Review

Mechanisms of CD4$^+$ T lymphocyte cell death in human immunodeficiency virus infection and AIDS

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AIDS, caused by the retroviruses human immunodeficiency virus type 1 and type 2 (HIV-1 and HIV-2), has reached pandemic proportions. Therefore, it is critical to understand how HIV causes AIDS so that appropriate therapies can be formulated. Primarily, HIV infects and kills CD4$^+$ T lymphocytes, which function as regulators and amplifiers of the immune response. In the absence of effective anti-retroviral therapy, the hallmark decrease in CD4$^+$ T lymphocytes during AIDS results in a weakened immune system, impairing the body's ability to fight infections or certain cancers such that death eventually ensues. The major mechanism for CD4$^+$ T cell depletion is programmed cell death (apoptosis), which can be induced by HIV through multiple pathways. Death of HIV-infected cells can result from the propensity of infected lymphocytes to form short-lived syncytia or from an increased susceptibility of the cells to death. However, the apoptotic cells appear to be primarily uninfected bystander cells and are eradicated by two different mechanisms: either a Fas-mediated mechanism during activation-induced cell death (AICD), or as a result of HIV proteins (Tat, gp120, Nef, Vpu) released from infected cells stimulating apoptosis in uninfected bystander cells. There is also evidence that as AIDS progresses cytokine dysregulation occurs, and the overproduction of type-2 cytokines (IL-4, IL-10) increases susceptibility to AICD whereas type-1 cytokines (IL-12, IFN-γ) may be protective. Clearly there are multiple causes of CD4$^+$ T lymphocyte apoptosis in AIDS and therapies that block or decrease that death could have significant clinical benefit.

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus that causes AIDS, and currently infects 42 million individuals worldwide (WHO, 2002). One of the principal cellular targets of HIV infection is the CD4$^+$ T helper lymphocyte (Th). Due to their central role in controlling immune responses, Th lymphocytes have become a focus of study with cytomegalovirus (Zeevi *et al.*, 1999), leishmaniasis (Lehmann & Alber, 1998), cancer (Ohmi *et al.*, 1999), transplantation (Schirren *et al.*, 2000) and allergy (Robinson *et al.*, 1993; Macaubas *et al.*, 1999). The Th immune response can be grouped according to which arm of the immune system is activated. While not absolute, type-1 helper responses are critical in controlling intracellular infections via cytotoxic T lymphocyte (CTL)-mediated mechanisms whereas type-2 helper responses protect against pathogens neutralized by antibodies (Maloy *et al.*, 2000). CD8$^+$ T cells represent a major arm of the cellular immune response and their differentiation into effector cells usually requires Th cell stimulation. The importance of CTL in clearing virus infections is well established and has been

demonstrated for several viruses (Lukacher *et al.*, 1984; Murray *et al.*, 1992; Lehmann-Grube *et al.*, 1993). The role of Th cells in the generation and maintenance of functional virus-specific CTL is not fully understood and some evidence suggests that CD4$^+$ and CD8$^+$ T cells can mount specific immune responses independently (Kasaian *et al.*, 1991). However, studies on the impact of Th lymphocytes in CTL responses to a chronic murine lymphocytic choriomeningitis virus (LCMV) infection demonstrated that, when Th cells were depleted, the LCMV-specific memory CTL responses decreased significantly, resulting in reduced protection and a persistent virus infection (Matloubian *et al.*, 1994; von Herrath *et al.*, 1996). An HIV-1 study demonstrated that a strong p24-specific Th proliferative response correlated with the magnitude of the Gag-specific CTL response, and the control of viraemia (Kalams *et al.*, 1999). In contrast, some *in vitro* data demonstrated strong CTL responses in people with high HIV virus loads (Koenig *et al.*, 1995). This discrepancy suggests a difference between immune response dynamics *in vivo* compared to *in vitro*. In addition to stimulating CTL activity, Th cells may also control HIV infection by producing, along with CD8$^+$ T cells, β-chemokines that competitively inhibit HIV attachment and down-regulate the HIV co-receptor chemokine receptor proteins (Kinter *et al.*, 1998; Saha *et al.*, 1998).

Th cells are thought to be important in controlling HIV infection both in the acute (Copeland & Heeney, 1996; Rosenberg & Walker, 1998) and in the chronic phases of the disease with a high HIV-specific $CD4^+$ response being noted in long-term non-progressing subjects (Rosenberg et al., 1997). Primary HIV infection presents with a high HIV titre that is initially controlled by a $CD8^+$ CTL response, along with anti-HIV antibodies (Clark et al., 1991; Daar et al., 1991). The virus plasma load reaches a set point that is maintained at a plateau during the asymptomatic phase of 2–10 years (Fig. 1). This homeostasis becomes unbalanced, resulting in a gradual decrease in the Th lymphocyte cell number concomitant with an increase in virus load, signalling the onset of AIDS. A hallmark of HIV infection is the progressive loss of Th lymphocytes as HIV disease progresses. When CD4 levels drop from a normal of 1000 cells mm^{-3} of whole blood to less than 200 mm^{-3}, immune dysregulation and opportunistic infections result. Evidence to date suggests that Th responses play a major role in the control of HIV infection (Rosenberg et al., 1997; Rosenberg & Walker, 1998; Phenix & Badley, 2002). In addition, our studies demonstrating HIV-specific Th responses in highly exposed persistently seronegative individuals suggest this may play a role in the prevention of HIV infection (Fowke et al., 2000). In HIV disease, the mechanism for deletion of Th cells remains unknown, although several processes probably contribute. As the loss of Th lymphocytes is central to AIDS pathogenesis, we will examine the possible mechanisms of their death and how their loss contributes to disease progression. Although the pathways of $CD4^+$ T cell death in HIV infection are many, they mainly result in programmed cell death (apoptosis).

Regulation of apoptosis: an overview

In HIV infection, disease progression correlates with both increased virus load (Furtado et al., 1995) and elevated levels of apoptosis (Gougeon et al., 1996). In particular, Th cell levels inversely correlate with levels of apoptosis (Fowke et al., 1997). In understanding how HIV contributes to the depletion of the immune system, one needs to be familiar with the various cell death pathways and to determine how HIV modulates them. Apoptosis is tightly regulated and occurs in response to either receptor-mediated (Fas, TNFR1, DR3, DR4 and DR5) or non-receptor-mediated (UV irradiation, DNA damage, granzymes, etc.) signals (Evan & Littlewood, 1998; Budihardjo et al., 1999). It has a role in many normal physiological processes, including homeostasis of lymphocyte populations, tissue differentiation (Golstein, 1998) and elimination of tumorigenic, mutated or virus-infected cells (Everett & McFadden, 1999). The response to death signals varies depending on cell type, activation or developmental stage of the cell, as well as the chemical or physical environment.

The apoptotic pathway involves two families of proteins, the effectors and the regulators (Los et al., 1999; Gross, 2001; Zimmermann et al., 2001). Upon receiving a death signal, the effector family of serine proteases called caspases (cysteine-dependent aspartate-specific proteases) are activated to catalyse a cascade of molecular events resulting in the activation or inactivation of numerous cellular proteins (Fig. 2). This results in the classical apoptotic morphological and biochemical changes such as plasma membrane blebbing, mitochondrial dysfunction and DNA fragmentation. The regulators of the apoptotic pathway are found in a second group of proteins, the Bcl-2 family (Strasser et al., 2000). These proteins contain anti-apoptotic (Bcl-2, Bcl-XL) and pro-apoptotic (Bax, Bid) members that exert their function primarily at the mitochondrion by either preventing or inducing mitochondrial dysfunction (Korsmeyer et al., 2000; Alimonti et al., 2001). Anti-apoptotic molecules localize to the outer mitochondrial membrane whereas the pro-apoptotic molecules are sequestered in the cytoplasm by a variety of mechanisms. Upon receiving a death signal, the pro-apoptotic proteins translocate to the mitochondrion to interact with the anti-apoptotic molecules, thereby promoting mitochondrial dysfunction. The relative level of these proteins is important, as increased amounts of anti-apoptotic proteins are able to override even larger numbers of pro-apoptotic signals. Activation of caspase 3 is the point
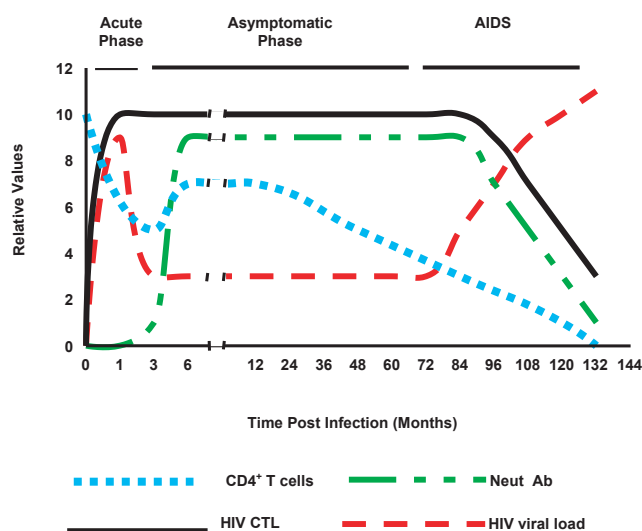
Fig. 1. Kinetics of HIV disease. This is a representation of a typical HIV/AIDS disease progression in the absence of anti-retroviral therapy. The initial spike in HIV load in the acute phase is accompanied by an increase in HIV-specific CTLs and a decrease in the number of $CD4^+$ T cells. Within 1 to 2 months the virus load is reduced and maintained at a new lower threshold by the immune response. The lower virus load is concomitant with an increase in $CD4^+$ T cells, and at 2 to 3 months seroconversion occurs as HIV-specific antibodies appear. This new asymptomatic state is generally maintained for a number of years. Gradually the $CD4^+$ T cell counts decrease to below 200 mm^{-3} in the blood signalling the onset of AIDS and increased susceptibility to opportunistic infections. This is followed by an increase in the virus load, along with a decrease in the HIV-specific CTL, and neutralizing antibodies.
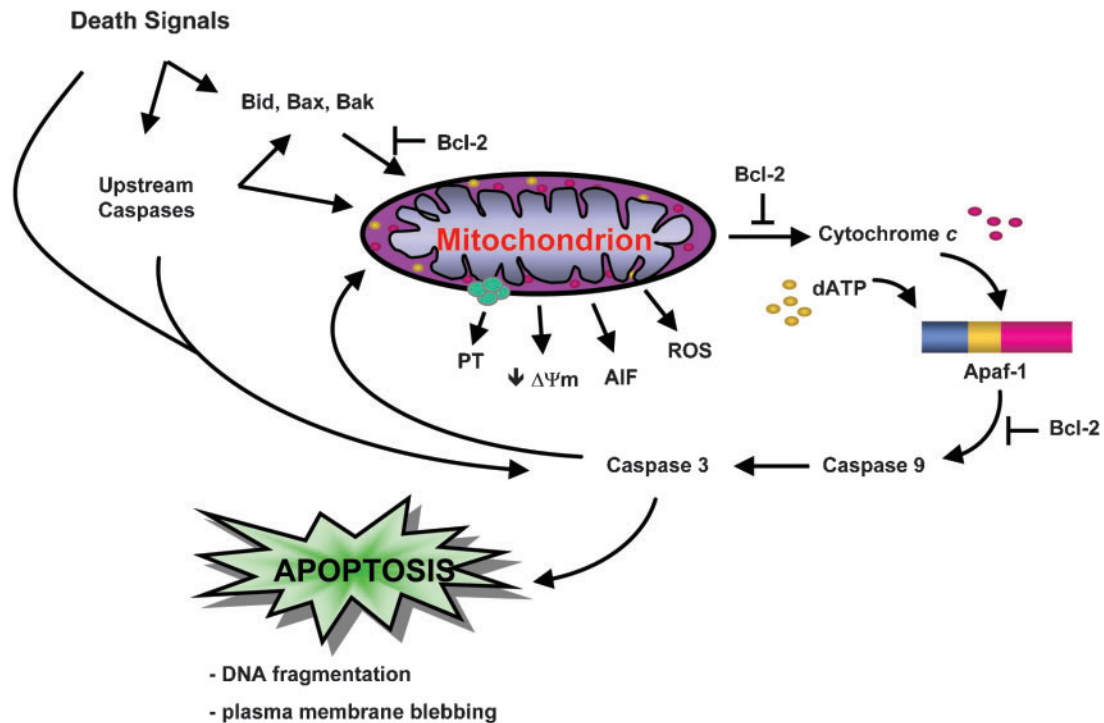
Fig. 2. The classical apoptotic pathway. Cells receive either a receptor-mediated or a non-receptor-mediated death signal to initiate the apoptotic pathway. Constitutive upstream caspases (i.e. caspase 8) and pro-apoptotic Bcl-2 family proteins (i.e. Bid, Bax) are activated, resulting in a cascade of molecular events that act at the mitochondrion. They can induce a loss of mitochondrial membrane potential ($\Delta\psi$m), production of reactive oxygen species (ROS), permeability transition (PT) due to opening of the permeability pore, mitochondrial swelling and ultimately release of apoptosis-inducing factor (AIF) and cytochrome *c*. Release of cytochrome *c* is a point of no return as cytochrome *c* forms a complex with caspase 9, Apaf-1 and dATP resulting in the autoactivation of caspase 9. Caspase 9 proceeds to cleave the downstream effector caspases (caspase 3, 6, etc.) that in turn act on many cellular proteins to give the typical biochemical and morphological features such as membrane blebbing and DNA fragmentation. Some apoptotic pathways are able to induce cell death in a mitochondrial-independent manner that is not inhibited by Bcl-2. In these cases, pro-apoptotic upstream molecules can activate caspase 3 directly. However, there is a feedback loop in which the activated caspase 3 acts on the mitochondrion to induce dysfunction at later stages of apoptosis.

of no return and results in the initiation of many downstream effector functions leading to the typical apoptotic features such as membrane blebbing and DNA fragmentation.

Apoptosis and the homeostasis of T lymphocytes

The Th cell's ability to become activated, proliferate and function is critical to an effective immune response. For CD4$^+$ T lymphocytes to function properly they must first become activated (Fig. 3) by antigen-presenting cells (APC) via a two signal mechanism (Bretscher, 1999). T cell receptor (TCR) engagement without the second co-stimulatory signal results in Th cell death. In fact, during HIV infection the expression of several co-stimulatory molecules is altered and may contribute to increased levels of T cell apoptosis (Kammerer *et al.*, 1996; Chougnet *et al.*, 1998; Sousa *et al.*, 1999).

A specific immune response must be able to expand rapidly

to process foreign antigens, but once the danger has been removed large numbers of antigen-specific T cells are induced to die because they are no longer needed. Receptor-mediated apoptosis has an essential role in maintaining the homeostasis of T lymphocyte numbers, so at the conclusion of the immune response mature antigen-specific activated lymphocytes undergo primarily Fas-mediated apoptosis (Lenardo *et al.*, 1999; Wolf & Green, 1999) (Fig. 4). Studies initially performed in *gld* and *lpr* knockout mice have revealed that molecules such as Fas (CD95/Apo-1) and Fas-ligand (FasL), respectively, function to down-regulate the immune response (Cohen & Eisenberg, 1992; Nagata & Suda, 1995), or otherwise massive and lethal lymphoproliferation occurs. Fas expression on activated T cells does not guarantee a FasL-induced death (Irmler *et al.*, 1997). FLIP protects cells from FasL-induced cell death during the initial phase of T cell activation, but not during the later stages. This period of Fas susceptibility is consistent with the time when the immune system is down-regulating an immune
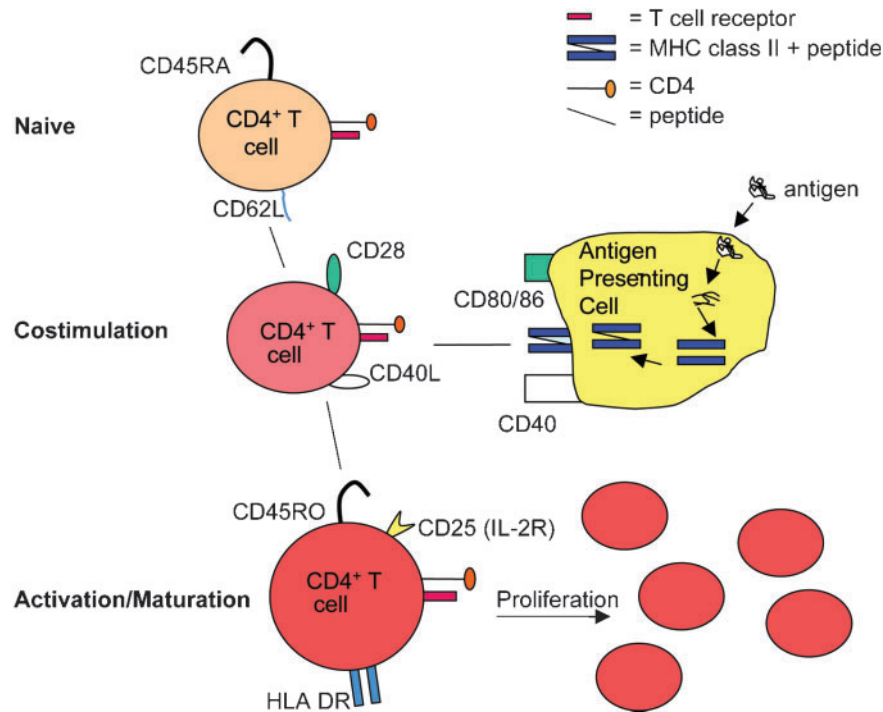
Fig. 3. Activation of T lymphocytes. Naive T cells (CD45RA) require a series of signals in order to become activated (CD45RO). The first signal involves the recognition of a peptide in the pocket of the MHC on the antigen-presenting cell (APC) by the T cell receptor (TcR) on the T lymphocyte. Other signals involve the interaction of CD28 and CD40L costimulatory molecules on the T cell with CD80/86 and CD40, respectively, on the APC.

response. In HIV infection, T cell apoptosis is a complex process that involves both HIV-infected and uninfected cells.

Cell death in HIV-infected cells

The devastating effect HIV has on the immune system is a result of infecting and killing the immune-regulating Th lymphocytes. HIV infects and replicates primarily in activated CD4$^+$ T lymphocytes and to a lesser extent in macrophages and dendritic cells. The cell specificity is due to the HIV envelope (Env) glycoprotein binding CD4, along with chemokine receptors CXCR4 or CCR5, for cell entry (Hogan & Hammer, 2001a, b). Once in the cytoplasm the viral RNA is reverse-transcribed into DNA and randomly integrated into the host cell genome. Activation of the host cell enhances the production of viral proteins, which assemble upon budding out of the cell, utilizing the plasma membrane as the viral envelope. HIV plasma levels during all stages of infection range from 50 to 11×10^6 virions ml^{-1} (Piatak et al., 1993). The half-life of most HIV-infected T cells in vivo is 12–36 h (Ho et al., 1995; Wei et al., 1995; Perelson et al., 1996), with the mechanisms of cell death being virus- or receptor-mediated apoptosis.

There are several mechanisms by which HIV can induce cell death directly in the cell it infects. CXCR4-tropic HIV isolates, generally found in the later stages of HIV infection,

preferentially infect T cells and induce membrane fusion between cells to form a giant multinucleated cell called a syncytium. Although syncytium formation is not necessary for the progression to AIDS, syncytia have a short lifespan, and the emergence of CXCR4-tropic strains correlates with an increased depletion of T cells (Richman & Bozzette, 1994; Sylwester et al., 1997; Kimata et al., 1999). In addition, cell viability can be compromised because the plasma membrane becomes disrupted or more permeable due to the continuous budding of the virion (Fauci, 1988), or due to specific HIV proteins such as Vpu that can induce membrane permeability (Gonzalez & Carrasco, 2001). Virus replication in the cell also has terminal consequences as cellular toxicity increases due to a build up of un-integrated linear viral DNA (Shaw et al., 1984; Levy, 1993). Also, through cleavage, the HIV protease can inactivate anti-apoptotic Bcl-2 while simultaneously activating pro-apoptotic procaspase 8, making the cell more susceptible to mitochondrial dysfunction in response to internal or external death signals (Korant et al., 1998; Nie et al., 2002).

Both CD4$^+$ and CD8$^+$ T lymphocytes are more susceptible to Fas-induced apoptosis in HIV$^+$ individuals, and this is related to the regulation of surface levels of CD95 (Fas) and FasL. Peripheral blood mononuclear cells (PBMC) from HIV$^+$ individuals express higher levels of CD95 (Silvestris et al., 1996), and the proportion of these T lymphocytes
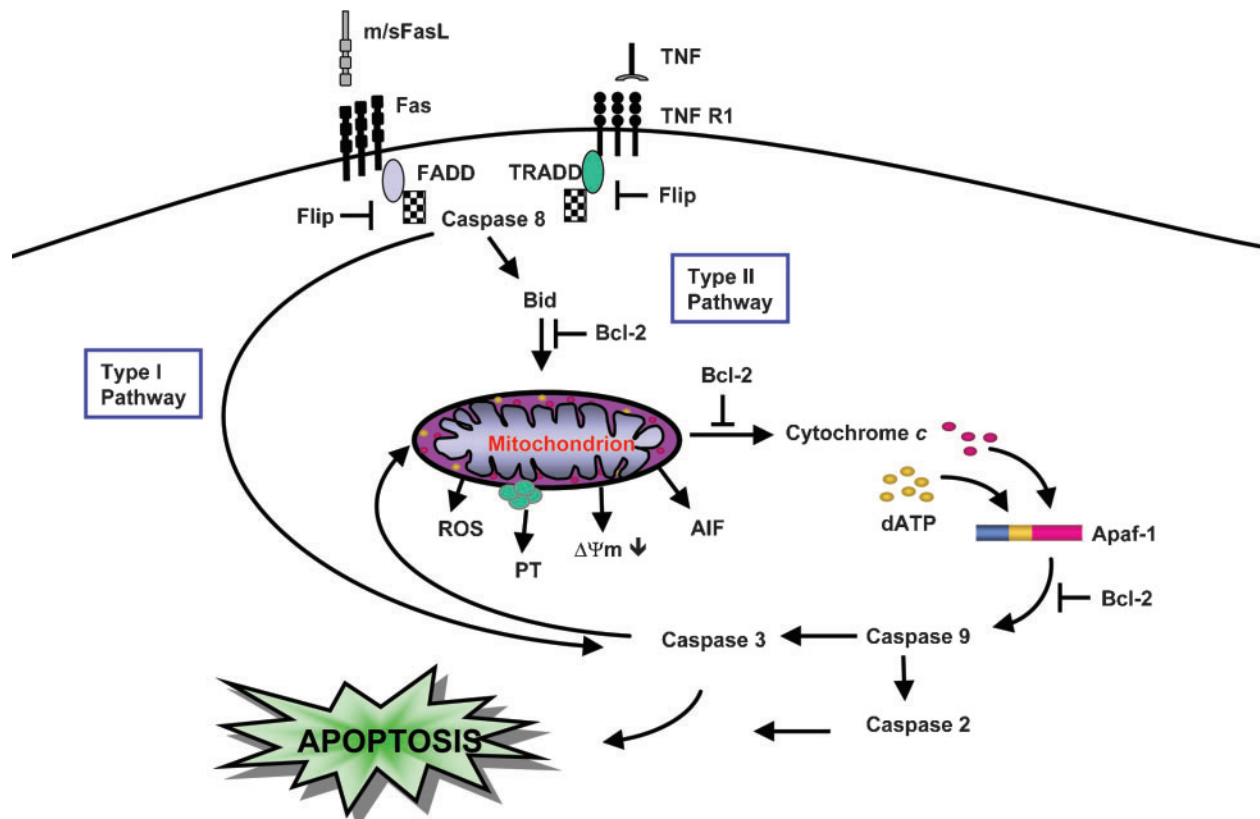
Fig. 4. The Fas cell death pathway. There are five receptors (Fas, TNFR1, DR3, DR4 and DR5) in the receptor-mediated death pathway. Fas-induced cell death requires the binding of either membrane-bound or soluble Fas ligand (m/sFasL) to the Fas receptor on the cell surface. This initiates the formation of the death-inducing signalling complex (DISC), which includes Fas, FADD and caspase 8, and ultimately results in the activation of caspase 8. The interaction of FADD with caspase 8 can be blocked by the cellular inhibitory protein Flip and can therefore block Fas-mediated apoptosis. There are two types of Fas-mediated death pathways. Type 1 is mitochondrial-independent and therefore not inhibited by the anti-apoptotic protein Bcl-2. It involves the direct activation of effector caspase 3 by the activated caspase 8. In contrast, type 2 proceeds via the mitochondria, resulting in mitochondrial dysfunction and cytochrome *c* release, to eventually activate caspase 3. Since Bcl-2 functions primarily at the mitochondria this pathway can be inhibited by Bcl-2.

increases with disease progression (Aries *et al.*, 1995; Baumler *et al.*, 1996; Estaquier *et al.*, 1996). The HIV proteins Nef (Zauli *et al.*, 1999), Env (Oyaizu *et al.*, 1994; Tateyama *et al.*, 2000) and Tat (Ensoli *et al.*, 1990; Westendorp *et al.*, 1995) have also been implicated in increasing CD95 and FasL levels, which presumably enhances susceptibility to Fas-mediated killing. Nef is thought to induce FasL by interacting specifically with the zeta chain of the TCR complex (Xu *et al.*, 1999). In addition, there are pro-apoptotic influences on other aspects of the Fas death pathway. Tat upregulates initiator caspase 8, resulting in more caspase 8 activity (Bartz & Emerman, 1999). HIV proteins also act on the central regulators of the death pathway, the Bcl-2 family members, that can either induce or prevent apoptosis. The cell normally has an appropriate balance of pro- versus anti-apoptotic proteins to ensure cell survival but HIV can alter the balance, resulting in the disruption of mitochondrial function. Levels of anti-apoptotic Bcl-2 are significantly lower in

HIV-infected individuals (Re *et al.*, 1998). In particular two HIV proteins, Tat (Sastry *et al.*, 1996) and HIV protease (Strack *et al.*, 1996), have been shown to decrease Bcl-2 levels. At the same time, Tat also has the ability to increase pro-apoptotic Bax (Sastry *et al.*, 1996) and Bim (Chen *et al.*, 2002). Other evidence demonstrates that deletion of Vpu partially increases survival in response to CD95 cross-linking in Jurkat cells and PBMCs (Casella *et al.*, 1999). This could be due to the ability of Vpu to suppress NF-κB-dependent expression of anti-apoptotic factors like Bcl-XL (Akari *et al.*, 2001).

CTLs are responsible for the removal of virus-infected cells from the body, and for inducing apoptosis in HIV-infected CD4$^+$ cells. However, like many other viruses, HIV has developed mechanisms to prevent, or simply delay, apoptosis of the cells it infects. The TCR on CTLs is used to recognize infected cells through the recognition of a non-self viral peptide in conjunction with major histocompatibility

complex class I (MHC I). The down-regulation of MHC I surface expression by Nef (Schwartz *et al.*, 1996) and Tat (Howcroft *et al.*, 1993) avert this recognition and can prevent CTL-mediated killing of the HIV-infected cell. If the cell were to receive a death signal, HIV has a secondary defence already in place. Vpr protein, which is expressed at low levels in the cell, is responsible for the increase in anti-apoptotic Bcl-2, while simultaneously decreasing pro-apoptotic Bax (Conti *et al.*, 1998). In contrast, other evidence indicates that Vpr induces a prolonged G_2 cell cycle delay followed by death in M phase (Watanabe *et al.*, 2000), and that apoptosis is mediated through interaction of Vpr with the mitochondrion permeability transition pore, which opens the pore causing mitochondrial swelling and release of cytochrome *c* (Jacotot *et al.*, 2001; Roumier *et al.*, 2002). The pro-apoptotic functions of Vpr may be secondary effects dependent upon induction of cell cycle arrest. At the same time, Vpr transactivates the viral promoter, the long terminal repeat (LTR), to increase virus replication (Gummuluru & Emerman, 1999). The cytoprotective effects of Vpr may play a role during early infection, allowing productive virus replication, but ultimately it promotes apoptosis toward the later stages. Finally, Nef, Vpu and Env all decrease CD4 on the surface of infected cells (Crise *et al.*, 1990; Willey *et al.*, 1992; Salghetti *et al.*, 1995). The fact that three HIV proteins have this function implies a critical role in the survival and replication of the virus. Down-regulation of CD4 would prevent a super infection and Env-induced apoptosis through the CD4 molecule. Overall, HIV has evolved multiple mechanisms to promote survival for long enough to ensure a productive infection and this may be supported by the fact that infected cells do not undergo apoptosis as readily as uninfected bystander cells (Finkel *et al.*, 1995). However, even infected cells have a shortened lifespan and, therefore, partially contribute to the overall decrease in Th cells (Ho *et al.*, 1995; Wei *et al.*, 1995; Perelson *et al.*, 1996).

Cell death in uninfected cells

Apoptosis plays a major role in killing uninfected cells. Although the Th cells infected by HIV can be directly killed by the virus, or by HIV-specific CTL, there are generally more apoptotic cells than infected cells (Embretson *et al.*, 1993). This is confirmed by direct evidence in lymph nodes where apoptosis was seen primarily in the uninfected bystander cells (Finkel *et al.*, 1995). There are two mechanisms by which uninfected cells could be killed: either by HIV proteins released from infected cells acting on neighbouring uninfected cells, or by activation-induced cell death (AICD).

Effect of apoptotic HIV proteins on uninfected cells

Inactivated HIV virions (Esser *et al.*, 2001) and HIV proteins released into the extracellular environment can have dramatic effects on uninfected cells. HIV proteins such as

gp120, Tat, Nef and Vpu have been shown to induce cell death in uninfected cells.

Soluble gp120 induces apoptosis in uninfected Th cells through cross-linking of the CD4 molecule (Banda *et al.*, 1992). The binding initiates part of the T cell activation pathway; however, in the absence of the TCR being specifically activated, this signal results in apoptosis or inhibition of antigen-induced cell activation (Marschner *et al.*, 2002). Soluble and membrane-bound gp120 induce, through cell receptors such as CD4, CXCR4 and CCR5, both Fas-dependent (upregulation of Fas/FasL, decreased FLIP) and Fas-independent (increased Bax, decreased Bcl-2) apoptotic pathways (Arthos *et al.*, 2002). Recently, CCR5-tropic viruses were shown to induce Fas and caspase 8-dependent apoptosis of uninfected Th cells (Algeciras-Schimnich *et al.*, 2002). Cell surface presentation of gp120 can induce a bystander cell death that requires close cell-to-cell contact and gp41 function (Blanco *et al.*, 2003). gp120 also has an inhibitory effect when cells at the G_0/G_1 phase of the cell cycle, such as naive T cells, are most sensitive to gp120-mediated negative signalling, whereas memory T cells are less affected.

The HIV protein Tat, secreted from infected cells (Chang *et al.*, 1997), can be endocytosed by neighbouring cells (Ensoli *et al.*, 1990; Mann & Frankel, 1991; Zagury *et al.*, 1998). Tat upregulates caspase 8 (Bartz & Emerman, 1999) and FasL (Li-Weber *et al.*, 2000), and induces apoptosis in neurons (New *et al.*, 1997) and Th cells (Li *et al.*, 1995). A Fas-independent Tat-mediated mechanism of bystander T cell death has also been suggested (Zhang *et al.*, 2001). Tat can upregulate TNF-related apoptosis-inducing ligand (TRAIL) on monocytes which may then interact with uninfected T cells to induce apoptosis (Zhang *et al.*, 2001). However, Tat also protects T cells from TRAIL-induced apoptosis (Gibellini *et al.*, 2001). Other anti-apoptotic effects of exogenous Tat include upregulation of Bcl-2, which was observed in the Jurkat cell line and PBMCs (Zauli *et al.*, 1995).

HIV Nef protein released into the extracellular matrix induces death in neuronal cells (Trillo-Pazos *et al.*, 2000) and a wide range of blood cells by a Fas-independent mechanism (Okada *et al.*, 1997, 1998). Much of the toxicity of Nef is likely due to its myristylated N terminus, which can insert into the plasma membrane and induce cell death in uninfected $CD4^+$ and $CD4^-$ T cells (Azad, 2000). It has been suggested that Nef plays a role in allowing HIV-infected cells to evade the immune response by inducing cytotoxic activity in uninfected $CD8^+$ T cells (Silvestris *et al.*, 1999) and down-regulating CD4 expression in neighbouring $CD4^+$ T cells (Pugliese *et al.*, 1999). Also, the extracellular addition of Vpu, or its C terminus, can cause membrane disruption and induce cell death in $CD4^+$ and $CD4^-$ cells (Azad, 2000). Clearly a number of extra-cellular HIV products are capable of inducing apoptosis in uninfected cells.

Activation-induced cell death (AICD) in uninfected cells

One interesting feature of apoptosis is that many of the molecular steps required for apoptosis are shared by cellular activation pathways. For example, the nuclear condensation that occurs in programmed cell death is similar to that which occurs prior to cell proliferation. Also, caspase activation, long known to be associated with apoptosis, occurs when cells become activated and proliferate (Alam et al., 1999; Kennedy et al., 1999). With these shared similarities it may not be surprising that activation and cell death are closely linked. AICD is a normal multi-step regulatory mechanism that primes a cell for death to limit an activated immune response (Hanabuchi et al., 1994). Priming can be achieved either by repeated stimulation through CD3/TCR (Kabelitz et al., 1995), sole stimulation through the CD4 receptor (Banda et al., 1992) or activation without co-stimulation (Borthwick et al., 2000). Although not normally expressed at high levels on resting T cells, Fas and FasL can be induced (Suda et al., 1995). In fact, allo-stimulation induces the expression of Fas and FasL on the surface of T cells (O'Flaherty et al., 2000). In HIV infection excessive immune activation has been suggested to induce apoptosis through Fas/FasL (Badley et al., 1998, 1999; Dockrell et al., 1999).

The binding of Env to the CD4 molecule renders the CD4$^+$ cell more susceptible to Fas-mediated killing (Algeciras et al., 1998) and also causes an increase in effector caspase 3, 6 and 8 activity, which can be blocked with soluble CD4 (Cicala et al., 1999, 2000; Algeciras-Schimnich et al., 2002). This suggests a CD4-dependent signalling mechanism, which is also known to block antigen-specific activation of primary lymphocytes (Marschner et al., 2002). T cells acquire an AICD-resistant phenotype when correctly activated by antigen. Engagement of CD4 by Env before TCR signalling prevents the upregulation of the Fas death pathway regulator FLIP, changing T cells from Fas-resistant to Fas-sensitive (Somma et al., 2000).

There are other HIV proteins that do not increase CD95 expression but still sensitize the cells to Fas-induced death. The Tat protein has the ability to increase pro-apoptotic proteins caspase 8 and Bax, while simultaneously decreasing the anti-apoptotic Bcl-2 (Sastry et al., 1996; Bartz & Emerman, 1999). Recent data have suggested that elevated levels of AICD in HIV infection may also be the result of altered FasL levels. Tat upregulates FasL expression through Egr transactivation of the FasL promoter resulting in increased AICD (Yang et al., 2002).

Cytokine responses in HIV disease and their effect on apoptosis

It is not surprising, given the death of Th lymphocytes in HIV infection, that there is a significant dysregulation of cytokine responses, which likely influences Th cell apoptosis susceptibility. It has been hypothesized that as HIV disease progresses there is a shift in the cytokine response from a predominantly type-1 cellular immune response (IFN-γ, TNF-α, IL-12), to a type-2 humoral response (IL-4, IL-5, IL-10, IL-13) (Clerici & Shearer, 1994). However, additional studies of cytokine profiles and HIV disease progression fail to completely corroborate these findings (Galli et al., 2001). Type-1 cytokine responses decrease as HIV disease progresses. While there is no clear evidence that type-2 responses increase, there is no concurrent decrease in type-2 responses. One possible explanation for the decrease in type-1 responses may be the increased susceptibility of cells expressing IFN-γ or TNF-α to AICD, presumably due to decreased Bcl-2 expression in these type-1 Th cells (Ledru et al., 1998). Thus, during HIV disease progression, dysregulation of cytokine responses results in a diminished ability to mount effective type-1 cytokine responses.

Resistance to apoptosis in vitro is associated with a predominant type-1 response (Gougeon & Montagnier, 1993). Therefore, deficiencies in type-1 responses could have drastic effects on apoptotic events regulating normal T cell homeostasis, and on HIV-induced AICD. IL-12 can protect against Fas-mediated apoptosis and AICD in HIV-infected patients (Estaquier et al., 1995). In addition, AICD in lymphocytes isolated from HIV-infected patients was blocked by the addition of recombinant IL-12 and IFN-γ, whereas type-2 cytokines IL-4 and IL-10 were shown to increase susceptibility to AICD (Clerici et al., 1996). Recent gene expression data demonstrated that IFN-α can strongly induce a number of pro-apoptotic genes in the TNF superfamily (Stylianou et al., 2002), suggesting that susceptibility to apoptosis may not exactly fit the type-1/-2 paradigm. Regardless of the assignation of these cytokines to a type-1 or type-2 phenotype, these data strongly suggest that cytokine dysregulation plays an important role in HIV-induced apoptosis. As HIV disease progresses, not only is apoptosis of Th lymphocytes enhanced due to the dual effects of pro-apoptotic HIV proteins and increased AICD, but cytokine dysregulation is likely to amplify these effects by providing a pro-apoptotic environment.

Although some of the strongest pro-apoptotic cytokine signals are TNF-α and TNF family members, their role in HIV-induced apoptosis is not clear. It is apparent that regulation of TNF is severely dysregulated in HIV-infected patients (Zangerle et al., 1994), as both HIV proteins and HIV infection can induce strong TNF responses in lymphocytes (Capobianchi, 1996). In fact, HIV infection of lymphocytes, or monocytes, induces TNF that in turn activates NF-κB, which induces high levels of HIV and TNF transcription (Han et al., 1996). In addition, serum TNF levels have been shown to be elevated in HIV-symptomatic but not asymptomatic individuals (Hober et al., 1996). TNF and TNF family members can initiate apoptosis immediately via membrane-bound receptors, and although it is apparent that TNF levels are indeed dysregulated in HIV infection, there is limited evidence for TNF-mediated apoptosis of infected cells directly, or by acting on bystander lymphocytes. Apoptosis of bystander Th cells can be reduced

by the addition of soluble TNF receptor decoys (Srivastava et al., 1999) and can protect against HIV protein-induced apoptosis (Cicala et al., 2000). However, clinical trials of anti-TNF therapies have failed to show improvement in either immunological or clinical outcome (Walker et al., 1996), making the role of TNF in HIV-induced apoptosis unclear. This is not surprising considering the multifactorial role of TNF in inducing cell activation and death. It is likely that the role of anti-apoptotic signals that negatively regulate TNF and TNF family member signalling such as caspase 8 may play a more important role in determining whether or not a particular cell undergoes apoptosis. The redundancy in cytokine function makes elucidation of a role in induction of apoptosis difficult.

The role of T cell growth factors IL-2 and IL-15 in HIV-induced apoptosis is also unclear. IL-2 displays both pro- and anti-apoptotic properties depending on the cellular microenvironment or activation status of a particular cell. Treatment with recombinant IL-2 or IL-15 can increase or decrease a cell's susceptibility to apoptosis. These discrepancies may be due to the activation status of the cells undergoing apoptosis. IL-2 and IL-15 were shown to increase susceptibility to CD95-mediated apoptosis (Naora & Gougeon, 1999) while protecting against spontaneous apoptosis (Adachi et al., 1996). The latter effect may be due to the ability of IL-2 and IL-15 to upregulate Bcl-2 expression (Akbar et al., 1994). Clinically, IL-2 has been useful in the treatment of HIV infection under certain conditions. IL-2 can increase Th cell survival independent of HIV replication, presumably by decreasing the apoptosis of bystander Th cells. Also, treatment with recombinant IL-2 can reduce overall levels of apoptosis in HIV-infected, but not uninfected, individuals (Adachi et al., 1996), further underscoring the importance of cellular activation, and the cytokine environment in the action of these apoptotic mediators. IL-2 and IL-15 share many similarities in their action due to utilization of common receptor elements (Giri et al., 1995). Of considerable interest is the observation that IL-15 may be a more potent inhibitor of apoptosis, which is likely because IL-15 (unlike IL-2) does not appear to induce HIV replication (Kovacs et al., 2001). Also, the relative levels of each cytokine in the apoptotic microenviroment are crucial to their ability to induce, or alternately suppress, apoptosis. A mouse model on senescence suggests that Th cells incapable of producing sufficient levels of IL-2 were unable to proliferate and were susceptible to apoptosis, but could be rescued by the addition of exogenous IL-2 (Nishimura et al., 2002). Thus, the role of IL-2 and IL-15 in apoptosis is likely to be complex due to the immuno-regulatory roles for these cytokines in cellular activation. Determination of whether a cell undergoes apoptosis will depend not only on the levels of IL-2 and IL-15, but also on the presence or absence of other appropriate pro- or anti-apoptotic signals such as TNF, other cytokines or Bcl-2 expression. These molecules may act directly through mediation of cellular activation, indirectly through the induction of other anti-apoptotic mediators such as Bcl-2 or

even through more downstream regulatory events such as the abilities of these cytokines to induce mediators like IFN-γ that regulate apoptosis in a more direct manner.

It is clear that cytokines play a multifactorial role in apoptosis during HIV disease pathogenesis. The loss of anti-apoptotic cytokines IFN-γ and IL-12 during disease progression and the increase in pro-apoptotic cytokines IL-4 and IL-10 are likely to amplify the role of apoptosis in the loss of Th cells. TNF-α and the TNF family of cytokines can induce apoptosis in a direct manner by inducing the appropriate (or inappropriate) apoptotic signals, while IL-2 and IL-15 appear to act in a more indirect manner via mediation of cell activation. Cytokines play an important role in apoptosis, and this is likely to be even more important during HIV infection when normal cytokine responses become dysregulated.

CONCLUSION

HIV causes AIDS and the loss of Th lymphocytes is a central factor in the progression of the disease. Due to the vital role of these cells in regulating and amplifying the immune response, any decline in their number results in deficits in both humoral and cell-mediated immunity. Understanding how these cells are eliminated is critical to the development of new, effective therapies for AIDS. However, difficulties arise as there are multiple mechanisms involved in the death of the Th lymphocytes. HIV-infected Th lymphocytes have a shortened lifespan due to syncytia formation, lysis by CTL and direct cytopathic effects of HIV, but the number of apoptotic cells in infected individuals greatly exceeds the number of HIV-infected cells. This suggests that HIV has additional detrimental effects on the uninfected bystander Th cell population. These bystander cells are attacked by two differing mechanisms. Firstly, several HIV proteins, whether attached to the virion or released by infected cells, can utilize a number of disparate death pathways to initiate apoptosis in uninfected cells. It is unclear in the in vivo situation what the relative contribution that gp120, Tat or Nef makes to the overall level of apoptosis; however, a therapy may be developed that would require the neutralization of all of their effects. On the other hand, there may be a point further along at which the HIV-induced apoptotic pathways converge. If we were to identify this point then blocking death may require intervention at a single focal point. Clearly more understanding of the death pathways initiated by these proteins is required. The second mechanism of bystander killing is AICD. HIV-infected individuals have higher levels of immune activation, which have been suggested to contribute to increased apoptosis. The dominant death pathway in AICD is through Fas and, therefore, inhibiting this path would be a logical point for therapeutic intervention. Currently, it is unknown whether the HIV proteins or AICD kill the majority of uninfected cells. Further studies of AICD in models of HIV and chronic immune stimulation could help determine its significance in disease progression.

Local environmental factors can also influence cell survival and the effectiveness of the HIV-specific immune response. During HIV infection the switch from the production of some type-1 cytokines to other type-2 cytokines could be very important since, respectively, they are associated with either protection from or enhancement of AICD.

This review has highlighted the many different modes by which HIV induces apoptotic cell death in both infected and uninfected Th cells. One of the ultimate goals of research in this field is to prevent or minimize death in Th cells. If this were achieved it may be possible to convert HIV infection from a progressively immunosuppressive and ultimately fatal disease to a chronic manageable infection.

ACKNOWLEDGEMENTS

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