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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-1 α and MIP-1 β 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-1 α and MIP-1 β . 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 immune 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 asymptotically 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 asymptotically 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/mm³, 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.

References

1. Akbar AN, Borthwick NJ, Salmon M, Gombert W, Bofill M, Shamsadeen N, Pilling D, Pett S, Grundy JE, Janossy G (1993) The significance of low bcl-2 expression by CD45RO T cells in normal individuals and patients with acute viral infections. The role of apoptosis in T cell memory. *J Exp Med* 178:427
2. Akbar AN, Salmon M, Savill J, Janossy G (1993) A possible role for bcl-2 in regulating T cell memory – a 'balancing act' between cell death and survival. *Immunol Today* 14:526
3. Ameisen JC, Capron A (1991) Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. *Immunol Today* 12:102
4. Asjo B, Albert J, Karlsson A, Morfeldt-Månson L, Biberfeld G, Lidman K, Fenyo EM (1986) Replicative properties of human immunodeficiency virus from patients with varying severity of HIV infection. *Lancet* II:660
5. Autran B, Legac E, Blanc C, Debré P (1995) A Th0/Th2-like function of CD4⁺CD7⁻ helper cells from normal donors and HIV-infected patients. *J Immunol* 154:1408
6. Badley AD, McElhinny JA, Leibson PJ, Lynch DH, Alderson MR, Paya CV (1996) Upregulation of Fas ligand expression by human immunodeficiency virus in human macrophages mediates apoptosis of uninfected T lymphocytes. *J Virol* 70:199
7. Baier M, Werner A, Bannert N, Metzner K, Kurth R (1995) HIV suppression by interleukin-16. *Nature* 378:563
8. Ballet JJ, Couderc LJ, Rabian-Herzog C, Duval-Roy C, Janier M, Danon F, Clauvel JP, Seligmann M (1988) Impaired T-lymphocyte-dependent immune responses to microbial antigens in patients with HIV-1-associated persistent generalized lymphadenopathy. *J Acquir Immune Defic Syndr* 2:291
9. Banda NK, Bernier J, Kurahara DK, Kurrel R, Haigwood N, Sekaly R-P, Helman Finkel T (1992) Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. *J Exp Med* 176:1099
10. Barcellini W, Rizzardi GP, Borghi MO, Fain C, Lazzarin A, Meroni PL (1994) Th1 and Th2 cytokine production by peripheral blood mononuclear cells from HIV-infected patients. *J Acquir Immune Defic Syndr* 8:757
11. Bell SJD, Cooper DA, Kemp BE, Doherty RR, Penny R (1992) Heterogenous effects of exogenous IL-2 on HIV-specific cell-mediated immunity (CMI). *Clin Exp Immunol* 90:6

12. Bishop CJ, Moss DJ, Ryan JM, Burrows SR (1985) T lymphocytes in infectious mononucleosis. II. Response in vitro to interleukin-2 and establishment of T cell lines. *Clin Exp Immunol* 60:70
13. Bishop SA, Gruffydd-Jones TJ, Harbour DA, Stokes CR (1993) Programmed cell death (apoptosis) as a mechanism of cell death in peripheral blood mononuclear cells from cats infected with feline immunodeficiency virus (FIV). *Clin Exp Immunol* 93:65
14. Blatt SP, Hendrix CW, Butzin CA, Freeman TM, Ward WW, Hensley RE, Melcher GP, Donovan DJ, Boswell RN (1993) Delayed type hypersensitivity skin testing predicts progression to AIDS in HIV-infected patients. *Ann Intern Med* 119:177
15. Bofill M, Gombert W, Borthwick NJ, Akbar AN, McLaughlin JE, Lee CA, Johnson MA, Pinching AJ, Janosy G (1995) Presence of CD3⁺CD8⁺Bcl-2^{low} lymphocytes undergoing apoptosis and activated macrophages in lymph nodes of HIV-1⁺ patients. *Am J Pathol* 146:1542
16. Bonyhadi ML, Rabin L, Salimi S, Brown DA, Kosek J, McCune JM, Kaneshima H (1993) HIV induces thymus depletion in vivo. *Nature* 363:728
17. Boudet F, Lecoœur H, Gougeon M (1996) Apoptosis associated with ex vivo down-regulation of Bcl-2 and upregulation of Fas in potential cytotoxic CD8⁺ T lymphocytes during HIV-1 infection. *J Immunol* 156:2282
18. Brinchmann JE, Gaudernack G, Vartal F (1990) CD8⁺ T cells inhibit HIV replication in naturally infected CD4⁺ T cells. *J Immunol* 144:2961
19. Brinchmann JE, Gaudernack G, Vartdal F (1990) CD8⁺ T cells inhibit HIV replication in naturally infected CD4⁺ T cells. Evidence for a soluble inhibitor. *J Immunol* 146:2961
20. Buttke TM, Sandstrom PA (1994) Oxidative stress as a mediator of apoptosis. *Immunol Today* 15:7
21. Cameron PU, Pope M, Gezelter S, Steinman RM (1994) Infection and apoptotic cell death of CD4⁺ T cells during an immune response to HIV-1 pulsed dendritic cells. *AIDS Res Hum Retroviruses* 10:61
22. Cao Y, Qin L, Zhang L, Safrit JT, Ho DD (1995) Virologic and immunologic characterization of long-term survivors of Human Immunodeficiency Virus type 1 infection. *N Engl J Med* 332:201
23. Carbonari M, Cibati M, Pesce AM, Sbarigia D, Grossi P, D'Offizi G, Luzi G, Fiorilli M (1995) Frequency of provirus-bearing CD4⁺ cells in HIV type 1 infection correlates with extent of in vitro apoptosis of CD8⁺ but not of CD4⁺ cells. *AIDS Res Hum Retroviruses* 11:789
24. Castro BA, Walker CM, Eichberg JW, Levy JA (1991) Suppression of human immunodeficiency virus replication by CD8⁺ cells from infected and uninfected chimpanzees. *Cell Immunol* 132:246
25. Castro BA, Homsy J, Lennette E, Murthy KK, Eichberg JW, Levy JA (1992) HIV-1 expression in chimpanzees can be activated by CD8⁺ cell depletion or CMV infection. *Clin Immunol Immunopathol* 65:227
26. Cefai D, Debre P, Kaczorek M, Idziorek T, Autran B, Bismuth G (1990) Human immunodeficiency virus-1 glycoproteins gp120 and gp160 specifically inhibit the CD3/T cell-antigen receptor phosphoinositide transduction pathway. *J Clin Invest* 86:2117
27. Chehimi J, Starr SE, Frank I, Rengaraju M, Jackson SJ, Llanes C, Kobayashi M, Perussia B, Young D, Nickbarg E, Wolf SF, Trinchieri G (1992) Natural killer (NK) cell stimulatory factor increases the cytotoxic activity of NK cells from both healthy donors and human immunodeficiency virus-infected patients. *J Exp Med* 175:789
28. Chehimi J, Starr SE, Frank I, D'Andrea A, Ma X, MacGregor RR, Sennelier J, Trinchieri G (1994) Impaired interleukin 12 production in human immunodeficiency virus infected patients. *J Exp Med* 179:1361
29. Cheng-Mayer C, Seto D, Tateno M, Levy JA (1988) Biologic features of HIV-1 that correlate with virulence in the host. *Science* 240:80
30. Clerici M, Shearer GM (1993) A T_H1 → T_H2 switch is a critical step in the etiology of HIV infection. *Immunol Today* 14:107
31. Clerici M, Shearer GM (1994) The Th1-Th2 hypothesis of HIV infection: new insights. *Immunol Today* 15:575
32. Clerici M, Via CS, Lucey DR, Roilides E, Pizzo PA, Shearer GM (1991) Functional dichotomy of CD4⁺ T helper lymphocytes in asymptomatic human immunodeficiency virus infection. *Eur J Immunol* 21:665
33. Clerici M, Hakim FT, Venzon DJ, Blatt S, Hendrix CW, Wynn TA, Shearer GM (1993) Changes in interleukin-2 and interleukin-4 production in asymptomatic human immunodeficiency virus-seropositive individuals. *J Clin Invest* 91:759

34. Clerici M, Lucey DR, Berzofsky JA, Pinto LA, Wynn TA, Blatt SP, Dolan MJ, Hendrix CW, Wolf SF, Shearer GM (1993) Restoration of HIV- specific cell-mediated immune responses by interleukin-12 in vitro. *Science* 262:1721
35. Clerici M, Sarin A, Coffman RL, Wynn TA, Blatt SP, Hendrix CW, Wolf SF, Shearer GM, Henkart PA (1994) Type 1/type 2 cytokine modulation of T cell programmed cell death as a model for human immunodeficiency virus pathogenesis. *Proc Natl Acad Sci USA* 91:11811
36. Clerici M, Wynn TA, Berzofsky JA, Blatt SP, Hendrix CW, Sher A, Coffman RL, Shearer GM (1994) Role of interleukin-10 (IL-10) in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus (HIV-1). *J Clin Invest* 93:768
37. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P (1995) Identification of RANTES, MIP-1 α , and MIP-1 β as the major HIV-suppressive factors produced by CD8⁺ T cells. *Science* 270:1811
38. Cohen J (1995) Clinical trials. IL-12 deaths: explanation and a puzzle. *Science* 270:908
39. Connor RI, Mohri H, Cao Y, Ho DD (1993) Increased viral burden and cytopathicity correlate temporally with CD4⁺ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1 infected individuals. *J Virol* 67:1772
40. Critchfield JM, Racke MK, Zúñiga-Pflücker JC, Cannella B, Raine CS, Goverman J, Lenardo MJ (1994) T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science* 263:1139
41. Denis M, Ghadirian E (1994) Dysregulation of interleukin 8, interleukin 10, and interleukin 12 release by alveolar macrophages from HIV type 1-infected subjects. *AIDS Res Hum Retroviruses* 10:1619
42. Diaz-Mitoma F, Kumar A, Karimi S, Kryworuchko M, Daftarian MP, Creery WD, Filion LG, Cameron W (1995) Expression of IL-10, IL-4 and interferon-gamma in unstimulated and mitogen-stimulated peripheral blood lymphocytes from HIV-seropositive patients. *Clin Exp Immunol* 102:31
43. Dröge W, Eck HP, Mihm S (1992) HIV-induced cysteine deficiency and T cell dysfunction – a rationale for treatment with *N*-acetylcysteine. *Immunol Today* 13:211
44. Eckwalanga M, Marussig M, Dias Tavares M, Bouanga JC, Hulier E, Pavlovitch JH, Minoprio P, Portnoi D, Rénia L, Mazier D (1994) Murine AIDS protects mice against experimental cerebral malaria: down-regulation by interleukin 10 of a T-helper type 1 CD4⁺ cell-mediated pathology. *Proc Natl Acad Sci USA* 91:8097
45. Emilie D, Fior R, Llorente L, Marfaing-Koka A, Peuchmaur M, Devergne O, Jarousse B, Wijdenes J, Boue F, Galanaud P (1994) Cytokines from lymphoid organs of HIV-infected patients: production and role in the immune disequilibrium of the disease and in the development of B lymphomas. *Immunol Rev* 140:5
46. Ennen J, Findelee H, Dittmar MT, Norley S, Ernst M, Kurth R (1994) CD8⁺ T lymphocytes of african green monkeys secrete an immunodeficiency virus-suppressing lymphokine. *Proc Natl Acad Sci USA* 91:7207
47. Estaquier J, Idziorek T, De Bels F, Barré-Sinoussi F, Hurtrel B, Aubertin A-M, Venet A, Mehtali M, Muchmore E, Michel P, Mouton Y, Girard M, Ameisen JC (1994) Programmed cell death and AIDS: Significance of T cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. *Proc Natl Acad Sci USA* 91:9431
48. Estaquier J, Idziorek T, Zou W, Emilie D, Farber C-M, Bourez J- M, Ameisen JC (1995) T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO- 1)-mediated apoptosis of CD4⁺ T cells from human immunodeficiency virus- infected persons. *J Exp Med* 182:1759
49. Evans LA, McHugh TM, Stites DP, Levy JA (1987) Differential ability of HIV isolates to productively infect human cells. *J Immunol* 138:3415
50. Eyster ME, Gail MH, Ballard JO, Al-Mondir H, Goedert JJ (1987) Natural history of human immunodeficiency virus infections in hemophiliacs: effects of T cell subsets, platelet counts, and age. *Ann Intern Med* 107:1
51. Fan J, Bass HZ, Fahey JL (1993) Elevated IFN-gamma and decreased IL-2 gene expression are associated with HIV infection. *J Immunol* 151:5031
52. Finkel TH, Tudor-Williams G, Banda NK, Cotton MF, Curiel T, Monks C, Baba TW, Ruprecht RM, Kupfer A (1995) Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. *Nature Med* 1:129

53. Fouchier RAM, Groenink M, Kootstra NA, Tersmette M, Huisman HG, Miedema F, Schuitemaker H (1992) Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. *J Virol* 66:3183
54. Fouchier RAM, Broersen SM, Brouwer M, Tersmette M, Van 't Wout AB, Groenink M, Schuitemaker H (1995) Temporal relationship between elongation of the HIV-1 gp120 V2 domain and the conversion towards a syncytium inducing phenotype. *AIDS Res Hum Retroviruses* 11:1473
55. Frissen PHJ, Van der Ende ME, Ten Napel CHH, Weigel HM, Schreij GS, Kauffmann RH, Koopmans PP, Hoepelman AIM, De Boer JB, Weverling GJ, Haverkamp G, Dowd P, Miedema F, Schuurman R, Boucher CAB, Lange JMA (1994) Zidovudine and interferon- α combination therapy versus zidovudine monotherapy in subjects with symptomatic human immunodeficiency virus type 1 infection. *J Infect Dis* 169:1351
56. Gazzinelli RT, Bala S, Stevens R, Baseler M, Wahl L, Kovacs J, Sher A (1995) HIV infection suppresses type 1 lymphokine and IL-12 responses to *Toxoplasma gondii* but fails to inhibit the synthesis of other parasite-induced monokines. *J Immunol* 155:1565
57. Gendelman HE, Baldwin T, Baca-Regen L, Swindells S, Loomis L, Skurkovich S (1994) Regulation of HIV1 replication by interferon alpha: from laboratory bench to bedside. *Res Immunol* 145:679
58. Giorgi JV, Fahey JL, Smith DC, Hultin LE, Cheng HL, Mitsuyasu RT, Detels R (1987) Early effects of HIV on CD4 lymphocytes in vivo. *J Immunol* 138:3725
59. Gougeon M, Garcia S, Heeney J, Tschopp R, Lecoeur H, Guetard D, Rame V, Dauguet R, Montagnier L (1993) Programmed cell death in AIDS-related HIV and SIV infections. *AIDS Res Hum Retroviruses* 9:553
60. Graziosi C, Pantaleo G, Gant KR, Fortin J-P, Demarest JF, Cohen OJ, Sékaly RF, Fauci AS (1994) Lack of evidence for the dichotomy of Th1 and Th2 predominance in HIV-infected individuals. *Science* 265:248
61. Groux H, Torprier G, Monté D, Mouton Y, Capron A, Ameisen JC (1992) Activation-induced death by apoptosis in CD4⁺ T cells from human immunodeficiency virus-infected asymptomatic individuals. *J Exp Med* 175:331
62. Gruters RA, Terpstra FG, De Jong R, Van Noesel CJM, Van Lier RAW, Miedema F (1990) Selective loss of T cell functions in different stages of HIV infection. *Eur J Immunol* 20:1039
63. Hall SS (1994) IL-12 holds promise against cancer, glimmer of AIDS hope. *Science* 263:1685
64. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M (1995) Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373:123
65. Ho DD, Perelson AS, Shaw GM (1995) HIV results in the frame (Reply). *Nature* 375:198
66. Hofmann B, Orskov Lindhardt B, Gerstoft J, Sand Petersen C, Platz P, Ryder LP, Odum N, Dickmeiss E, Nielsen PB, Ullman S, Svejgaard A (1987) Lymphocyte transformation response to pokeweed mitogen as a marker for the development of AIDS and AIDS related symptoms in homosexual men with HIV antibodies. *Br Med J* 295:293
67. Hsieh C-S, Macatonia SE, Tripp CS, Wolf ST, O'Garra A, Murphy KM (1993) Development of Th1 CD4⁺ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260:547
68. Hsueh FW, Walker CM, Blackbourn DJ, Levy JA (1994) Suppression of HIV replication by CD8⁺ cell clones derived from HIV-infected and uninfected individuals. *Cell Immunol* 159:271
69. Hyjek E, Lischner HW, Hyslop T, Bartkowiak J, Kubin M, Trinchieri G, Kozbor D (1995) Cytokine patterns during progression to AIDS in children with perinatal HIV infection. *J Immunol* 155:4060
70. Katsikis PD, Wunderlich ES, Smith CA, Herzenberg LA, Herzenberg L (1995) Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals. *J Exp Med* 181:2029
71. Keet IPM, Krol A, Klein MR, Veugelers P, De Wit J, Roos MTL, Koot M, Goudsmit J, Miedema F, Coutinho RA (1994) Characteristics of long-term asymptomatic infection with the human immunodeficiency virus Type 1 with normal and low CD4⁺ cell counts. *J Infect Dis* 169:1236
72. Klein MR, van Baalen CA, Holwerda AM, Kerkhof-Garde SR, Bende RJ, Keet IPM, Eeftink Schattenkerk JKM, Osterhaus ADME, Schuitemaker H, Miedema F (1995) Kinetics of Gag-specific CTL responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. *J Exp Med* 181:1365
73. Koot M, Keet IPM, Vos AHV, De Goede REY, Roos MThL, Coutinho RA, Miedema F, Schellekens PThA, Tersmette M (1993) Prognostic value of human immunodeficiency virus type 1 biological phenotype for rate of CD4⁺ cell depletion and progression to AIDS. *Ann Intern Med* 118:681

74. Koot M, Van 't Wout AB, Kootstra NA, De Goede REY, Tersmette M, Schuitemaker H (1996) Relation between changes in cellular load, evolution of viral phenotype, and the clonal composition of virus populations in the course of human immunodeficiency virus type 1 infection. *J Infect Dis* 173:349
75. Korsmeyer SJ (1992) Bcl-2: a repressor of lymphocyte death. *Immunol Today* 13:285
76. Kovacs JA, Baseler M, Dewar RJ, Vogel S, Davey RT Jr, Falloon J, Polis MA, Walker RE, Stevens R, Salzman NP, Metcalf JA, Masur H, Lane HC (1995) Increases in CD4 T lymphocytes with intermittent courses of interleukin-2 in patients with human immunodeficiency virus infection. *N Engl J Med* 332:567
77. Laurent-Crawford AG, Krust B, Muller S, Rivière Y, Rey-Cuillé MA, Béchet J-M, Montagnier L, Hovanessian AG (1991) The cytopathic effect of HIV is associated with apoptosis. *Virology* 185:829
78. Lewis DE, Ng Tang DS, Adu-Oppong A, Schober W, Rodgers JR (1994) Anergy and apoptosis in CD8⁺ T cells from HIV-infected individuals. *J Immunol* 153:412
79. Li CJ, Friedman DJ, Wang C, Metelev V, Pardee AB (1995) Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. *Science* 268:429
80. Lifson JD, Benike CJ, Mark DF, Kothe K, Engleman EG (1984) Human recombinant interleukin-2 partly reconstitutes deficient in vitro immune responses of lymphocytes from patients with AIDS. *Lancet* i:698
81. Lu Y-Y, Koga Y, Tanaka K, Sasaki M, Kimura G, Nomoto K (1994) Apoptosis induced in CD4⁺ cells expressing gp160 of human immunodeficiency virus type 1. *J Virol* 68:390
82. Macho A, Castedo M, Marchetti P, Aguilar JJ, Decaudin D, Zamzami N, Girard PM, Uriel J, Kroemer G (1995) Mitochondrial dysfunctions in circulating T lymphocytes from human immunodeficiency virus-1 carriers. *Blood* 86:2481
83. Mackewicz CE, Yang LC, Lifson JD, Levy JA (1994) Non-cytolytic CD8 T cell anti-HIV responses in primary HIV-1 infection. *Lancet* 344:1671
84. Maggi E, Macchia D, Parronchi P, Mazzetti M, Ravina A, Milo D, Romagnani S (1987) Reduced production of interleukin-2 and interferon-gamma and enhanced helper activity for IgG synthesis by cloned CD4⁺ T cells from patients with AIDS. *Eur J Immunol* 17:1685
85. Maggi E, Giudizi MG, Biagiotti R, Annunziato F, Manetti R, Piccinini M-P, Parronchi P, Sampognaro S, Giannarini L, Zuccati G, Romagnani S (1994) Th2-like CD8⁺ T cells showing B cell helper function and reduced cytolytic activity in human immunodeficiency virus type 1 infection. *J Exp Med* 180:489
86. Maggi E, Mazzetti M, Ravina A, Annunziato F, De Carli M, Pesce AM, Del Prete G, Romagnani S (1994) Ability of HIV to promote a Th1 to Th0 shift and to replicate preferentially in Th2 and Th0 cells. *Science* 265:244
87. Manca F, Habeshaw JA, Dalglish AG (1990) HIV envelope glycoprotein, antigen specific T cell responses, and soluble CD4. *Lancet* 335:811
88. Manetti R, Parronchi P, Giudizi MG, Piccinini M-P, Maggi E, Trinchieri G, Romagnani S (1993) Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 177:1199
89. Manetti R, Gerosa F, Giudizi M-G, Biagiotti R, Parronchi P, Piccinini M-P, Sampognaro S, Maggi E, Romagnani S, Trinchieri G (1994) Interleukin-12 induces stable priming for interferon-gamma (IFN-gamma) production during differentiation of human T helper (Th) cells and transient IFN-gamma production in established Th2 cell clones. *J Exp Med* 179:1273
90. Margolick JB, Muñoz A, Donnenberg AD, PARK LP, Galai N, Giorgi JV, O'Gorman MRG, Ferbas J, for the Multicenter AIDS Cohort Study (1995) Failure of T cell homeostasis preceding AIDS in HIV-1 infection. *Nature Med* 1:674
91. Markowitz N, Hansen NI, Wilcosky TC, Hopewell PC, Glassroth J, Kvale PA, Mangura BT, Osmond D, Wallace JM, Rosen MJ, Reichman LB (1993) Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. *Ann Intern Med* 119:185
92. Martin SJ, Mearns PM, Vyakarnam A (1994) HIV-1 infection of human CD4⁺ T cells in vitro. Differential induction of apoptosis in these cells. *J Immunol* 152:330
93. Mastino A, Grelli S, Piacentini M, Oliverio S, Favalli C, Perno CF, Garaci E (1993) Correlation between induction of lymphocyte apoptosis and prostaglandin E2 production by macrophages infected with HIV. *Cell Immunol* 152:120
94. Meyaard L, Otto SA, Jonker RR, Mijster MJ, Keet RPM, Miedema F (1992) Programmed death of T cells in HIV-1 infection. *Science* 257:217

95. Meyaard L, Otto SA, Keet IPM, Roos MThL, Miedema F (1994) Programmed death of T cells in HIV-1 infection: no correlation with progression to disease. *J Clin Invest* 93:982
96. Meyaard L, Otto SA, Keet IPM, Van Lier RAW, Miedema F (1994) Changes in cytokine secretion patterns of CD4⁺ T cell clones in HIV-1 infection. *Blood* 84:4262
97. Meyaard L, Hovenkamp E, Otto SA, Miedema F (1996) IL-12- induced IL-10 production by human T cells as a negative feedback for IL-12- induced immune responses. *J Immunol* 156:2776
98. Meyaard L, Hovenkamp E, Pakker N, Van der Pouw Kraan TCTM, Miedema F (1996) IL-12 production in whole blood cultures from HIV-infected individuals studied in relation to IL-10 and PGE2 production. *Blood* (in press)
99. Meyaard L, Hovenkamp E, Keet IPM, Hooibrink B, De Jong IH, Otto SA, Miedema F (1996) Single cell analysis of IL-4 and IFN-gamma production by T cells from HIV infected individuals: decreased IFN-gamma in the presence of preserved IL-4 production. *J Immunol* (in press)
100. Miedema F, Petit AJC, Terpstra FG, Schattenkerk JKME, De Wolf F, Al BJM, Roos MThL, Lange JMA, Danner SA, Goudsmit J, Schellekens PTA (1988) Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4⁺ T helper cell depletion occurs. *J Clin Invest* 82:1908
101. Miedema F, Tersmette M, Van Lier RAW (1990) AIDS pathogenesis: a dynamic interaction between HIV and the immune system. *Immunol Today* 11:293
102. Miedema F, Meyaard L, Klein MR (1993) Protection from HIV-1 infection or AIDS? *Science* 262:1074
103. Miedema F, Meyaard L, Koot M, Klein MR, Roos MThL, Groenink M, Fouchier RAM, Van 't Wout AB, Tersmette M, Schellekens PThA, Schuitemaker H (1994) Changing virus-host interactions in the course of HIV-1 infection. *Immunol Rev* 140:35
104. Miyawaki T, Uehara T, Nibu R, Tsuji T, Yachie A, Yonehara S, Taniguchi N (1992) Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood. *J Immunol* 149:3753
105. Moss AR, Bachetti P, Osmond D, Krampf W, Chaisson RE, Stites D, Wilber J, Allain JP, Carlson J (1988) Seropositivity of HIV and the development of AIDS or AIDS-related conditions: Three year follow-up of the San Francisco General Hospital cohort. *Br Med J* 269:745
106. Moss DJ, Bishop CJ, Burrows SR, Ryan JM (1985) T lymphocytes in infectious mononucleosis. I. T cell death in vitro. *Clin Exp Immunol* 60:61
107. Muro-Cacho CA, Pantaleo G, Fauci AS (1995) Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden. *J Immunol* 154:5555
108. Murray HW, Hillman JK, Rubin BY, Kelly CD, Jacobs JL, Tyler LW, Donnelly DM, Carriero SM, Godbold JH, Roberts RB (1985) Patients at risk for AIDS-related opportunistic infections. Clinical manifestations and impaired gamma interferon production. *N Engl J Med* 313:1504
109. Murray HW, Scavuzzo DA, Kelly CD, Rubin BY, Roberts RB (1988) T4⁺ cell production of interferon gamma and the clinical spectrum of patients at risk for and with acquired immunodeficiency syndrome. *Arch Intern Med* 148:1613
110. Navikas V, Link J, Wahren B, Persson C, Link H (1994) Increased levels of interferon-gamma (IFN-gamma), IL-4 and transforming growth factor- β (TGF- β) mRNA expressing blood mononuclear cells in human HIV infection. *Clin Exp Immunol* 96:59
111. Newell MK, Haughn LJ, Maroun CR, Julius MH (1990) Death of mature T cells by separate ligation of CD4 and the T cell receptor for antigen. *Nature* 347:286
112. Newman GW, Guarnaccia JR, Vance III EA, Wu J-Y, Remold HG, Kazanjian PH Jr (1994) Interleukin-12 enhances antigen-specific proliferation of peripheral blood mononuclear cells from HIV-positive and negative donors in response to *Mycobacterium avium*. *AIDS* 8:1413
113. Nye KE, Knox KA, Pinching AJ (1990) HIV infection of H9 lymphoblastoid cells chronically activates the inositol polyphosphate pathway. *AIDS* 4:41
114. Orange JS, Wolf SF, Biron CA (1994) Effects of IL-12 on the response and susceptibility to experimental viral infections. *J Immunol* 152:1253
115. Orange JS, Salazar-Mather TP, Opal SM, Spencer RL, Miller AH, McEwen BS, Biron CA (1995) Mechanism of interleukin 12-mediated toxicities during experimental viral infections: role of tumor necrosis factor and glucocorticoids. *J Exp Med* 181:901

116. Oyaizu N, Chirmule N, Kalyanaraman VS, Hall WW, Good RA, Pahwa S (1990) Human immunodeficiency virus type 1 envelope glycoprotein gp120 produces immune defects in CD4⁺ T lymphocytes by inhibiting interleukin 2 mRNA. *Proc Natl Acad Sci USA* 87:2379
117. Oyaizu N, McCloskey TW, Coronese M, Chirmule N, Kalyanaraman VS, Pahwa S (1993) Accelerated apoptosis in peripheral blood mononuclear cells (PBMCs) from human immunodeficiency virus type-1 infected patients and in CD4 cross-linked PBMCs from normal individuals. *Blood* 82:3392
118. Paganelli R, Scala E, Ansotegui IJ, Ausiello CM, Halapi E, Fanales-Belasio E, D'Offizi G, Mezzaroma I, Pandolfi F, Fiorilli M, Cassone A, Aiuti F (1995) CD8⁺ T lymphocytes provide helper activity for IgE synthesis in human immunodeficiency virus-infected patients with Hyper-IgE. *J Exp Med* 181:423
119. Paganin C, Frank I, Trinchieri G (1995) Priming for high interferon-gamma production induced by interleukin-12 in both CD4⁺ and CD8⁺ T cell clones from HIV-infected patients. *J Clin Invest* 96:1677
120. Palacio J, Souberbielle BE, Shattock RJ, Robinson G, Manyonda I, Griffin GE (1994) In vitro HIV-1 infection of human cervical tissue. *Res Virol* 145:155
121. Pandolfi F, Oliva A, Sacco G, Polidori V, Liberatore D, Mezzaroma I, Kurnick JT, Aiuti F (1993) Fibroblast-derived factors preserve viability in vitro of mononuclear cells isolated from subjects with HIV-1 infection. *J Acquir Immune Defic Syndr* 7:323
122. Paxton WA, Martin SR, Tse D, O'Brien TR, Skurnick J, Vandevanter NL, Padian N, Braun JF, Kotler DP, Wolinsky SM, Koup RA (1996) Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures. *Nature Med* 2:412
123. Purvis SF, Jacobberger JW, Sramkoski RM, Patki AH, Lederman MM (1995) HIV type 1 Tat protein induces apoptosis and death in Jurkat cells. *AIDS Res Hum Retroviruses* 11:443
124. Razvi ES, Welsh RM (1993) Programmed cell death of T lymphocytes during acute viral infection: a mechanism for virus-induced immune deficiency. *J Virol* 67:5754
125. Richman DD, Bozzette SA (1994) The impact of the syncytium-inducing phenotype of human immunodeficiency virus on disease progression. *J Infect Dis* 169:968
126. Roos MTL, Miedema F, Koot M, Tersmette M, Schaasberg WP, Coutinho RA, Schellekens PTA (1995) T cell function in vitro is an independent progression marker for AIDS in human immunodeficiency virus (HIV)-infected asymptomatic individuals. *J Infect Dis* 171:531
127. Saag MS, Hahn BH, Gibbons J, Li Y, Parks ES, Parks WP, Shaw GM (1988) Extensive variation of human immunodeficiency virus type I in vivo. *Nature* 334:440
128. Salazar-Gonzalez JF, Moody DJ, Giorgi JV, Martinez-Maza O, Mitsuyasu RT, Fahey JL (1985) Reduced ecto-5'-nucleotidase activity and enhanced OKT 10 and HLA-DR expression on CD8 lymphocytes in the acquired immune deficiency syndrome: evidence of CD8 cell immaturity. *J Immunol* 135:1778
129. Salk J, Bretcher PA, Salk PL, Clerici M, Shearer GM (1993) A strategy for prophylactic vaccination against HIV. *Science* 260:1270
130. Salmon M, Pilling D, Borthwick NJ, Viner N, Janossy G, Bacon PA, Akbar AN (1994) The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis. *Eur J Immunol* 24:892
131. Schellekens PTA, Roos MTL, De Wolf F, Lange JMA, Miedema F (1990) Low T cell responsiveness to activation via CD3/TCR is a prognostic marker for AIDS in HIV-1 infected men. *J Clin Immunol* 10:121
132. Schellekens PTA, Tersmette M, Roos MTL, Keet R, De Wolf F, Coutinho RA, Miedema F (1992) Biphasic rate of CD4⁺ cell decline during progression to AIDS correlates with HIV-1 phenotype. *J Acquir Immune Defic Syndr* 6:665
133. Schnittman SM, Psallidopoulos MC, Lane HC, Thompson L, Baseler M, Massari F, Fox CH, Salzman NP, Fauci AS (1989) The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* 245:305
134. Schuitemaker H, Kootstra NA, De Goede REY, De Wolf F, Miedema F, Tersmette M (1991) Monocytotropic human immunodeficiency virus 1 (HIV-1) variants detectable in all stages of HIV infection lack T cell line tropism and syncytium-inducing ability in primary T cell culture. *J Virol* 65:356
135. Schuitemaker H, Koot M, Kootstra NA, Dercksen MW, De Goede REY, Van Steenwijk RP, Lange JMA, Eeftink Schattenkerk JKM, Miedema F, Tersmette M (1992) Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T cell-tropic virus populations. *J Virol* 66:1354

136. Schuitemaker H, Meyaard L, Kootstra NA, Otto SA, Dubbes R, Tersmette M, Heeney JL, Miedema F (1993) Lack of T cell dysfunction and programmed cell death in human immunodeficiency type-1 infected chimpanzees correlates with absence of monocytotropic variants. *J Infect Dis* 168:1140
137. Seder RA, Grabstein KH, Berzofsky JA, McDyer JF (1995) Cytokine interactions in human immunodeficiency virus-infected individuals: Roles of interleukin (IL)-2, IL-12 and IL-15. *J Exp Med* 182:1067
138. Shearer GM, Bernstein DC, Tung KSK, Via CS, Redfield R, Salahuddin SZ, Gallo RC (1986) A model for the selective loss of major histocompatibility complex self-restricted T cell immune responses during the development of acquired immune deficiency syndrome (AIDS). *J Immunol* 137:2514
139. Sheppard HW, Lang W, Ascher MS, Vittinghoff E, Winkelstein W (1993) The characterization of non-progressors: long-term HIV-1 infection with stable CD4⁺ T cell levels. *J Acquir Immune Defic Syndr* 7:1159
140. Staal FJT, Ela SW, Roederer M, Anderson MT, Herzenberg LA (1992) Glutathione deficiency and human immunodeficiency virus infection. *Lancet* 339:909
141. Stites DP, Moss AR, Bacchetti P, Osmond D, McHugh TM, Wang YJ, Hebert S, Colfer B (1989) Lymphocyte subset analysis to predict progression to AIDS in a cohort of homosexual men in San Francisco. *Clin Immunol Immunopathol* 52:96
142. Tamaru Y, Miyawaki T, Iwai K, Tsuji T, Nibu R, Yachie A, Koizumi S, Taniguchi N (1993) Absence of bcl-2 expression by activated CD45RO⁺ T lymphocytes in acute infectious mononucleosis supporting their susceptibility to programmed cell death. *Blood* 82:521
143. Tepler H, Kaplan G, Smith K, Cameron P, Montana A, Meyn P, Cohn Z (1993) Efficacy of low doses of the polyethylene glycol derivative of interleukin-2 in modulating the immune response of patients with human immunodeficiency virus type 1 infection. *J Infect Dis* 167:291
144. Tepler H, Kaplan G, Smith KA, Montana AL, Meyn P, Cohn ZA (1993) Prolonged immunostimulatory effect of low-dose polyethylene glycol interleukin 2 in patients with human immunodeficiency virus type 1 infection. *J Exp Med* 177:483
145. Terai C, Kornbluth RS, Pauza CD, Richman DD, Carson DA (1991) Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1. *J Clin Invest* 87:1710
146. Tersmette M, De Goede REY, Al BJM, Winkel IN, Gruters RA, Cuypers HTM, Huisman HG, Miedema F (1988) Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J Virol* 62:2026
147. Tersmette M, Lange JMA, De Goede REY, De Wolf F, Eeftink Schattenkerk JKM, Schellekens PThA, Coutinho RA, Huisman JG, Goudsmit J, Miedema F (1989) Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. *Lancet* I:983
148. Tindall B, Cooper DA (1991) Primary HIV infection: host responses and intervention strategies. *J Acquir Immune Defic Syndr* 5:1
149. Uehara T, Miyawaki T, Ohta K, Tamaru Y, Yokoi T, Nakamura S, Taniguchi N (1992) Apoptotic cell death of primed CD45RO⁺ T lymphocytes in Epstein-Barr virus induced infectious mononucleosis. *Blood* 80:452
150. Van 't Wout AB, Kootstra NA, Mulder-Kampinga GA, Albrecht MA, Scherpbier HJ, Veenstra J, Boer K, Coutinho RA, Miedema F, Schuitemaker H (1994) Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral and vertical transmission. *J Clin Invest* 94:2060
151. Van den Berg AP, Meyaard L, Otto SA, Van Son WJ, Klompmaier IJ, Mesander G, De Leij LHF, Miedema F, The TH (1995) Cytomegalovirus infection associated with a decreased proliferative capacity and increased rate of apoptosis of peripheral blood lymphocytes. *Transplant Proc* 27:936
152. Von Briesen H, Becker WB, Henco K, Helm EB, Gelderblom HR, Brede HD, Rubsamen-Waigmann H (1987) Isolation frequency and growth properties of HIV variants: multiple simultaneous variants in a patient demonstrated by molecular cloning. *J Med Virol* 23:51
153. Vyakarnam A, Matear P, Meager A, Kelly G, Stanley B, Weller I, Beverley P (1991) Altered production of tumour necrosis factors alpha and beta and interferon gamma by HIV-infected individuals. *Clin Exp Immunol* 84:109
154. Walker CM, Levy JA (1989) A diffusible lymphokine produced by CD8⁺ T lymphocytes suppresses HIV replication. *Immunology* 66:628

155. Walker CM, Erickson AL, Hsueh FC, Levy JP (1991) Inhibition of human immunodeficiency virus replication in acutely infected CD4⁺ cells by CD8⁺ cells involves a noncytotoxic mechanism. *J Virol* 65:5921
156. Walker CM, Thomson-Honniebier GA, Hsueh FC, Erickson AL, Pan LZ, Levy JA (1991) CD8⁺ T cells from HIV-1 infected individuals inhibit acute infection by human and primate immunodeficiency viruses. *Cell Immunol* 137:420
157. Wang R, Murphy KM, Loh DY, Weaver C, Russell JH (1993) Differential activation of antigen-stimulated suicide and cytokine production pathways in CD4⁺ T cells is regulated by the antigen-presenting cell. *J Immunol* 150:3832
158. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, Lifson JD, Bonhoeffer S, Nowak MA, Hahn BH, Saag MS, Shaw GM (1995) Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 373:117
159. Wesselborg S, Janssen O, Kabelitz D (1993) Induction of activation-driven death (apoptosis) in activated but not resting peripheral blood T cells. *J Immunol* 150:4338
160. Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, Debatin K-M, Krammer PH (1995) Sensitization to CD95-mediated apoptosis by HIV-1 Tat and gp120. *Nature* 375:497
161. Wiviott LD, Walker CM, Levy JA (1990) CD8⁺ lymphocytes suppress HIV production by autologous CD4⁺ cells without eliminating the infected cells. *Cell Immunol* 128:628
162. Wood R, Montoya JG, Kundu SK, Schwartz DH, Merigan TC (1993) Safety and efficacy of polyethylene glycol-modified interleukin-2 and zidovudine in human immunodeficiency virus type 1 infection: a phase I/II study. *J Infect Dis* 167:519
163. Yssel H, De Waal Malefyt R, Roncarolo MG, Abrams JS, Lahesmaa R, Spits H, De Vries JE (1992) IL-10 is produced by subsets of human CD4⁺ T cell clones and peripheral blood T cells. *J Immunol* 149:2378
164. Yssel H, Fasler S, De Vries JE, De Waal Malefyt R (1994) IL-12 transiently induces IFN-gamma transcription and protein synthesis in human CD4⁺ allergen-specific Th2 T cell clones. *Int Immunol* 6:1091
165. Zauli G, Vitale M, Re MC, Furlini G, Zamai L, Falcieri E, Gibellini D, Visani G, Davis BR, Capitani S, La Placa M (1994) In vitro exposure to human immunodeficiency virus type 1 induces apoptotic cell death of the factor-dependent TF-1 hematopoietic cell line. *Blood* 83:167
166. Zhang M, Gong J, Iyer DV, Jones BE, Modlin RL, Barnes PF (1994) T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. *J Clin Invest* 94:2435
167. Zinkernagel RM, Hengartner H (1994) T cell mediated immunopathology versus direct cytolysis by virus: implications for HIV and AIDS. *Immunol Today* 15:262