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Immune dysregulation and CD4$^+$ T cell loss in HIV-1 infection

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Introduction

Acquired immunodeficiency syndrome (AIDS) is the clinical outcome of human immunodeficiency virus type 1 or 2 (HIV-1, HIV-2) infection. Acute infection with HIV, which may present with influenza- or mononucleosis-like symptoms [148], is followed by an asymptomatic period of a few months to more than 13 years. This asymptomatic period is characterized by declining numbers of circulating CD4$^+$ T helper (Th) cells, eventually leading to immunodeficiency and clinical symptoms defining the AIDS diagnosis. The symptoms observed may be lymphadenopathy, infections with opportunistic pathogens, neoplasms such as Kaposi sarcoma and non-Hodgkin lymphoma, neurological symptoms and dementia. The opportunistic infections are mainly caused by intracellular pathogens such as viruses, mycobacteria, protozoa and fungi which, as the malignancies observed, are indicative for failing cellular immunity.

T cell turnover in HIV infection

The hallmark event in AIDS pathogenesis is the loss of CD4$^+$ cells. The risk for development of AIDS-associated symptoms increases with declining CD4$^+$ T cell numbers [50, 105]. The very gradual loss of circulating CD4$^+$ T cells has long been regarded as a sign that the disease process had slow dynamics with relatively little virus activity in the asymptomatic phase. New insights into virus-host interaction in HIV infection came with the novel results on viral dynamics reported by the groups of Ho and Shaw [64, 158]. They showed that treatment of relatively late stage patients with anti-viral drugs, results in rapid clearance of free virus in plasma which is accompanied by a surprisingly quick increase of the number of CD4$^+$ T cells in

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peripheral blood even in patients with very low $CD4^+$ T cell counts before treatment. Mathematical modelling of the kinetic data showed that turnover of plasma virions and virus-infected cells was very fast. Assuming a steady state before treatment, plasma virion half-life is now estimated to be in the order of 6 h. $CD4^+$ T cell production was estimated to be in the order of 2×10^9 cells per day. Thus, the gradually increasing small net loss of $CD4^+$ T cells as observed in the course of HIV infection has, since that study appeared, been interpreted to be an increasing difference between two main factors, namely the clearance and renewal of $CD4^+$ T cells.

The interpretation of these studies, however, is hampered by our lack of quantitative information on $CD4^+$ T cell turnover in non-infected healthy subjects. Moreover, it has been argued by others that the rapid increase in peripheral blood $CD4^+$ T cell numbers after anti-viral treatment might be due to redistribution of cells coming from lymphoid tissues and is not due to newly formed cells derived from peripheral or thymic precursors. Preliminary results from Ho et al. [65] of phenotypic analyses of the repopulating $CD4^+$ T cells after anti-viral therapy, in an attempt to determine where they originate from, based on the expression of activation markers or homing receptors, showed that most of the $CD4^+$ T cells were cycling and had an activated memory phenotype which they have interpreted as evidence for post-thymic proliferation. It remains to be seen whether this interpretation is correct.

Programmed death of T cells in HIV infection

Before these studies appeared, several other observations have supported the idea of increased turnover of T cells in HIV infection through apoptosis, or programmed cell death (PCD). Ameisen and Capron [3] were the first to propose that in HIV infection interaction of soluble gp120 with CD4, previously shown to lead to impaired lymphocyte function [26], could prime $CD4^+$ T cells for PCD, which might provide a mechanism for $CD4^+$ T cell loss. Indeed, peripheral blood mononuclear cells (PBMC) from HIV-infected individuals die due to PCD in vitro [59, 61, 78, 94, 95, 117, 121]. PCD can be enhanced by activation in vitro with T cell receptor (TCR)/CD3 monoclonal antibodies (mAb), lectins, superantigens or ionomycin [59, 61, 94, 117]. PCD occurs in both $CD4^+$ and $CD8^+$ T cells and phenotypical analysis suggests that higher percentages of $CD8^+$ cells are dying [23, 59, 94, 95].

In primary HIV infection the increased percentage of T cells dying due to apoptosis after overnight culture is high (up to 60%) and parallels transient increases of $CD8^+$ cell numbers. Because they form the largest fraction of T cells, numerically the majority of cells dying during primary infection are activated, $CD8^+CD45RO^+$ cells. However, all $CD8^+$ T cell subsets contain cells dying due to PCD and there is no evidence for preferential death in one specific subset of cells [78, 95]. In the asymptomatic phase of HIV infection there is a variable but, compared to HIV-negative controls, consistently increased percentage of cells dying due to PCD [59, 94, 95]. We and others have shown that PCD does not correlate with $CD4^+$ T cell numbers in asymptomatic individuals, nor with viral load, arguing against dramatic changes in the extent of PCD with progression to disease [82, 95, 107].

Mechanisms of apoptosis of T cells in HIV infection

In HIV-infected chimpanzees, that do not develop clinical symptoms, the proportion of T cells dying due to PCD does not exceed that in non-infected animals [59, 136]. This could imply either a function for PCD in HIV pathogenesis in humans, or that PCD is a reflection of immunopathogenic events. Several hypotheses on the cause of increased PCD of T cells in human HIV infection and the contribution to AIDS pathogenesis have been proposed, including direct virus infection of cells, CD4 ligation by gp120 and excessive immune activation.

Direct viral infection. In vitro infection of T cells and T cell lines with HIV results in cell death associated with apoptosis [77, 92, 145], and pulsing of dendritic cells with HIV results in infection and apoptosis of co-cultured CD4+ T cells [21]. The capacity of HIV to induce apoptosis in vitro is related to the cell line and virus strain used and is, at least in part, associated with the efficiency of virus replication in these cells [92].

Direct virus-induced cell death, however, can be excluded as the main cause of PCD of peripheral T cells in asymptomatic HIV infection. Not only is the frequency of infected cells during asymptomatic infection too low to explain the cell death observed, there seems to be no clear cut relation between elevated virus load during both acute and asymptomatic infection and increases in PCD [95]. However, in later stages of infection, with a high viral burden in T cells in lymph nodes, direct infection of cells leading to apoptosis might contribute to CD4+ T cell depletion. Arguing against this is the finding that in HIV- and simian immunodeficiency virus-infected lymph nodes apoptosis occurs predominantly in bystander cells and not in the productively infected cells themselves [52]. However, HIV infection of thymocytes might lead to increased apoptotic death in the thymus, thereby affecting regeneration of the peripheral T cell compartment [16].

Apoptosis induced by viral proteins. The initial hypothesis regarding PCD in HIV infection was that interaction of soluble HIV envelope protein (gp120) with CD4 could prime T cells for PCD [3]. Mature murine lymphocytes die from PCD after stimulation via TCR/CD3 when CD4 has been previously ligated by CD4 antibodies [111]. Furthermore, addition of gp120 in vitro impairs T cell function [26, 87, 116]. Indeed in human cells, cross-linking of CD4 mAb or bound gp120 on human CD4+ T cells followed by signaling through the TCR results in apoptosis in vitro [9, 117]. Expression of gp160 in a CD4+ T cell line causes down-regulation of CD4 and single-cell killing due to apoptosis [81] and in vitro exposure to HIV, without infection, of a CD34+ hematopoietic progenitor cell line induces apoptotic cell death [165]. In addition to the viral envelope, the virally encoded protein Tat was demonstrated to induce cell death in vitro in T cell lines and PBMC [79, 123], possibly by up-regulation of Fas expression on T cells [160].

These data all point to a role for viral proteins in inducing T cell deficiency and apoptosis. gp120-CD4 ligation might be a mechanism for apoptosis of CD4+ T cells in vivo.

Immune activation. A CD4-dependent mechanism for PCD is not likely to be the only phenomenon in HIV infection. First, both CD4+ and CD8+ cells, with a preference for CD8+ cells, are dying due to apoptosis [23, 59, 94, 107] and secondly, during primary HIV infection, the number of cells dying exceeds by far the percentage of CD4+ cells present at that time [95]. CD8+ T cells from HIV-infected individuals have

increased expression of activation markers as CD38, HLA-DR and CD57, suggestive for continuous immune activation [128, 141]. In lymph nodes from HIV-infected individuals a relation between apoptosis and immune activation but not viral load has been reported [107]. Because the percentage of cells dying due to PCD in primary HIV infection parallels the $CD8^+$ T cell expansion, it is tempting to speculate that PCD in HIV infection reflects turn over of activated immune cells, although PCD is not confined to a specific subset expressing activation markers [78, 95].

PCD as a result of massive immune activation following acute virus infection is not specific for HIV infection since it was also demonstrated for cytomegalovirus infection in man [151] and acute lymphocytic choriomeningitis virus (LCMV) infection in mice [124], correlating with hypo- responsiveness as a result of hyperactivation of T cells in vivo. Although it was argued by Estaquier et al. [47] that $CD4^+$ cell death is specific for HIV infection, in Epstein-Barr virus (EBV) infection in humans, both $CD4^+$ and $CD8^+$ cells also die upon culture [106, 149]. Dying cells were confined to the $CD45RO^+$ population and cell death could be prevented by culture in the presence of cytokines such as interleukin (IL)-2 [12, 149]. Under these conditions, it was suggested that PCD affects the population of activated T cells that expands during the acute phase of the infection.

We propose that PCD in acute HIV infection is a reflection of immune activation leading to high turnover of cells, as is observed in acute virus infections in general. High numbers of apoptotic cells in the early stage of infection are followed by moderately increased numbers of cells dying during the asymptomatic phase, as seen in the asymptomatic phase of feline immunodeficiency virus infection in cats [13]. In asymptomatic HIV infection, PCD reflects a continuous activation leading to priming for death and deletion of responding T cells.

Turnover of activated T cells by apoptotic cell death

The mechanism by which T cells in HIV infection are driven towards apoptosis might reflect a general phenomenon of termination of the immune response upon activation. Wesselborg et al. [159] reported that while freshly isolated T cells from healthy individuals are resistant to PCD, the susceptibility of these cells for induced death increases upon activation and culture. In agreement with the observations in HIV infection, in these experiments no correlation between susceptibility for death and the expression of a specific activation marker could be demonstrated.

Several cascades of events can be envisaged by which the immune system will set stop at an initiated immune response. As suggested by findings in murine LCMV infection [124] and experimental autoimmune encephalomyelitis [40], T cell death might be a physiological response to prolonged IL-2 stimulation after massive immune activation or high antigen dose. The apoptosis-related Fas antigen is known to be preferentially expressed on previously activated or memory T cells [104]. Fas-Fas ligand (FasL) interaction might play a role by the elimination of excessive immune cells, since $CD45RO^+$ cells in acute EBV infection, known to undergo apoptosis, have increased expression of Fas [149]. In HIV-infected individuals, T cells also have an increased expression of Fas and increased percentages of cells dying in culture upon Fas ligation with anti- Fas antibodies [70].

The proto-oncogene *bcl*-2 has been identified as a controller of PCD in a variety of cell types [75]. It was proposed that the regulation of *bcl*-2 expression within the

CD45RO+ T cell population regulates cell death and survival and is a mechanism for the removal of unwanted T cells after resolution of viral disease [2]. After repeated stimulation, primed T cells lose *bcl*-2 expression, gain Fas expression and become highly susceptible for death [130], which has also recently been demonstrated for CD8+ T cells from HIV-infected individuals [15, 17]. Furthermore, a relation between low *bcl*-2 expression and enhanced cell death has been demonstrated in other viral infections [1, 142].

Importantly, we and others have observed that in HIV infection, T cells die irrespective of the expression of activation markers [78, 95]. Massive immune activation could lead to exhaustion of growth and survival factors and subsequently result in PCD. Our finding that, in vitro, growth factors could not prevent the death of cells from HIV-infected individuals, does not exclude such a mechanism but may indicate that cells from HIV-infected individuals are already irreversibly primed for PCD in vivo [95]. Other groups, however, have reported rescue of cells from HIV-infected individuals from apoptosis by combinations of growth factors [59, 61, 121]. Furthermore, apoptosis has been reported to be differentially influenced by Th1 and Th2 cytokines. Th1 cytokines and anti-Th2 cytokine antibodies were reported to inhibit programmed cell death of T cells from HIV-1-infected individuals in vitro [35, 48].

Oxidative stress has been proposed as a mediator of apoptosis [20]. Activated CD4+ and CD8+ T cells from HIV-infected individuals have glutathione deficiency [43, 140] and might be less capable of withstanding oxidative stress and, thereby, death due to PCD. Antigen-presenting cell (APC) function, regulating either proliferation and cytokine production or cell death of the responding T cell, was proposed as a mechanism to shape a given immune response [157]. Increased prostaglandin E2 production by HIV-infected human macrophages induces apoptosis in co-cultured non-infected lymphocytes [93]. Infection with HIV in vitro leads to increased expression of FasL on monocytes, which leads to cell death of co-cultured uninfected lymphocytes [6].

In conclusion, we propose that, as in other acute non-persistent viral infections, PCD in acute HIV infection is a reflection of immune activation. The increased numbers of cells dying during the asymptomatic phase might be the result of continuous activation priming for death. Although PCD in early asymptomatic infection is merely reflecting the activated immune system rather than being a dominant pathogenic mechanism, virus-induced apoptosis might contribute to CD4+ cell depletion. First by infection of precursor cells or accessory cells affecting the renewal of the T cell compartment and, secondly, when the viral burden increases in late stages directly by HIV-induced apoptosis of peripheral CD4+ T cells.

Biological variation of HIV

One feature of HIV-1 is its great variability with respect to biological properties such as replication rate and cytotropism [4, 29, 49, 146, 152]. The HIV-1 biological phenotype is believed to be an important determinant in the variable clinical course of the infection. The asymptomatic phase of HIV-1 infection is characterized by low frequencies of infected cells in peripheral blood [39, 74], and the predominant HIV-1 variants replicate both in primary T cells and macrophages and are non-syncytium inducing (NSI) [135]. HIV-1-infected macrophages in the tissue compartment are believed to be the viral reservoir because peripheral blood T cells, in the asymptomatic

phase of infection, carry preferentially macrophage-tropic viruses compatible with recent infection of these T cells by HIV-1 derived from macrophages [134, 135].

Virus phenotype and kinetics of CD4$^+$ T cell loss

Several groups have analyzed the kinetics of CD4$^+$ T cell counts in progression to AIDS [73, 90, 132, 147]. These studies all showed that between 18 and 24 months before onset of AIDS, CD4$^+$ T cell counts rapidly decline, often paralleled by CD8$^+$ T cell counts. It has been shown that this precipitous drop in CD4$^+$ T cell counts is strongly associated with SI variants and is much less pronounced in patients that develop AIDS with NSI variants [73, 125, 132]. This suggests that SI variants and to some extent late stage NSI variants induce progressively increasing CD4$^+$ T cell turnover and rapid collapse of the immune system. It has been suggested that this could be due to progressive exhaustion of CD4$^+$ T cell renewal.

These SI variants in general are absent in the asymptomatic phase of infection. Since macrophages are the main target cells for the virus to enter upon transmission [120, 150], in the first phase of infection only NSI variants are present and a period of time is needed to generate the specific mutations correlated with the SI phenotype [53, 54]. Until now it is not understood why it takes so long to generate these mutants and why this only happens in a fraction of infected individuals.

SI are escape variants from HIV-1-suppressive chemokines

An interesting component of host immunity that has been ascribed an important role in AIDS pathogenesis is the non-cytolytic inhibition of virus replication by CD8$^+$ T lymphocytes in a major histocompatibility complex class I non-restricted way [18, 127, 161]. Inhibition has been demonstrated to be mediated at least in part by a soluble factor [83, 154, 155]. This suppressive activity has been demonstrated in CD8$^+$ T cells from both HIV-1-positive and -negative individuals as well as from non-human primates [19, 24, 25, 46, 68, 156]. In addition, CD8$^+$ lymphocytes from long-term asymptomatic HIV-1-infected individuals show vigorous HIV-1 suppressive activity and from most of these individuals infectious virus can only be isolated after CD8$^+$ T cell depletion [22]. Next to these CD8$^+$ T cell-derived factors, two groups recently reported cloning of HIV inhibitory factors, revealing its identities as IL-16 in one report [7] and as a combination of RANTES, MIP-1α and MIP-1β in another [37]. Cocchi et al. [37] showed that these chemokines acted predominantly on laboratory adapted, so-called SI non-macrophage tropic isolates. Paxton et al. [122] described two patients that appeared to have CD4$^+$ T cells insusceptible to infection with primary macrophage tropic NSI isolates and the CD4$^+$ T cells of both patients produced high amounts of the three chemokines. The patients cells could be readily infected with SI primary isolates, suggesting that the chemokines did not inhibit SI variants. To us, this all suggests that in early infection the predominant viruses present are NSI that are sensitive to inhibition and control by the three chemokines, but during the course of infection, SI variants emerge that have become insensitive to inhibition by RANTES, MIP-1α and MIP-1β. Indeed studies from our laboratory have shown relative insensitivity of SI viruses compared to NSI viruses isolated from a single individual to CD8$^+$-derived inhibitory factors (Kootstra et al., submitted for

publication). This indicates that SI variants have to be seen as escape variants from immune pressure, initially RANTES but later on from other inhibitory factors as well.

Altered T cell function and cytokine network

Already prior to the loss of CD4+ T cells in later stages of infection, HIV-1 infection is characterized by functional defects of T cells in vitro, which are observed from early infection on. Proliferation of T cells in response to ligation of the TCR/CD3 complex is impaired [8, 58, 100, 138] and IL-2 production decreased [32, 62]. In addition, delayed-type hypersensitivity (DTH) reactions are decreased in vivo [14, 91]. In our view this early imune dysfunction is instrumental in paving the way for the virus to be ultimately capable of inducing immune collapse and severe immune deficiency [101].

The fact that both CD4+ and CD8+ T cells of HIV-1-infected individuals are disturbed in their function [62, 100] in a stage of infection where the number of infected T cells is low [133], asks for a systemic explanation for the observed T cell dysfunction. When T cell dysfunction in HIV-1-infected individuals is studied in detail, it is clear that functional properties ascribed to Th1 cells are specifically disturbed. Since 1993 when Clerici and Shearer [30] proposed that T cell dysfunction in HIV-1 infection was associated with a change in cytokines secreted by Th cells from Th1 to Th2 cells, many studies on cytokine secretion patterns of T cells from HIV-1-infected individuals have been reported.

Dysregulated cytokine patterns in HIV-1 infection

One approach to study the capacity of patient T cells to secrete a certain cytokine is to generate T cell clones. Maggi et al. [84] reported already in 1987 a reduced number of T cell clones producing IL-2 and interferon (IFN)-γ in AIDS patients. Recently, the same group generated a large panel of CD4+ T cell clones specific for purified protein derivative of tuberculin or *Toxoplasma gondii* and observed a significant increase in the production of Th2 cytokines by clones generated from HIV-1-infected individuals, resulting in an increased percentage of Th0 type clones [86]. In agreement with this, T cell clones generated by random cloning procedures from CD4+ memory cells from asymptomatically HIV-1-infected individuals comprised increased numbers of Th0 clones [96]. In addition to this increased percentage of Th0/Th2 CD4+ T cell clones, CD8+ T cell lines and clones have been generated from symptomatic HIV-1-infected individuals that can provide helper activity for IgE synthesis [118] and show decreased cytolytic activity [85]. These CD8+ T cells indeed have Th2-like cytokine secretion patterns [85, 118].

Next to analyses of T cell lines and T cell clones, studies on cytokine production after stimulation of PBMC in vitro have been published. Decreased IL-2 production by T cells from HIV-1-infected individuals in vitro has been extensively documented [10, 33, 51, 56, 69, 99, 166]. However, for other Th1 and Th2 cytokines, conflicting results have been reported. A clear-cut shift to Th2 responses in bulk cultures, has so far only been reported by Clerici et al. [30], who showed a decreased IL-2 production induced by recall antigen but an increased mitogen-induced IL-4 production by PBMC from HIV-1-infected individuals in certain phases of infection. Autran et al. [5] described

a specific subset of $CD4^+$ T cells, lacking CD7 expression, which have a Th2-like cytokine secretion pattern and are increased in number in HIV-1-infected individuals. However, increased IL-4 production as reported by Clerici et al. [30] has only been reported by two other groups [69, 110], while most reports show unchanged [44, 45, 113, 166] or decreased [10, 42, 48, 86, 99] IL-4 production. It should be noted that most studies on PBMC cultures show a general decrease of all cytokines studied, probably due to the fact that T cells from HIV-1-infected individuals respond less well to a polyclonal T cell activation signal in vitro [10, 86, 99]. Thus, in agreement with a decrease in Th1 cytokine secretion, decreased IFN-γ production was published in very early [108, 109] and more recent studies [10, 56, 69, 99, 166]. On the other hand, increased IFN-γ production, probably originating from mainly $CD8^+$ T cells, and possibly reflecting activated cellular immunity, has been reported [45, 51, 60, 110, 153].

IL-10, a clear Th2 cytokine in mice, is produced in humans by both Th1- and Th2-type T cells [163], but might be considered 'Th2' because of its immunosuppressive effects [31]. IL-10 can be produced by different cell types, which may explain the conflicting data on IL-10 production in HIV-1 infection reported so far. In line with other monokines, the increase in IL-10 production in HIV-1-infected individuals [10, 36, 41, 51, 60] might be due to production by mainly monocytes [45]. Unresponsiveness of T cells might be the cause of the observation that IL-10 production is decreased or unaltered in other studies [28, 42, 48, 99, 166].

One should keep in mind that the various results on cytokine secretion in HIV-1-infected individuals might be related to the stage of infection of the patients studied, in addition to differences in cell preparations, mode of activation of cells and, not least, in cytokine assays. Furthermore, one must be careful in comparing data on cytokine responsiveness obtained by T cell cloning with results of bulk PBMC cultures. By generating T cell clones the potential of T cells to secrete a certain cytokine is studied under optimal circumstances. By analyzing PBMC stimulated in bulk, the intrinsic decreased responses to the stimulus by cells from HIV-1-infected subjects might bias the observation as to whether the cells have a commitment in vivo to secrete a certain cytokine. Furthermore, cross-regulation of different cell types in bulk cultures might influence the outcome. Single-cell analysis of cytokine secretion by T cells from HIV-1-infected individuals gives insight in the number of cells that are capable of producing either IFN-γ or IL-4, regardless the amount of cytokines they are producing. Indeed, on single-cell analysis by intracellular staining, we observed a significant decrease in the ratio of cells producing IFN-γ and IL-4 in HIV-1-infected individuals, in agreement with the findings in T cell clones [99].

The conclusion from our data and those discussed here might be that there is a decrease in Th1-type cytokine production causing a disturbance of the balance between Th1 and Th2 responses, leading to a Th0-like cytokine profile. In view of this, cytokines produced by APC that are critical for polarization of Th cell responses are of interest. The main cytokines in this context are IL-10 and IL-12. Since IL-10 can be produced by different cell types, including T cells, this cytokine has been discussed in the previous paragraph. IL-12 plays a critical role in Th1 cell differentiation [67]. Chehimi et al. [28] were the first to publish that PBMC from HIV-1-infected individuals are impaired in IL-12 p40 and p70 production upon stimulation with *Staphylococcus aureus* antigen (SAC). A similar finding was obtained with stimulation with *T. gondii* antigen for IL-12 p40 [56]. Alveolar macrophages from asymptomatically infected individuals, however, produce increased amounts of IL-12 p70 when stimulated

with bacterial antigens and a decreased IL-12 secretion was only found in patients with AIDS [41]. In a whole blood culture system, we demonstrated decreased IL-12 p40 and p70 production upon SAC but not lipopolysaccharide stimulation in HIV-infected persons. No relation with IL-10 or prostaglandin E2, potential inhibitors of IL-12 production, was found in these individuals [98]. These results at this time are suggestive of an underlying failure to produce the required amount of IL-12 to mount proper Th1 responses.

Cytokines and immune modulation

Proliferative responses to recall antigen are already disturbed early in HIV-1 infection and can be restored in vitro by IL-2 [80, 138]. IL-2 is also capable of restoring HIV-1-specific proliferative responses in vitro [11, 137]. In addition, the recently described IL-2-like cytokine IL-15 seems to have similar and synergistic effects to IL-2 on T cell proliferation from HIV-1-infected individuals and controls [137].

In recent years, in view of the publications on Th1/Th2 cytokine disturbances, in vitro manipulation of proliferative responses by various cytokines or anti-cytokines antibodies has been widely studied. IL-12 is capable of enhancing proliferation of T cells from HIV-1-infected individuals in response to influenza, HIV-1 peptides [34], *Mycobacterium avium* [112, 137] and polyclonal T cell stimulators [137]. Furthermore IFN-γ production in response to several T cell stimulators in vitro is enhanced upon IL-12 addition [34, 137, 166]. When T cells from HIV-1-infected individuals are cloned in the presence of IL-12, an increased outgrowth of IFN-γ-producing cells is observed [119]. Thus, IL-12 seems capable of restoring T cell defects in vitro. However, it should be noted that in most publications it was reported that T cells from non-infected controls are also enhanced in their proliferative responses and IFN-γ production in the presence of IL-12 [112, 137]. The enhanced outgrowth of IFN-γ-producing T cells in the presence of IL-12 is also observed when T cells from non-infected controls are used [88, 89, 119, 164]. This suggests that IL-12 does not restore a disturbed balance, but, at least in vitro, just enhances Th1-mediated responses, which is also observable in normal conditions. In agreement with this, a general enhancing effect of IL-12 on natural killer cell function was observed in both HIV-1-infected individuals and controls [27].

IL-10 has also been implied to play a major role in HIV-1-induced immune deficiency. Indeed, addition of neutralizing antibodies to T cell cultures enhances proliferative responses [36, 166]. In the original studies by Clerici et al. [33], neutralizing anti-IL-4 mAb were shown to be capable of restoring proliferative responses, but seemed to be much less efficient than anti-IL-10 antibodies [36].

Since T cells from HIV-1-infected individuals are impaired in their capacity to produce IL-2, needed for intact T cell proliferative responses, suppletion with IL- 2 would be a logical form of immune therapy in HIV-1-infected individuals. Indeed, several studies on IL-2 therapy have been published in the last few years, all in individuals that were simultaneously treated with anti-viral drugs, in most cases zidovudine. Low-dose IL-2 treatment was demonstrated in several studies to increase immune functions, especially in individuals with higher CD4+ T cell counts, without increases in viral load [143, 144, 162]. Prolonged treatment with IL-2 recently demonstrated beneficial effects in individuals with CD4+ T cell counts above 200/mm^3, while pa-

tients with less $CD4^+$ T cells showed little immunological improvement but did show evidence of increased viral activation and suffered from toxic effects [76].

Another candidate for immune therapy is IFN-α, not primarily because of its immune modulatory potential but because of its anti-viral capacities [57]. IFN-α is produced in increased amounts during natural HIV-1 infection, and resistance to IFN-α of HIV-1 variants was demonstrated in the course of infection. Thus, it was proposed that IFN-α therapy would only be useful in early stages of HIV-1 infection [57]. A phase I trial of IFN-α in combination with IL-2 showed few side effects and preliminary evidence for immune modulation and anti-viral activity [55]. IFN-α together with zidovudine had beneficial, though transient, effects on $CD4^+$ T cell numbers and T cell function when compared with zidovudine alone [55].

Recently, IL-12 has been greeted enthusiastically as a potential immune modulator to administer to HIV-1-infected individuals [63]. Although single-dose treatment induced little or no adverse effects, in clinical trials in cancer patients, repetitive infusions inducing episodes of severe toxicity have been reported, which points to caution in using IL-12 as a therapeutic agent [38]. Furthermore, although there are many reports on beneficial effects of IL-12 in animal models of parasitic and bacterial infections, there is still relatively little known of the effects on IL-12 on viral infections in animal models. Whereas low-dose treatment with IL-12 is capable of enhancing the cellular immune response against LCMV infection in mice, high-dose IL-12 treatment results in enhanced virus burden in these animals [114]. IL-12-mediated cytotoxicity in these high-dose IL-12 treated animals might be explained by the increased production of tumor necrosis factor (TNF)-α upon IL-12 treatment [115]. In addition, in human T cells IL-12 is capable of inducing the production of the immunosuppressive cytokine IL-10 in vitro [97]. Thus, prolonged IL-12 treatment might lead to immunosuppression due to increased production of cytokines as TNF-α and IL-10 and lead to an increased viral burden and failure of the immune system to prevent this.

Concluding remarks

The rationale behind immune modulation in HIV-1 infected individuals should not only be correction of cytokines that are over- or underproduced in the course of infection. To estimate the chance of success of immune modulation in vivo, a more basic question has to be answered first: how important is the gradual loss of cellular immunity in immunopathogenesis of HIV-1 infection? One may reason that with a properly functioning immune system, HIV-1 will never get the chance to give rise to overt virus replication and severe $CD4^+$ T cell loss. Some findings indeed make a case for an important role of the failing immune system in progression to AIDS. First, the loss of T cell function is predictive for rapid progression to AIDS independent from $CD4^+$ T cell numbers [66, 126, 131] and, secondly, in individuals who remain asymptomatic for a prolonged period of time, immune function is preserved [71, 139].

To understand the role of a deteriorating immune system in AIDS pathogenesis, many questions remain to be answered, one of the most important being the contribution to protection from disease made by cytotoxic T lymphocytes (CTL) [100]. There is some controversy as to the role of HIV-1-specific CTL in controlling virus infection [167]. Studies in long-term asymptomatic individuals have demonstrated preserved HIV-1 gag-specific CTL responses [72], suggestive of a contribution in controlling virus load. However, despite the presence of strong CTL responses, viral

load did increase in people who progressed rapidly to AIDS [72]. Based on these results, one may conclude that HIV-1-specific CTL do not play a critical role in determining the rate of progression to AIDS. Alternatively, one could argue that in vivo, although in these individuals CTL precursors are present, they are not capable of exerting their function properly. The CTL precursor frequency in this study was determined by limiting dilution culture and propagation of cells under optimal in vitro conditions. Defective presentation of HIV-1 antigens, lack of IL-12 or increased IL-10 production and decreased Th1 responses in vivo may all lead to the failure of cellular immune responses. This would point to an important role for APC and Th cells. Lack of cellular immunity would lead to lack of CTL responses and increasing viral loads. Cellular immunity has even been proposed to be correlated with protection from HIV-1 infection [129], but clear evidence to formally proof this point is lacking [102].

To be able to adequately and successfully intervene by immunotherapy in HIV-1 infection, we need to gain more insight into why the host fails to successfully handle this virus infection. Given the complexity of virus-host interactions, integrated viro-immunological studies are required to determine what the critical event(s) is/are that eventually lead to loss of CD4+ T cells and progression to disease in HIV-1-infected individuals [103]. Hopefully, this will lead to the development of therapeutic strategies leading to prevention of disease progression.

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