The immunopathology of HIV infection

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Fourteen years into the global epidemic of the acquired immunodeficiency syndrome, the exact mechanisms by which the human immunodeficiency virus (HIV) causes the destruction of the immune system remain unresolved. Infection with HIV is characterized by both continual virus replication and a vigorous immune response. The length of time from initial infection to the almost inevitable loss of CD4 positive T helper lymphocytes averages 10 years, indicating the dramatic and prolonged interplay of the virus and the host immune response. In this article we discuss many of the leading hypotheses for both direct and indirect mechanisms that have been proposed to explain the loss of CD4 cells. Current evidence suggests strongly that direct infection of CD4 cells is adequate to explain their loss, but that cofactors and indirect mechanisms may contribute to the overall process. This leads to the conclusion that the immunopathology of HIV infection can be most effectively countered by using antiretroviral chemotherapy.

Introduction

The immunopathology of human immunodeficiency virus (HIV) infection may be defined either as the damage wrought by HIV upon the immune system, or alternatively, as the pathology resulting from the host immune response to the infection. Infection with HIV almost invariably leads to the destruction of the CD4-positive T-helper lymphocytes (CD4 cells). The means by which HIV causes CD4 cell destruction has been the subject of intense debate, with many alternative mechanisms proposed (Table). Some investigators support the hypothesis of a direct mechanism of destruction, with viral lysis of infected CD4 cells. Others have postulated indirect methods of cell death, through host immune mechanisms targeted against infected cells or against viral proteins on the surface of uninfected CD4 cells. While there may be no consensus as to which mechanism(s) contribute to the destruction of CD4 cells in vivo, most would agree that CD4 cell loss is central to the development of the acquired immunodeficiency syndrome (AIDS).

The natural course of HIV infection is shown graphically in the Figure, and serves as a foundation on which to discuss the immunopathology of HIV. Initial infection with HIV is often characterized by clinical signs of fever, rash and lymphadenopathy (Kinloch-de Loës et al., 1993), and is associated with a phase of replication of a very
homogeneous virus population (Clark et al., 1991; Daar et al., 1991; Zhu et al., 1993). With the onset of the immune response there is a decrease, though not cessation, of virus replication (Daar et al., 1991; Koup et al., 1994). During the ensuing stage of clinical latency there is evidence of ongoing virus replication and vigorous humoral and cellular immune responses (Ho, Moudgil & Alam, 1989; Koup et al., 1991; Goudsmit, 1992). The virus populations within the patient are continually evolving during this period (Delwart et al., 1994), presumably in response to immune pressure, and the overall virus load is increasing (Connor et al., 1993). The CD4 cell numbers are usually declining, even during the period when the patient is asymptomatic. After a period as short as a few months to as long as 12 years or more, the CD4 count decreases to a level below which the host can no longer maintain effective immunity against new pathogens or those it had contained for many years. This leads to the emergence of multiple opportunistic infections and tumours, and ultimately, death.

The intricate interplay of HIV and the immune response eventually leading to depletion of CD4 cells, forms the basis of the scientific debate on the immunopathology of HIV. We will discuss both indirect and direct mechanisms for the immunopathology of HIV infection. This article will also touch on some of the more intensely debated theories of HIV pathogenesis. This review should not be considered a comprehensive account, and for more details the reader should refer to several excellent reviews, (Levy, 1993; Pantaleo, Graziosi & Fauci, 1993*; Poli, Pantaleo & Fauci, 1993; Weiss, 1993; Fauci, 1994).

**Indirect mechanisms of CD4 cell loss**

Researchers who first isolated HIV observed that it was extremely difficult to propagate *in vitro*, probably because it rapidly destroyed the cells which it infected (Barre-Sinoussi et al., 1983; Gallo et al., 1984; Popovic et al., 1984). This was in sharp contrast to their experiences with HTLV-I, a previously described human retrovirus, which transformed the cells that it infected producing immortalized cell lines. Investigators also observed that the CD4 molecule served as the necessary cell surface receptor for HIV (Dalgleish et al., 1984), leading to the conclusion that infection of CD4 cells resulted in their destruction both *in vitro* and *in vivo*.

The theory that HIV infection directly caused lysis of CD4 cells was challenged when investigators initially failed to recover virus from every HIV-seropositive patient, even those who had evidence of CD4 cell destruction (Andrews et al., 1987). In addition, early in-situ hybridization studies identified viral genes in only one in every 10,000 peripheral blood mononuclear cells (PBMC) (Harper et al., 1986). Intuitively, it seemed that such a small percentage of infected cells *in vivo* could not explain the massive destruction of these cells observed over the course of disease. These inconsistencies, which, in retrospect, were probably due to a lack of sensitivity in early virus detection methodology, led researchers to postulate a number of indirect mechanisms of CD4 cell destruction as a cause of AIDS.

**Apoptosis**

Clonal selection during the process of cellular maturation in lymphoid tissues requires that senescent or aberrant cells undergo a process of programmed cell death (apoptosis)
characterized by nuclear condensation and DNA fragmentation. Several investigators believe that this process of normal cell death is accelerated to a pathological degree in HIV infection, and is at least partially responsible for the destruction of CD4 cells (Ameisen, 1992; Meyaard et al., 1992; Finkel & Banda, 1994). Experiments have suggested that the envelope glycoprotein in some strains of HIV can induce apoptotic changes in CD4 cells in vitro in the absence of infection (Banda et al., 1992). Data suggest that viral envelope glycoprotein can directly induce these changes through a variety of mechanisms which may include abnormal activation after cross-linking of CD4 cells by gp120 and antibody (Banda et al., 1992; Groux et al., 1992). These conclusions are controversial, and it is uncertain if these phenomena occur in vivo.

It has been suggested that HIV infection itself may prevent apoptosis of the infected cells, thus preserving a chronic state of viral production (Finkel & Banda, 1994). Several HIV proteins have been implicated in this process, including nef, tat and vpu (Finkel & Banda, 1994). Recent data, however, suggest that another viral protein, vpr, acts by arresting cells in the G2 phase of the cell cycle (Rogel, Wu & Emerman, 1995). This could allow for greater virus production by limiting apoptosis of these infected cells. The implication of these results is that, if HIV-induced apoptosis plays a major role in CD4 cell depletion in AIDS, then it is the uninfected CD4 cells that are being depleted, not the infected ones (Finkel & Banda, 1994). The overall contribution of this potential mechanism of CD4 cell depletion in AIDS, however, remains unknown.

**Superantigen effects**

Some superantigens act by binding to and cross-linking common determinants on MHC class II molecules with specific Vβ determinants on the T cell receptor (TCR), thereby leading to activation, anergy and depletion of class-II-specific (CD4-bearing) cells expressing certain Vβ determinants. This activation and depletion bypasses the normal antigen-specific recognition of class-II-bearing cells which requires the contribution of both the α and β chains of the TCR. Certain bacterial and viral pathogens have been shown to contain antigens with superantigenic properties which lead to profound perturbations of Vβ repertoires during infection (White et al., 1989; Choi, Kappler & Marrack, 1991; Woodland et al., 1991). It has been postulated that HIV, like some murine retroviruses, may contain a superantigen which will deplete CD4 cells expressing specific Vβ determinants (Imberti et al., 1991). In order to investigate this hypothesis, several groups have studied the frequency of Vβ families in cohorts of HIV-infected patients, either by PCR techniques or by using monoclonal antibody staining of cells. The results have been inconclusive, with some studies indicating a depletion in specific Vβ families on CD4 cells from infected patients (Imberti et al., 1991), while others have failed to identify Vβ perturbations in infected individuals (Posnett et al., 1993).

The problems inherent in these studies are many. Firstly, the patients being studied are likely to be infected with several opportunistic pathogens, making it difficult to separate an HIV-induced perturbation in Vβ repertoire from one mediated by the latter. Secondly, unlike studies in mice where the subjects are genetically identical, studies in humans must take into account the MHC diversity that may alter the specificity of the superantigenic effect. One study attempted to control for this by using identical twins in which one twin was HIV-infected and the other was uninfected, but the small numbers of available subjects that can be studied in this manner, and again the inability to control for opportunistic pathogens remain problematic (Rebai et al., 1994). Since
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a superantigenic effect would only lead to depletion of a small percentage of CD4 cells (those expressing the specific V\(\beta\) family), and it is known that HIV infection ultimately leads to loss of all CD4 cells, one must conclude that a superantigenic effect of HIV, if operative, is a minor contributor to the overall loss of CD4 cells in AIDS. It is interesting that HIV appears to replicate preferentially in CD4 cells expressing a specific V\(\beta\) family (V\(\beta\)12) (Laurence, Hodtsev & Posnett, 1992), suggesting that activation by a superantigen, present in either HIV or an opportunist, may lead to enhanced viral replication rather than anergy and depletion of the CD4 cells expressing V\(\beta\)12.

**Immune-mediated destruction of CD4 cells**

There is experimental evidence in humans and animals that the immune response to virus infection may lead to pathological consequences. Lymphocytic choriomeningitis virus (LCMV) is cytolytic for murine cells in vitro, but can establish a chronic infection of little to no pathological consequence in vivo. Adoptive transfer of cytotoxic T lymphocytes (CTL) to the central nervous systems of infected mice, however, results in acute choriomeningitis (Klavinskis, Tishon & Oldstone, 1989). Likewise, in hepatitis B infection, there is a striking association between a local infiltration of CTL and chronic
active hepatitis (Eggink et al., 1984). These are both examples of a pathological immune response to virus leading to disease.

Could a similar scenario be operative in HIV infection whereby an immune response could effect the lysis of either uninfected CD4 cells or infected CD4 cells that would otherwise not be lysed by HIV itself? It has clearly been demonstrated that uninfected CD4 cells coated with gp120 can be lysed by antibody-armed killer cells through a mechanism reminiscent of antibody-dependent cellular cytotoxicity (ADCC), but which has been termed cell-mediated cytotoxicity (CMC) in the immunology of HIV infection (Lyerly et al., 1987; Weinhold et al., 1988). Amino acid sequence similarities between HIV protein and MHC class II molecules has led some investigators to suggest that HIV infection may lead to the generation of antibodies that react with self antigens, resulting in loss of class II-expressing cells (Blackman, et al., 1991; Dalgleish, 1993). Similarly, gp120 can be taken up and processed by CD4 cells, rendering them susceptible to lysis by CD4+ CTL (Germain, 1988; Siliciano et al., 1988). That these mechanisms of uninfected cell lysis are possible is not in dispute; their overall contribution to the destruction of CD4 cells, however, remains to be determined. By most estimates, the amount of free gp120 in the extracellular compartment is small and the frequency of CD4 bearing HIV-specific CTL is low (Koup et al., 1991), thereby indicating that these potential mechanisms of CD4 cell destruction are probably minor.

It has also been suggested that HIV, while cytolytic for CD4 cells in vitro, may be non-cytolytic in vivo (Zinkernagel & Hengartner, 1994). If this were the case then it would follow that the vigorous CTL response by CD8-bearing cells present in most infected patients (Walker et al., 1987, 1988; Koup et al., 1989; Reviere et al., 1989) would constitute a major mechanism of CD4 cell destruction in vivo. Two pieces of evidence, however, argue against this hypothesis. Firstly, there is not an inverse correlation between the magnitude of the CTL response to HIV and the rate of CD4 cell decline (Carmichael et al., 1993; Klein et al., 1995). Indeed, patients with the most stable CD4 cell counts often exhibit the greatest CTL responses to HIV (Harrer et al., 1995). Secondly, HIV has been demonstrated to be cytopathic in vivo in two mouse models of HIV, the hu-peripheral blood lymphocyte-severe combined immunodeficiency (SCID) model (Mosier et al., 1993) and the SCID-hu model (Aldrovandi et al., 1993; Bonyhadi et al., 1993). While the mechanisms of CD4 cell decline in the two models may differ, no immune response to HIV is generated in either model; CD4 cells are lost as a result of HIV infection alone. These results strongly argue that HIV is cytolytic in vivo and that the magnitude of the CTL response is associated with a good rather than a bad outcome.

Direct mechanisms of CD4 cell loss

Viral infection

As mentioned briefly above, early efforts to quantify HIV in infected patients were hampered by difficulties in culturing the virus, and relatively insensitive detection methods, leading to the conclusion that indirect mechanisms of CD4 cell destruction must be involved in HIV pathogenesis. Improvement in the sensitivity of viral detection methods and better culture techniques began to yield estimates of far greater viral load.

Two seminal studies published in 1989 showed that, in contrast to previous reports, a high proportion of HIV-infected cells could be demonstrated in symptomatic
individuals (one in 100 peripheral blood mononuclear cells) (Coombs et al., 1989; Ho et al., 1989). These two groups were also able to detect significant levels of virus in plasma and cells of asymptomatic patients proving that there was no true latent viral state as emphasized in an accompanying editorial by Baltimore & Feinberg (1989). Subsequent improvements in the methods for detecting HIV have indicated that even asymptomatic patients can carry $10^3$–$10^5$ copies of HIV RNA per mL of plasma, and symptomatic patients can carry $10^4$–$10^5$ copies/mL (Piatak et al., 1993; Cao et al., 1995).

The coupling of in-situ hybridization with polymerase chain reaction (PCR) technology has led to a re-estimation of the number of infected cells in the peripheral blood by several orders of magnitude (Bagasra et al., 1992; Patterson et al., 1993). In addition, lymphoid tissue is an additional large and previously underestimated reservoir of HIV (Embretson et al., 1993a,b; Pantaleo et al., 1993a). Finally, there is a direct correlation between the amount of HIV, the in-vitro cytopathic effect of the HIV, and the rate of CD4 cell depletion in infected patients (Connor et al., 1993). All of these studies indicate that there is probably an adequate level of active infection of CD4 cells at all stages of disease to account for the loss of CD4 cells on the basis of a direct viral cytolytic mechanism alone.

Two recently published studies have independently attempted to quantify viral and CD4 cell turnover rates in HIV infection in order to understand better the direct role of HIV in CD4 cell loss. These reports (Ho et al., 1995; Wei et al., 1995) independently arrived at very similar conclusions and cast new light on the relationship between the virus and the human host, provoking new insights into the nature of this infection, development of disease, and therapeutic strategies (Coffin, 1995). Both studies used antiviral drugs to shut down new virus production in HIV infected patients. Rates of HIV loss and CD4 cell increases were measured. Both groups concluded that after virus production is halted, the level of HIV in the plasma is reduced by half every 2 days. This suggested that, before initiating antiviral therapy, approximately one-third of the total plasma virus load was being replaced through new virus production each day in order to maintain the steady state. Wei et al. (1995) demonstrated that, when patients developed strains of HIV resistant to the antiretroviral agent, this resistant virus multiplied rapidly, and virtually replaced the non-resistant virus after 2 to 4 weeks of therapy. They concluded that CD4 cell turnover was about $2 \times 10^5$ cells per day (approximately 5% of the total CD4 cell population depending upon the stage of illness). Ho et al. (1995) similarly calculated that the entire population of CD4 cells would be replaced in about 2 weeks. Both groups concluded that this rapid cell replacement would result in constantly replenishing the pool of cells susceptible to virus infection.

These studies provide a vivid demonstration that there is an extraordinarily large amount of HIV being produced during human infection, and that the immune system is highly stressed in its effort to replenish the CD4 cells that are constantly being lost. The calculated rates of virus and CD4 cell turnover are consistent with the hypothesis that HIV is directly cytopathic to CD4 cells and that the immune system has a great capacity to regenerate the lost cells (Coffin, 1995). The magnitude of the CD4 cell regeneration in response to the HIV infection may be partially responsible both for the increased levels of activation markers on these cells, and the increased levels of apoptosis that have been observed by some investigators (Ho et al., 1995).
Potential contributing factors in the immunopathology of AIDS

While many investigators feel that the destruction of CD4 cells is both necessary and sufficient to explain the pathogenesis of HIV infection, there is a large body of work that suggests that other immune dysfunctions contribute to the clinical syndrome AIDS. We will touch upon some of these theories below.

Lymphoid tissue

What is known of the natural history of HIV infection has, for the most part, been based upon studies of cells and virus isolated from peripheral blood. Recently, more studies have begun to focus on lymphoid tissue as a site important in the immunopathology of HIV. One such study has determined that lymph nodes of HIV-infected patients are the sites of massive covert replication of HIV that is not apparent in the peripheral blood (Embretson et al., 1993b). The histopathological changes in lymph nodes over the course of infection have led others to conclude that follicular dendritic cells are the major contributors to viral trapping and clearance (Pantaleo et al., 1993a; Pantaleo et al., 1994). These cells were progressively depleted with advancing disease, possibly resulting in less efficient viral clearance and increased peripheral viral loads (Pantaleo et al., 1994). These conclusions however, have been challenged by failure to demonstrate different rates of plasma viral clearance in patients with high CD4 cell counts (500 cells/µL) when compared with those with low CD4 cell counts < 50 cells/µL (Ho et al., 1995). Finally, dendritic cells have been implicated in the spread of HIV infection rather than in viral clearance. Dendritic cells may be responsible for transmitting HIV to CD4 cells, and simultaneously stimulating these cells to produce a rapidly cytopathic infection (Cameron et al., 1992; Pope et al., 1994).

Cytokines

Many studies have described perturbations of the cytokine profile in HIV infection which may contribute to immune dysfunction (Pantaleo et al., 1993b; Poli et al., 1993). An association between elevated plasma levels of tumor necrosis factor (TNF)-α and disease progression or disease-related cachexia has been described in HIV-infected individuals (Lähdevirta et al., 1988). TNF-α upregulates HIV expression in chronically infected cell lines in vitro (Folks et al., 1989). This has led some to propose the use of pentoxifylline and thalidomide, which inhibit TNF-α and other cytokines, as adjunctive therapy in HIV-infected patients (Fauci, 1993). A number of other cytokines (TNF-β, IFN-γ, granulocyte-macrophage colony stimulating factor) which appear to be dysregulated in HIV infection have all been shown to increase HIV expression in vitro (Pantaleo et al., 1993b). These observations have led to the conclusion that cytokine dysregulation is not only a consequence of HIV infection, but may actually augment HIV replication, further contributing to HIV pathogenesis.

In-vitro measurements of cytokines have also led some investigators to conclude that the cytokine profile shifts from a T helper 1 (Th1) pattern (increased IL-2 and IFN-α), which primarily stimulates cellular immune responses, to a Th2 pattern (elevated IL-4 and IL-10) which promotes humoral immunity, and that this shift is intimately involved in the pathogenesis of HIV. More detailed descriptions of the hypothesis and experimental evidence for this shift can be found elsewhere (Clerici & Shearer, 1994;
Romagnani et al., 1994). The observations that host CTL responses are strongly associated with initial control of HIV replication, and decreased CTL responses are associated with advancing disease, are consistent with the predictions of the theory (Koup et al., 1994; Klein et al., 1995); it remains, however, a controversial hypothesis. Indeed, a recent study of cytokine profiles in lymphoid tissues of HIV-infected patients failed to provide confirmatory evidence for this phenomenon (Graziosi et al., 1994).

Other cofactors

It has been shown that cells latently infected with HIV can be stimulated to produce virus through cellular activation pathways (Nabel & Baltimore, 1987). This has led to the hypothesis that cofactors that lead to cellular activation may increase virus replication. In support of this hypothesis, one study found increased HIV loads following acute influenza infection or vaccination with influenza antigens (Ho, 1992). These findings argue strongly for the role of opportunistic infections or antigenic stimulation as cofactors that may augment the rate of HIV replication and subsequent CD4 cell death. In response to these observations, it has been suggested that HIV-infected haemophilia patients should use highly purified clotting factor preparations (Seremetis et al., 1993), and that immunosuppressive agents such as cyclosporin be used as an adjunct in the treatment of HIV infection (Fauci, 1993).

Conclusions

Destruction of CD4 cells, and the many perturbations of the immune system that result, are pathognomonic of HIV infection. The immunopathology of HIV infection is most likely due to direct effects of the virus on CD4 cells. In addition, secondary effects such as derangements in production of cytokines or cellular development may contribute to the overall immunopathology. Current evidence suggests that the major contributor to CD4 cell loss in HIV infection is the virus itself, and that strategies aimed at inhibiting HIV replication should be the primary focus of therapeutic intervention (Coffin, 1995).

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