which transduce a signal into the cell after encounter with the cognate antigen. The α and β chains consist of a constant region and a variable region. The complementarity determining region 3 (CDR3) is a hypervariable region which determines the antigen binding specificity. This region is randomly generated during the maturation of T cells in the thymus, and is selected to bind with non-self, but not self-antigens. The random recombination of the CDR3 results in the generation of a T cell pool comprising a high variety of antigen-specific TCR's, which is
necessary to provide a specific T cell for each potential pathogen ever to be encountered.

After detection of a target cell, CD8+ T cells can perform several effector mechanisms against viruses. T cells can produce antiviral cytokines such as IFNγ and TNFα, which inhibit viral replication intracellularly (6,7) and Mip-1α/b, which can block HIV viral entry (8). In addition, T cells can exert cytolytic functions to kill the infected cell. CD8+ T cells can upregulate Fas ligand surface expression, which induces apoptosis of the target cell through binding with Fas, expressed on the target cell. Furthermore, cytolytic T cells may secrete perforin and granzymes to induce apoptosis. For these activities the CD8+ T cells are frequently called cytotoxic T lymphocytes (CTL), and this cytotoxicity makes them important in clearing viral infections.

The natural course of HIV-1 infection
During acute infection, viral load rapidly increases and may cause flu-like symptoms. After that, viraemia is suppressed and maintained at a level which may vary among individuals. The height of the viral load, viral set-point, is a determinant for clinical progression (9). After suppression of the acute infection most infected individuals experience a relatively stable period of clinical latency, although numbers of CD4+ T cells gradually decline and T cell reactivity decreases. Eventually viral load increases, CD4+ T cells become severely depleted until the immune system is degenerated and can not prevent the infection of opportunistic infections or occurrence of malignancies. This stage of immunodeficiency is defined as acquired immunodeficiency syndrome (AIDS). Individuals who develop AIDS quickly, are often called rapid progressors, whereas individuals who remain high CD4 T cell counts and low viral load for prolonged periods are referred to as long-term asymptomatics (LTA). Following AIDS diagnosis most patients die within 2 years of opportunistic infections or malignancies.

CTL in HIV-infection
In most HIV-1 infected individuals cytotoxic T lymphocyte (CTL) activity can be observed in chromium release assays (10-12). Ample results have been reported which strongly suggest a suppressive effect of CD8+ T cells on HIV replication. In the acute phase, the rise of HIV-specific T cells correlate with control of initial viral burst (13-15), and slower decline of CD4+ T cell numbers (16). Several escape mutations have been described which are likely selected by immune pressure exerted by HIV-specific CTL (17-22). In the chronic phase of infection, HIV-specific T cells inversely correlate with viral load (23-25), and high levels of HIV-specific T cells delay disease progression (26). Infusion of HIV-specific CD8+ T cells temporarily suppresses viral load (27). Finally, depletion of CD8+ T cells in SIV infected-macaques leads to severely increased viraemia (28,29). These lines of evidence are in contradiction with the eventual outcome, which is an increase in viral load and progression to AIDS in most individuals until now. Rapid progressors and long-term asymptomatics (LTA) do not significantly differ in the levels of CTL precursor frequencies (CTLp) (30) during the asymptomatic phase. Progressors initially can have strong CTL activity, but this activity declines concordant with an increase in viral load and a decrease in T cell reactivity (30-32). Apparently, the CTL response eventually declines and is incapable of controlling HIV-infection and preventing opportunistic infections or malignancies.

Aim of the thesis
HIV-specific CTL precursors are readily found in most HIV-infected individuals including rapid progressors. Given the fact that initially CTL responses are able to suppress viral replication and delay disease progression (13,16,33) it would be advantageous to keep a strong CTL response throughout the course of infection. However, CTLp frequencies ultimately decline leading to increased viral load and progression to AIDS in most individuals until now (34). As the clinical progression seems to correlate with the declining CTL activity it is important to understand the mechanism by which CTL activity is lost. We hypothesised that CTL activity is lost either due to physical deletion or due to functional anergy of HIV-specific CD8+ T cells. Depletion of antigen specific CD8+ T cells has been shown in LCMV infected mice (35,36). Transfused HIV-specific T cells in mice indeed can go into apoptosis due to antigen encounter (37) and after prolonged HIV infection telomere lengths of CD8+ T cells are shortened (38,39). Alternatively, HIV-infected individuals increasingly lost polyclonal T cell reactivity, whereas HIV-specific T-cell receptors were demonstrated in high frequencies throughout the course of infection (40-46). Furthermore, viral escape mutations may contribute to the failure of the CTL response (17-22). These possible mechanisms have been investigated in the
experiments described in this thesis. Insight in this process may lead to vaccination or immunotherapeutic strategies intended to improve the antiviral immune response.

Methods of research
To answer the question whether HIV-specific CD8+ T cells lose their functionality or are physically lost we intended to compare the presence and the function of CD8+ T cells. The presence of a T cell specific for a given antigen is deducted from its T cell receptor. Detection of the HIV-specific TCR was originally proposed by probing the unique CDR3 region as a clonotypic marker. For this we obtained the method of TCR-CDR3 specific polymerase chain reaction (PCR) in order to obtain TCR DNA fragments. This technique was used to assess TCR diversity in diverse clinical settings and T cell subsets.

Recently, however, a new technique was developed to detect antigen-specific CD8+ T cells: tetrameric peptide-HLA complexes (47). HIV-specific TCR's were detected by virtue of their specific binding to HIV-peptides complexed in HLA proteins. This allowed for direct phenotyping and functional analysis of antigen-specific T cells by flow-cytometry.

Phenotypic analysis was performed by combining tetramer staining with antibodies against various cell surface proteins, most notably CD27, a differentiation marker (48). In addition, T cell function was assessed by antigen induced IFNγ production. For this assay, T cells were incubated with HIV derived peptides. In vitro, these peptides exchange binding-sites with endogenously MHC-bound peptides, and activate CD8+ T cells specific for the given peptide (49). T cells that recognize the peptide may produce IFNγ in response. This cytokine is intracellularly detected, and defines antigen specific and responsive T cells.

In the first part of this thesis, T cells and T cell populations are analysed by the genetic composition of their TCR, whereas in part 2, antigen specific CD8+ T cells are selected by the tetrameric MHC-peptide complexes. These two methods approach the T-cell receptor from two different views in order to investigate T cell responses during the natural course of HIV infection and during antiretroviral treatment.