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HIV-specific CD4$^+$ T cells and viremia: who's in control?

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It has been proposed that HIV-specific CD4$^+$ T cells with a central memory phenotype might be involved in controlling HIV replication. Based on recent data (lack of protective effects of HIV-specific CD4$^+$ T-cell responses in acutely infected patients undergoing treatment interruptions; loss of initially strong T-helper cell responses in progressors to AIDS; and lack of prognostic value of HIV-specific CD4$^+$ T cells in a prospective study) we argue that the level of persistent viremia determines the fate of HIV-specific CD4$^+$ T cells. We postulate that, rather than the absence of HIV-specific T cells, it is the viral and immune activation set points that are major determinants of progression to AIDS. This influences ideas about the type of cellular immunity a protective HIV vaccine should induce.

Most chronic viral infections might have short periods of clinically manifest reactivations but normally have long periods of true viral latency. As HIV-1 preferentially infects and kills activated CD4$^+$ T cells, including those specifically activated by HIV [1,2], it has been suggested that HIV-specific CD4$^+$ T-helper responses might be irreversibly impaired during acute HIV-1 infection. Because of insufficient CD4$^+$ T-cell help [3] and progressively impaired CD8$^+$ T-cell responses, complete immune control and viral latency is never reached [4]. However, the role of HIV-specific CD4$^+$ T cells during HIV infection and more precisely their contribution to the failing immune control that is observed in progressive HIV infection is still unclear. Here, we hypothesize that the prevailing level of immune activation at the time when a steady-state level of viral replication is reached, rather than the overall magnitude of HIV-specific CD4$^+$ T-cell responses, is the major determinant of progression to AIDS.

HIV-specific CD4$^+$ T-cell function in the natural history of HIV-1 infection

HIV-specific CD4$^+$ T cells were initially detected using *in vitro* proliferation assays only in individuals with persistent low viremia such as individuals with non-progressing HIV-1 infection (long-term non-progressors, LTNPs) [5] and individuals who received anti-viral treatment during acute HIV-1 infection [1]. HIV-1 specific CD4$^+$ T-helper function was reported to be inversely related to viral load [5–8]. Using flow-cytometry-based assays to measure intracellular cytokines after stimulation of peripheral blood mononuclear cells (PBMCs) with recombinant HIV proteins or overlapping peptide pools, HIV-specific interferon-γ (IFNγ)-producing CD4$^+$ T cells were detected in all HIV-1 infected individuals, regardless of disease progression, although lower frequencies of IFNγ-producing CD4$^+$ T cells were observed in individuals that progress to AIDS (i.e. progressors compared with LTNPs) [9]. However, this difference was not confirmed by others [7,8,10]. Recent studies have shown that HIV-specific interleukin-2 (IL-2)-producing CD4$^+$ T cells and T-helper cells producing both IL-2 and IFNγ are detectable in LTNPs but not in progressors [7], and this was also shown to be the case in those individuals that control the disease versus those that do not [11]. Furthermore, the presence of these cells was found to be inversely related to viremia [8,10,12], which is consistent with proliferative CD4$^+$ T-cell responses in individuals with low viremia [5,6,13].

Effect of HAART on HIV-specific CD4$^+$ T-cell responses in chronic HIV infection

CD4$^+$ T-cell responses against recall antigens and mitogens are generally found to be restored by highly active antiretroviral therapy (HAART) [14]. With respect to HIV-specific CD4$^+$ T-cell responses, results might vary depending on which populations of cells are analysed and how long individuals were treated. The fraction of HIV-specific IFNγ-producing CD4$^+$ T cells has been shown to decrease after approximately one year of HAART [9,12], but other studies did not report HAART-induced effects on HIV-specific IFNγ-producing CD4$^+$ T-cells [10,15]. Interestingly, both IL-2-producing CD4$^+$ T cells and proliferative capacity [8,10,12,15] were found to be restored by one year of therapy in more recent studies, whereas previous studies did not report restoration of HIV-specific CD4$^+$ T-cell proliferative responses by HAART [14,16]. Moreover, despite a prior history of progressive disease, partial controllers on HAART had many immunological characteristics seen in controllers without HAART [11]. The effects of successful HAART on HIV-specific CD4$^+$ T-cell responses have been reported. After one year of therapy, numbers of HIV-specific CD4$^+$ IL-2$^+$ and IFNγ$^+$ and, to a lesser extent, IL-2$^+$ CD4$^+$ T cells were higher than

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pre-HAART levels, whereas a decrease in numbers of IFNγ$^+$ CD4$^+$ T cells was observed. However, after five years of treatment, all cytokine-producing HIV-specific CD4$^+$ T cells were decreased compared with pre-HAART levels [17].This could be a reflection of the persistent decrease in HIV load, as was proposed to explain the decrease in HIV-specific CD8$^+$ T-cell responses after long-term HAART. Interestingly, HIV-, cytomegalovirus (CMV)- and Epstein–Barr (EBV)-specific CD4$^+$ T-cell proliferative capacity, as well as polyclonal CD4$^+$ T-cell proliferative responses, increased after five years of HAART, suggesting that the improved proliferative response is not specific for HIV, but reflects a more general improvement of anti-viral immune responses that is induced by HAART.

HIV-specific CD4$^+$ T-cell responses during treatment of acute HIV infection

T-cell responses against mitogens, and recall and HIV antigens have been shown to be impaired within three months of acute HIV-1 infection [18]. Walker and colleagues [1] therefore suggested that early treatment limiting virus-induced damage to the immune system might preserve HIV-specific immune responses. With hindsight, the more recent reports on massive memory CD4$^+$ T-cell depletion in mucosal (gut) [19,20] and lymphoid tissues [21] during acute HIV infection support this line of thinking in that initial damage control by HAART during acute infection might significantly prevent the loss of functional CD4$^+$ T-cell populations. Indeed, several laboratories have shown preserved HIV-specific CD4$^+$ T-helper responses after early treatment (i.e. within 0–2 weeks of HIV-1 infection) with HAART during acute HIV-1 infection. This rescue of CD4$^+$ T-helper responses was initially associated with evidence of immune control of viremia after interruption of therapy in a fraction of the patients studied [5,22,23]. More recent studies showed that early treatment resulted in a preservation of total CD4$^+$ T-cell numbers rather than an increase in functionality of these cells. However, in four out of five patients who decided to stop therapy, this preservation was not associated with control of viremia upon treatment interruption, thus questioning the beneficial effect of early HAART and in particular the structured treatment interruptions during primary infection [24]. Lack of durable viral control despite detectable HIV-specific T cell responses was recently also reported by Walker and co-workers [25]. Upon long term follow up of their initial study group they found that, despite early signs of immune control, viremia rebounded and CD4$^+$ T-cell loss seemed faster than in untreated historic controls [25]. Thus, despite the initial promising results of the short-term early treatment during acute HIV-1 infection, it is now clear that this is only a transient benefit and might not be a therapeutic option for the future.

HIV-specific CD4$^+$ T-helper responses and viral load: cause and effect

Although the inverse relationship between HIV-specific CD4$^+$ T-cell function and viral load is widely accepted, there is no consensus regarding their causal relationship. Initially it was thought that loss of HIV-specific CD4$^+$ T cells in progressive HIV-1 infection allowed viral load increases and the subsequent development of AIDS [5]. Later it was proposed that the decrease in HIV-specific CD4$^+$ T-helper cells is caused by viremia and is part of the progressive deterioration of the immune system [8]. Iyasere *et al.* [26] described the presence of HIV-specific CD4$^+$ T cells with proliferative capacity in individuals in whom viremia was successfully suppressed by HAART. Interrupting therapy resulted in an increase in viral load, which was accompanied by loss of HIV-specific CD4$^+$ T-cell proliferation. Upon re-initiating therapy, viral load decreased again and HIV-specific CD4$^+$ T-cell proliferative capacity was rapidly restored [26]. The kinetics of this restoration of CD4$^+$ T-cell functionality was not compatible with restoration through *de novo* production of HIV specific CD4$^+$ T cells. This indicates that the level of viremia determines HIV-specific CD4$^+$ T-cell responses. Interestingly, not all studies that investigated HIV-specific CD4$^+$ T-cell function reported an inverse relation between HIV-specific IL-2$^+$ or proliferating CD4$^+$ T cells and viral load [7,15,24], underscoring the complexity of the relationship between viral load and HIV-specific CD4$^+$ T-cell function. On the basis of analyses in individuals who were treated during acute HIV-1 infection and subsequently decided to stop therapy [24], a bell-shaped relationship was proposed between plasma HIV-1 RNA and HIV-specific helper responses (Figure 1). Early in HIV infection, chronic exposure to viremia drives proliferation of CD4$^+$ T cells until the optimal relationship between plasma HIV-1 RNA and proliferation is found (the peak of the curve). This is the balance between maximal proliferation and plasma HIV-1 RNA. If the amount of virus further increases, proliferative capacity becomes progressively impaired and this results in a negative correlation between plasma HIV-1 RNA and proliferation. The level and persistence of viremia might

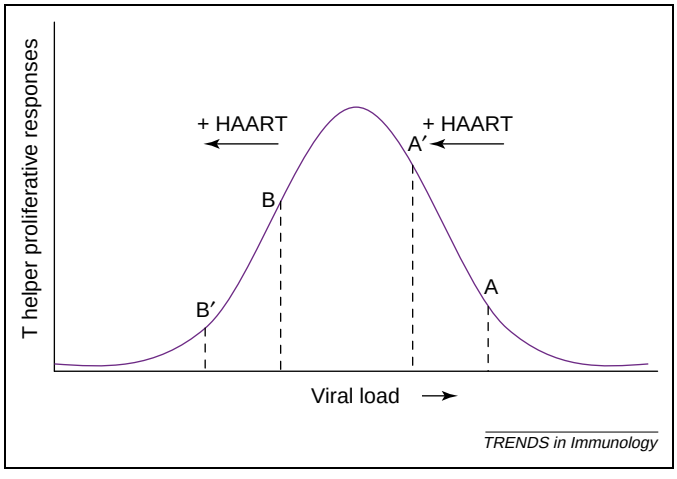

Figure 1. The relationship between viral load and proliferative responses of HIV-specific CD4$^+$ T-cell responses. Supposing that HIV-specific CD4$^+$ T-cell proliferative responses are driven by viral load [22], we propose the following model. Early in HIV infection, viral load will drive proliferation of T-helper cells until the optimal relation between viral load and proliferation is reached. If viral load further increases, proliferative capacity becomes impaired (A). Treatment with HAART will decrease viral load, which results in an increase in proliferation (A′). Treatment early in HIV-1 infection, when viral load is low (B), will result in a low-level proliferation of HIV-specific CD4$^+$ T cells (B′).

therefore determine the fate of the HIV-specific T helper cells, and thereby has an important role in HIV-1 pathogenesis.

Skewing of HIV-specific CD4$^+$ T cells towards the effector phenotype by persistent viremia

Several stages of CD8$^+$ and CD4$^+$ T-cell differentiation have been defined on the basis of a set of differentiation markers including CCR7, CD27, CD28, CD45RO and CD45RA [27,28]. A linear model of CD8$^+$ T-cell differentiation initially proposed by Lanzavecchia and Sallusto [29] has been extended to HIV-specific CD4$^+$ T cells to try to understand the impact of HIV-viremia on the size of memory and effector CD4$^+$ T-cell pools. In individuals with low levels of virus, central memory (Tcm: IL-2$^+$IFNγ^+CD45RA$^-$CCR7$^+$) and effector (Tem1: IL-2$^+$IFNγ^+CD45RA$^-$CCR7$^-$) CD4$^+$ T-cell pools are maintained by periods of reactivation and by virtue of their IL-2-dependent self-renewal capacity. In individuals with high viral load, HIV-specific naïve and resting memory CD4$^+$ T cells are continuously activated and preferentially induced to differentiate into IFNγ-only producing Tem2 cells (IL-2$^-$IFNγ^+CD45RA$^-$CCR7$^-$), which lack the capacity to produce IL-2 and proliferate, but produce IFNγ.

Thus in viremic individuals, HIV-specific CD4$^+$ T cells are continuously driven to the presumably short-lived IFNγ^+ Tem stage that lacks self-renewal capacity, whereas the IL-2-producing Tcm population diminishes. Suppressing viremia by anti-retroviral therapy might restore the Tcm population [12,13,17], which suggests that the differentiation state of antigen-specific CD4$^+$ T-cell responses is regulated by antigen exposure and antigen load [30,31].

HIV-specific CD4$^+$ T cells and protection from progression to AIDS

Studies in which HIV-specific CD4$^+$ T cells were analysed in LTNPs and individuals progressing to AIDS, clearly demonstrated higher percentages of IL-2$^+$ or IL-2$^+$IFNγ^+ CD4$^+$ T cells in individuals with non-progressing disease and low viral load [10,12,26]. This suggests an association between HIV-specific IL-2$^+$ CD4$^+$ Tcm cells and protection against progression to AIDS. The protective potential of IL-2$^+$ Tcm CD4$^+$ T cells in humans was supported by findings in HIV [32] and hepatitis C (HCV) infection [33], where these cells were only observed in individuals controlling viremia. Conclusions from these correlative studies in humans were to some extent corroborated by studies in mice showing that adoptive transfer of CD4$^+$ T-cells with Tcm characteristics could transfer protective immunity to naïve recipient mice in a Leishmania mice model [34]. Thus, in both humans and mice, Tcm$^+$ CD4$^+$ T cells appeared to be preferentially associated with protective immunity, suggesting that the lack of CD4$^+$ T cells of the memory phenotype could indeed be one of the major problems in lack of control of HIV-1 infection.

Both murine studies and recent studies in humans have now provided evidence of a role for CD4$^+$ cytotoxic T lymphocytes (CTLs) in the immune response [35–37]. However, their precise role and its importance is still unclear.

Most studies on HIV-specific CD4$^+$ T-cell function during HIV infection compared LTNPs with progressors, which makes it difficult to determine a causal relationship between the absence of HIV-specific CD4$^+$ T cells and progression to AIDS. It could, as discussed above, also be that the decrease in HIV-specific CD4$^+$ T-cell function is a consequence rather than a cause of progression to AIDS. In humans, only prospective studies in larger cohorts might solve this issue of causality.

A longitudinal analysis of HIV-specific CD4$^+$ T-cell function in six non-progressors and seven progressors to AIDS with well-defined clinical endpoints showed equal numbers of Gag-specific IFNγ-producing, IL-2-producing, and IL-2- and IFNγ-producing CD4$^+$ T cells in both groups one year after seroconversion [38]. Interestingly, in the course of HIV-1 infection, cytokine-producing CD4$^+$ T cells strongly decreased only in progressors and this was paralleled by a decrease in proliferative capacity. The results suggested that the presence of HIV-specific CD4$^+$ T-cell responses early in HIV-1 infection did not protect against progression to AIDS in treatment naive patients. These preliminary data prompted us to perform a prospective study in 96 HIV-infected participants of the Amsterdam cohort with known seroconversion dates and well-defined clinical endpoints in which HIV-specific cytokine producing CD4$^+$ T cells were analysed one year after seroconversion. HIV-specific CD4$^+$ T-cell numbers had no prognostic value for the development of AIDS [38]. The presence of HIV-1 specific CD4$^+$ T cells early in HIV-1 infection therefore did not appear to protect against progression to AIDS.

CD4$^+$ T-cell help and an effective CD8$^+$ T-cell response

Although HIV-specific CD4$^+$ T cells do not seem to protect from progression to AIDS, CD4$^+$ T-helper cells might have a role in maintaining HIV-specific CD8$^+$ T-cell responses. T-helper dependence on the generation and late stage maintenance and expansion of functional pathogen-specific CD8$^+$ T cells has been shown in mice [39,40] and humans [3,41]. Furthermore, p24-specific proliferative CD4$^+$ T-cell responses were found to correlate with levels of Gag-specific CTL precursors [3] and the fraction of HIV-specific IFNγ^+ CD8$^+$ T cells correlated with total numbers of CD4$^+$ T cells [42]. However, when cytokine-producing HIV-specific CD4$^+$ T cells were related to numbers and functionality of HIV-specific CD8$^+$ T cells, only numbers of Gag-specific IL-2$^+$-and-IFNγ^+ CD4$^+$ T cells were positively related to the fraction of IFNγ^+ tetramer$^+$ CD8$^+$ T cells one year after seroconversion (C.A. Jansen *et al.*, unpublished). This relationship was not observed late in HIV-1 infection or for the other populations of cytokine-producing CD4$^+$ T cells, the latter finding agrees with a previously described lack of correlation between Gag-specific IFNγ producing CD4$^+$ and CD8$^+$ T cells [43].

Lack of evidence for a role for HIV-specific CD4$^+$ T-cell help in a functional HIV-specific CD8$^+$ T-cell response might be explained by direct infection and priming of dendritic cells (DCs), followed by signalling through the

surface molecule CD40 on the antigen-presenting cells, as has been described for other pathogens [44]. Indeed, HIV-infection of DCs *in vitro* as well as loss and infection of DCs *in vivo* has been reported [45]. Thus, although lack of $CD4^+$ T-cell help has been suggested for many years to be one of the major causes for inadequate maintenance of HIV-specific $CD8^+$ T cells, no conclusive evidence in support of this assumption has been reported until now.

Concluding remarks

Progression to AIDS is a complex, multifactorial process, in which both viral and host factors are involved. Recently, HIV-1 has been demonstrated to be strongly adapted to human leukocyte antigen (HLA) restricted CTL responses at the population level [46]. This suggests a strong adaptation of HIV-1 to CTL responses most frequently encountered in the population, determined by the most frequent HLA alleles. The adaptation of the virus after an early encounter with HIV-specific $CD8^+$ T cells might result, depending on the strength and specificity of the initial HLA class I restricted CTL response, in a virus with reduced fitness and thus in a lower viral load set point. The initial interaction between virus and host during the first weeks of primary HIV-1 infection therefore determines the viral set point, but subsequently also determines the level of HIV-specific $CD4^+$ T cells and the so-called 'immune activation set point' (Figure 2). Given that immune activation has been reported to be a strong predictor for progression to AIDS [47] and increased immune activation has been shown in rapid progressors already early in HIV-1 infection [48], persistent activation of the immune system might be a major determinant of progression to AIDS [49]. In agreement with this, it has been shown that patients that had been exposed frequently to HIV but did not become infected might have relatively low levels of T-cell activation and reactivity [50].

Persistent immune activation might lead to exhaustion of the naïve CD4 and CD8 T-cell pool [48] and might cause replicative senescence and loss of $CD8^+$ T-cell function [51]. Diminished control by HIV-specific $CD8^+$ T cells will result in increased viral replication, which results in an increase in the occurrence of viral escape mutants. Because of viral escape, viral load will increase resulting in further loss of functional HIV-1-specific $CD4^+$ T-cells, and thus a progressive deterioration of the immune system. Taking these new insights together, it seems that the major determinant of progression to AIDS is not the absence of functional HIV-specific $CD4^+$ T cells, but a high immune activation set point early after the viremia has peaked and when the immunological effects of acute infection are resolved (Figure 2). The data show wide variation in the strength of immune activation between HIV infected patients in response to viral load, which is possibly determined by genetic factors that might determine the host immune response phenotype in general [52].

This view of AIDS pathogenesis is supported by many observations in SIV-infected sooty mangabeys (SMs) [53]. SMs are a natural host for SIV in that they do not show $CD4^+$ T-cell loss and do not progress to AIDS-like disease. Interestingly, SMs have high viral loads and appear to have numbers of SIV-infected cells comparable to numbers of infected cells reported in HIV-infected humans. Despite high viral load and SIV cytopathicity, little immune activation, as reflected in generalized leukocyte activation, T-cell division and death rates and activation of the cytokine network, is observed. It has been proposed that, by ignoring the high level SIV infection, the SMs have a minimal loss of $CD4^+$ T cells resulting from infection and are healthy despite infection with cytopatic SIV.

Given that the presence of functional HIV-specific CTL responses [54] and $CD4^+$ T-helper responses [38] do not protect against progression to AIDS in the chronic phase of HIV infection, a vaccine to improve HIV-specific cellular immune responses in individuals who are already infected with HIV-1 might not be effective. However, if the level of immune activation and the amount of virus (both major determinants of progression to AIDS) are determined by the effectiveness of the CTL response during the first days to weeks of acute infection, an effective vaccine should aim to elicit strong CTL responses to conserved epitopes

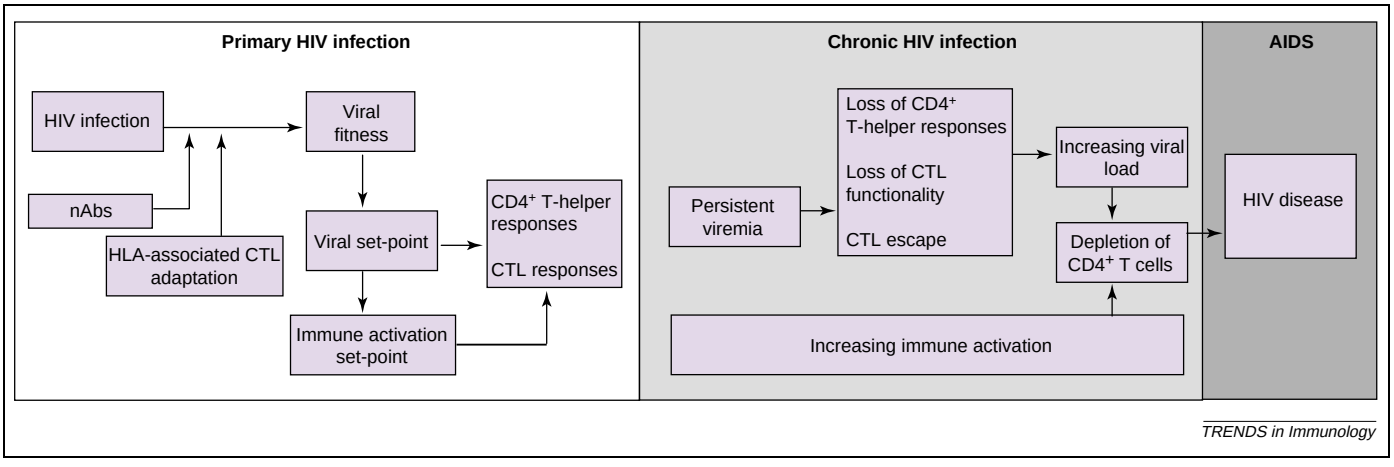

Figure 2. Events following infection with HIV, eventually leading to progression to AIDS. The HIV-specific $CD8^+$ T-cell response immediately after infection, as well as the presence of neutralizing antibodies (nAbs), determine the viral set point that is achieved after the peak in viral load observed in primary infection. Subsequently, the viral set point determines the level of immune activation (immune activation set point) and elicits cellular immune responses. In the chronic phase of infection, persistent viremia eventually results in loss of HIV-specific cellular immune responses, and a subsequent increase in viral load. This increased viral load, together with increased immune activation, will cause severe depletion of $CD4^+$ T cells, eventually leading to the development of AIDS.

known to be associated with severe fitness loss when forced to mutate under CTL pressure. Although some suggestions might be obtained from animal models, it remains unclear at this stage what type of class II restricted T helper response might be required to generate and sustain an optimal $CD8^+$ T-cell response and, in parallel, to elicit broadly neutralizing antibodies in humans.

Acknowledgements

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