HOW HUMAN IMMUNODEFICIENCY VIRUSES AND HERPESVIRUSES AFFECT APOPTOSIS

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Summary. – Eighty to hundred percent of patients positive for human immunodefficiency viruses 1 or 2 (HIV) may develop opportunistic viral infections. According to the National Institute of Health data, only in the USA the HIV patients are positive also for human cytomegalovirus (HCMV) in 25–40%, varicella-zoster virus (VZV) in 10%, herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), and Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8, KSHV, HHV-8) in 20%. HIV and herpesviruses express numerous different proteins that are able to influence interactions between the host and virus. One of the most interesting regulatory phenomenon is apoptosis which could play a significant role during both specific and non-specific antiviral response and latency. Apoptosis is an ordered cascade of precisely regulated enzymatic reactions which may be modulated or even controlled by viruses. Dramatic changes which occur during infection and which are exerted by HIV and certain herpesviruses on the mechanism of apoptosis may contribute to the pathogenesis of acquired immunodeficiency syndrome (AIDS).

Key words: apoptosis; herpesviruses; HIV

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Abbreviations: AIDS = acquired immunodeficiency syndrome; ANT = adenine nucleotide translocator; BHV-1 and BHV-4 = bovine herpeviruses 1 and 4; BL = Burkitt's lymphoma; CMV = cytomegalovirus; CNS = central nervous system; DED = death effector domain; EBNA = EBV nuclear antigen; EBV = Epstein-Barr virus; EHV-1 and EHV-4 = equine herpesviruses 1 and 4; FADD = the Fas and TNF receptor R1-associated, death-inducing cytoplasmic adaptor; HBV = herpesvirus B; HCMV = human CMV (human herpesvirus 5, HHV-5); HHV-6 = human herpesvirus 6; HIV = human immunodeficiency viruses 1 or 2; HSV-1 and HSV-2 = herpes simplex viruses 1 and 2; HVS = herpesvirus saimiri; KSHV = Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8, HHV-8); LANA = latency-associated nuclear antigen; LCL = lymphoblastoid cell line; LMP-1 = latent infection membrane protein 1; LR = latency-related; MCMV-1 = mouse CMV 1; ORF = open reading frame; PRV = pseudorabies virus; TNF = tumor necrosis factor; TNF-R = TNF receptor; VZV = varicella-zoster virus

Introduction

Apoptosis is a genetically controlled, energy-dependent process involved in the regulation of homeostasis, tissue development, and the elimination of immune cells that are no longer useful. Apoptosis also functions in removing aberrant cells generated by DNA damage or those infected by viruses (Golstein, 1997; Nagata, 1997; Sieg *et al.*, 1997; Teodoro and Branton, 1997; White, 1996). Apoptosis is characterized by cell shrinkage, plasma membrane blebbing, chromatin condensation, intranucleosomal cleavage and compartmentalization of dead cell into membrane-enclosed vesicles or apoptotic bodies that are phagocytosed by surrounding cells (Nagata, 1997; White, 1996). The relationship between viruses and apoptosis is very complex, in part because a successful infection requires not only an efficient production and spread of virus progeny but also avoidance of host's defense mechanisms that limit replication by direct killing of infected cells and also by immune and inflammatory processes. Infection with most

viruses triggers apoptosis since many of them have evolved proteins that are able to inhibit or delay protective action of the host immune system until sufficient viral yields are produced. Some viruses encode products that actively induce apoptosis as part of the strategy to enhance virus spread. In such case a delicate balance between suppression and induction of apoptosis is established by combination of viral products. Recognition of these mechanisms provides not only means for development of new antiviral agents but also points at consequences of viruses and their certain products on the outcome and treatment of virus infection. This review deals with strategies which herpesviruses and HIV can possibly use for modulation of apoptosis during simultaneous infection of the AIDS-developing host. Opportunistic diseases are the major clinical manifestation of HIV infection and the condition *sine qua non* of AIDS. Virtually, all HIV-related mortality is preceded by such secondary diseases. Herpesvirus infections have been shown to very common in AIDS-suffering patients. The most frequent are CMV, VZV, HSV-1 and KSHV (HHV-8) infections leading to herpetic keratitis, EBV-associated lymphomas, and other diseases.

The incubation period between a primary infection with HIV and the development of fulminant AIDS varies among individuals and correlates with plasma level of HIV. Factors modulating the viral load could affect positively or negatively the disease progression. There is a strong evidence that co-infection with unrelated viruses, particularly herpesviruses, can produce an intracellular milieu favourable for the replication of HIV. *In vitro* studies have shown that HSV, HHV-8, EBV and CMV can activate the HIV LTR (Davis *et al.*, 1987, Kenney *et al.*, 1988, Lusso *et al.*, 1989). Other studies have shown that in HIV patients with acute genital herpes infection a transient increase in HIV plasma load appeared, which returns to baseline once the episode has terminated (Mole *et al.*, 1997). However, there is also a contrary evidence that under certain circumstances herpesviruses can inhibit or severely suppress HIV propagation within the host. Bowen *et al.* (1996) demonstrated that active CMV infection can decrease HIV replication rate. Mechanisms of possible herpesvirus/HIV interactions on the molecular level include transactivation, induction of alternative HIV receptors, cytokine(s) release, up-regulation of CD4 and/or co-receptors expression, and last but not least, modulation of apoptosis.

Herpesviruses

The *Herpesviridae* family contains over 100 different viruses of which 8 are important human pathogens (Pringle, 1999; Roizmann *et al.*, 1992). All herpesviruses remain latent in specific cells, but the cell type and latency period may vary considerably. Members of the three subfamilies, *Alpha-, Beta- and Gammaherpesvirinae*, have been shown to induce or suppress apoptosis, depending on the target cell type and the conditions of the infection. The interactions between herpesviruses and pathways regulating apoptosis have a profound effect on the viral replication and the pathogenesis of the disease (Banks and Rouse, 1992; Kieff and Shenk, 1998; Stevens, 1989).

Alphaherpesviruses

Alphaherpesviruses have a wide host range and short replication cycle, and may efficiently destroy infected cells. They include HSV-1, HSV-2, VZV, bovine herpesviruses 1 and 2 (BHV-1 and BHV-2), equine herpesviruses 1 and 4 (EHV-1 and EHV-4), pseudorabies virus (PRV), and herpesvirus B (HBV).

HSV-1 and BHV-1 can *in vitro* induce apoptosis in activated human and bovine peripheral blood T lymphocytes, respectively, while VZV induces apoptosis in Vero cells (Griebel *et al.*, 1990; Ito *et al.*, 1997; Sadzot-Delvaux *et al.*, 1995). The mechanism for this selective induction of apoptosis in a certain cell type is yet to be understood. Activated T lymphocytes play an important role in the clearance of local herpesvirus infections (Martin *et al.*, 1988; Niemiałtowski *et al.*, 1994), so their destruction could delay virus elimination, facilitating the spread of HSV-1 and BHV-1 within the host.

Recently Koyama and Adachi (1997) showed that wild-type HSV-1 could drive cell into apoptosis by inhibition of viral protein synthesis *de novo*, suggesting that it is presumably an early event during infection; the induced HSV-1 proteins may specifically block apoptosis. Moreover, HSV-1 may also block cell death induced by sorbitol-mediated osmotic shock, hypothermia, thermal shock or exposure to ceramide, TNF and anti-Fas antibody (Koyama and Miwa, 1997; Leopardi *et al.*, 1996).

The HSV-γ34.5 gene encodes a protein that might inhibit the onset of apoptosis in virus-infected neuronal cells. This gene enables the virus to replicate and spread in central nervous system (CNS) mice and also in neuronal cells *in vitro* and blocks the phosphorylation of the eIF-2α transcription factor. When cultured neuronal or HeLa cells were infected with a HSV-γ 34.5 mutant, the onset of viral DNA replication coincided with a complete shutdown of viral and cellular protein synthesis (Chou and Roizman, 1992; He *et al.*, 1996). However, the cells infected with this mutant were not demonstrated to display any symptoms of apoptosis (He *et al.*, 1996).

At least two additional HSV-1 genes: Us3 and Us5, have been implicated in the inhibition of apoptosis. The Us3 gene encodes a serine/threonine protein kinase that phosphorylates both viral and cellular proteins. The Us5 gene

Table 1. Examples of viruses encoding proteins inducing or inhibits apoptosis

Virus	Product	Action	Reference
HSV-1	γ 34,5	Blocks phosphorylation of eIF-2α	Chou and Roizman (1992)
	Us3&Us5	Inhibits caspases 8 and 3	Leopardi *et al.* (1997)
BHV-1	LR	Inhibits S-phase entry	Ciacci-Zanella *et al.* (1999)
HCMV	IE1	Modulates p53 activity	Fortunato *et al.* (1997)
	IE2	Interacts with p53 and 105^{Rb}	Lukac and Alwine (1999)
	v-MIA	Inhibits cytochrome-c release	Goldmacher *et al.* (1999)
EBV	BHRF1	Bcl-2 homologue	Henderson *et al.* (1993)
	BALF1	Bcl-2 homologue	Marshall *et al.* (1999)
	LMP-1	Up-regulates p53 and cyclin D2 expression, intracellular levels of Bcl-2 and A20	Okan *et al.* (1995) Chen *et al.* (1998)
	EBNA-2	Up-regulates Bcl-2 expression	Henderson *et al.* (1993) Rowe *et al.* (1994)
HVS	ORF 16 (HVSBcl-2)	Bcl-2 homologue	Nava *et al.* (1997) Derfuss *et al.* (1998)
	ORF71	FLICE-inhibitory protein	Thome *et al.* (1997)
MHV-68	M11	Bcl-2 homologue	Wang *et al.* (1999)
	γHV68-v-cyclin	v-cyclin, promotes cell cycle progression	Van Dyk *et al.* (1999)
HHV-8	ORF 16 (KSbcl-2)	Inhibits Bax-mediated cell death	Cheng *et al.* (1997)
	ORF K 13	FLICE-inhibitory protein	Thome *et al.* (1997)
	LANA (ORF 73)	Interacts with tumor supressor protein p53 and represses its transcriptional activity	Friborg *et al.* (1999)
	KSHV-D-cyclin (ORF 72)	KSHV-D-cyklin forms complex with CDK6 and induces cell proliferation	Ojala *et al.* (1999) Li *et al.* (1997)
HIV-1	Nef	Transactivates c-kit promoter leading to apoptosis	He *et al.* (1997)
	Tat	Up-regulates FasL	Kaplan and Sieg (1998)
		Down-regulates Bcl-2	Zauli *et al.* (1993)
	gp120	Induces apoptosis upon binding with CD4	Herbein *et al.* (1998)
	Vpr	Supresses apoptosis via inhibition of NF-κB	Kaplan and Sieg (1998) Ayyavoo *et al.* (1997)

For abbreviations see their list on the first page of the paper.

contains a small open reading frame (ORF) predicted to encode a small, membrane-associated glycoprotein gJ of unknown function (Balan *et al.*, 1994). Viruses deleted for this gene were found to be phenotypically normal, including typical plaque formation and cell to cell spread. Both Us3 and Us5 contributed to protection of infected cells from UV- or anti-Fas-antibody-mediated apoptosis (Leopardi *et al.*, 1997). Data from experiments using Us3 and Us5 deletion mutants showed the ability of HSV-1 to inhibit both caspase-8 and caspase-3 activation after UV irradiation or anti-Fas antibody ligation. (Leopardi *et al.*, 1997; Jerome *et al.*, 1999).

BHV-1 was shown to induce apoptosis both *in vivo* and *in vitro*. It was hypothesized that latency-related (LR) gene products promote neuronal survival by inhibiting apoptosis. During productive infection, LR RNA is the only abundant viral transcript present in neurons. LR gene products inhibit the S phase entry, and LR is associated with cyclin-dependent kinase 2. Other studies demonstrated that LR gene products inhibit or delay apoptosis following transient transfection of CV-1 cells, low-passage human fibroblasts or mouse neuroblastoma cells (Ciacci-Zanella *et al.*, 1999; Winkler *et al.*, 1999).

Betaherpesviruses

Betaherpesviruses including HCMV (HHV-5), MCMV-1, and human herpesvirus 6 (HHV-6) have characteristically long replication period. There is some evidence that cytomegaloviruses inhibit the process of hematopoesis (Gibbons *et al.*, 1995); using the mouse as a model, it has been demonstrated that an acute MCMV infection prevents reconstitution of bone marrow cells (Mon *et al.*, 1997), they interfere with process of early hematopoesis (Gibbons *et*

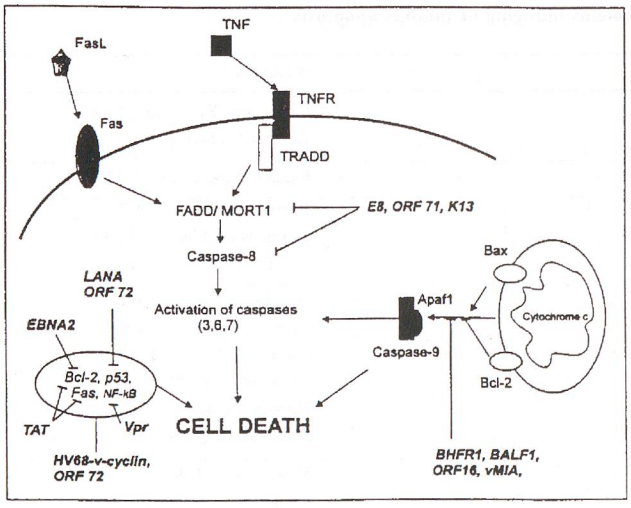

Fig. 1
Viral modulators of apoptosis

al., 1995), and causes significant decrease in the number of hematopoetic progenitor cells in the bone marrow. The remaining progenitor cells exhibited an increase in the level of cell-surface Fas-R and these cells (Fas-R^{III}) were sensitive to Fas-mediated apoptosis upon treatment with antibody to the Fas-R. This led to the notion that the MCMV infection might reduce the number of hematopoetic progenitor cells at least in part through Fas-mediated apoptosis. It has been also shown that the MCMV infection predisposes T cells to apoptosis or induces apoptosis in T cells of acutely or persistently infected mice (Koga *et al.*, 1994; Boppana and Britt, 1996). Elevated levels of Fas-R in B and T cells of acutely infected mice were only partially responsible for cell death, since mice deficient in FasR also displayed apoptosis as a result of the MCMV infection (Kaur *et al.*, 1996). On the other hand, HCMV can directly block at least some pathways of apoptotic response; transfection of HeLa cells with vectors expressing two immediate early viral genes, IEl and IE2, inhibited the TNF-α-induced apoptosis. Furthermore, HeLa cells constitutively expressing the IE1 or IE2 proteins were resistant to the induction of apoptosis by treatment with TNF-α (or by infection with the adenovirus mutant d13379) (Zhu *et al.*, 1995). The IE1 and IE2 mRNAs are differentially spliced products of the same primary HCMV transcript. IE2, a promiscuous transactivator is supposed to bind to the TATA-binding protein, transcription factor IIB, and Sp1 (Caswell *et al.*, 1993). IE1 activates transcription through NF-κB sites and affects synergistically with many IE2 promoters (Zhu *et al.*, 1995). Various studies have shown that IE1 and IE2 play important role in cell cycle and growth control and it seems likely that they block apoptosis by modulating the expression of cellular proteins that in turn regulate or mediate the cell death process. IEl and IE2 also up-regulate p53 but inhibit its ability to activate transcription (Muganda *et al.*, 1994). Both proteins can bind to p105/Rb and inhibit its ability to induce the flat-cell phenotype in Saos-2 cells (Lukac and Alwine, 1999).

HCMV has been recently shown to possess a novel mitochondria-located inhibitor of apoptosis, denoted vMIA, which is capable of inhibiting the Fas-mediated apoptosis at a point downstream of caspase-8 activation and Bid cleavage but upstream of cytochrome c release (Goldmacher *et al.*, 1999). An antiapoptotic vMIA is located close to the outer mitochondrial membrane and is associated with adenine nucleotide translocator (ANT), thus implicating its role in intracellular ATP depletion and mitochondrial efflux of cytochrome c into the cytoplasm. These functional properties are analogous to those of Bcl-2 but vMIA lacks any sequence homology and does not bind to Bax. This suggests that vMIA may be a biochemically distinct suppressor of apoptosis involved in stabilization of mitochondrial membrane functions (Goldmacheret *et al.*, 1999)

Gammaherpesviruses

Gammaherpesviruses generally exhibit the most limited host range as they are characterized by the tropism for B and/or T cells and are associated with B and T cell malignancies. In humans, infection with EBV may lead to development of Burkitt's lymphoma (BL) and Hodgkin's disease. Herpesvirus saimiri (HVS) and newly discovered KSHV (HHV-8) are known to establish long-term latency in lymphocytes (Nava *et al.*, 1997; Ohishima *et al.*, 1999; Spender *et al.*, 1999). Wild-type EBV has not been reported to induce apoptosis in resting human B lymphocytes. This is in part due to the fact that during latency period EBV up-regulates the expression of Bcl-2 in B lymphocytes (Pepperi *et al.*, 1998). Other antiapoptotic proteins of the Bcl-2 family include Mcl-1 and Bcl-xl and there is also a proapoptotic factor Bax – they all may be expressed in EBV-transformed B lymphocytes (Martin *et al.*, 1993; Simonian *et al.*, 1997). Gammaherpesviruses are known to encode Bcl-2 homologues of which most show conservation of sequences of both BH1 and BH2 domains with respect to Bcl-2 but have little homology with other regions. The viral Bcl-2 proteins have poorly conserved BH3 domains and lack BH4 domains. All viral homologues contain a strech of hydrophobic amino acids at their carboxy-termini, which is expected to mediate intracellular membrane localization. The BH1 and BH2 domains of cellular Bcl-2 are important for Bcl-2 and Bcl-xl to form heterodimers with pro-apoptotic Bax. The BH3 domain of Bak and Bax is critical for those to form heterodimers with Bcl-2 and Bcl-xl. The B4 domain of Bcl-2 is important for interactions with other cellular proteins. The herpesvirus Bcl-2 homologues are primarily active during

lytic replication cycle (Marshal *et al.*, 1999, Reed, 1997; Simonian *et al.*, 1997, Shimizu *et al.*, 1996).

EBV, HHV-8, HVS, mouse herpesvirus strain 68 (MHV-68), BHV-4, and equine herpesvirus 2 encode some Bcl-2 homologues but only EBV BHRF1, EBV BALF1, and HHV-8 ORF16 (Cheng *et al.*, 1997; Sarid *et al.*, 1997), HVS ORF 16 (Nava *et al.*, 1997; Derfuss *et al.*, 1998), and MHV-68 ORF M11 (Wang *et al.*, 1999) have been shown to inhibit apoptosis. EBV encodes two proteins, namely BHRF1 and BALF1, which have substantial colinear homology to Bcl-2 in BH1 and BH2 domains and associate with cellular Bax and Bak. BHFR1 is expressed early during the EBV replication cycle and it is the first viral protein that was recognized as related to Bcl-2 (Henderson *et al.*, 1993; Kieff and Shenk, 1998). BHRF1 expression can render cells somewhat resistant to T lymphocyte cytotoxic attack by inhibiting TNF- and Fas-induced cell death (Kawanishi, 1997). Analysis of the BALF1 sequence showed that there is closer similarity between BALF1, Bcl-xl, and Bcl-2 then between BHRF1 Bcl-xl, and Bcl-2. These results indicate that EBV encodes two operating v-bcl-2 genes (Marshall *et al.*, 1999; Reed, 1997). The level of homology of the human HVS and HHV-8 genes and the bcl-2 gene is 15–20%, just slightly less than that of BHRF1. In contrast to HHV-8 ORF16, HVS ORF 16 having BH1 and BH2 domains and MHV-68 M11 possibly encode a 171 amino acid protein that has a limited homology to Bcl-2. Expression of these viral Bcl-2 homologues protected cells from the apoptotic challenge of Sindbis virus (Shimizu *et al.*, 1996). The lack of BH2, BH3 and BH4 domains in M11 may make M11 a better inhibitor of apoptosis than Bcl-2 by preventing interactions of the viral protein with Bax and Bak (Wang *et al.*, 1999).

The EBV latent infection membrane protein 1 (LMP-1) is essential for EBV-mediated growth transformation of resting primary human B lymphocytes into indefinitely proliferating lymphoblastoid cell lines (LCLs) and has been implicated in many antiapoptotic activities of the virus in latently infected B cells. Primary infection of normal B cells with EBV rapidly increases p53 expression level about 10 times. This increase has been shown to be directly mediated by LMP-1 *via* activation of the nuclear NF-κB transcription factor (Chen and Cooper, 1996). In contrast, over-expression of p53 in a number of cell types blocks cell cycle progression at the G1/S boundary and leads to apoptosis. Hence, the virus must possess the mechanism to counteract the lethal over-expression of p53. The EBV membrane protein LMP-1 fulfills this role because it up-regulates intracellular levels of Bcl-2 as well as A20, another antiapoptotic protein (Hendersson *et al.*, 1991). LMP-1 also blocks apoptosis triggered by over-expression of p53 in EBV-negative BL cells-bearing mutant p53 (Okan *et al.*, 1995). However, EBV proteins inhibiting p53-mediated apoptosis lacked the ability to block the p53-induced cell cycle arrest at the G1/S or G2/M boundaries (Chen *et al.*, 1998). HHV-8 has also been shown to encode a protein that interacts with p53 and modulates its activity. The latency-associated nuclear antigen (LANA) encoded by ORF73 of the HHV-8 genome is a highly immunogenic protein that is expressed predominantly during viral latency in cell lines established from body-cavity-based lymphomas. LANA interacts with the tumor suppressor protein p53 and represses its transcriptional activity, thus inhibiting its ability to induce cell death (Friborg *et al.*, 1999). EBV nuclear antigens expressed in latent infection (EBNAs) have also been implicated in the induction of Bcl-2. In some Burkitt's lymphoma tumor cell lines, EBNA-2 remaining under control of a heterologous promoter partially up-regulated Bcl-2 expression (Henderson *et al.*, 1993; Rowe *et al.*, 1994).

It has been recently characterized that both FADD (the Fas and TNF receptor Rl-associated, death-inducing cytoplasmic adaptor) and caspase-8 have a homologous death effector domain (DED) used as a region of interaction that leads to the initiation of apoptosis (Teodoro and Branton, 1997).

Thome *et al.* (1994) found out that there are two similar regions within the genome of EHV-2, HHV-8, HVS, and BHV-4, which encode viral FLICE-like inhibitory proteins that (vFLIPs) contain two death-effector domains that interact with the adaptor protein FADD thus preventing recruitment and activation of FLICE. The EHV-2 E8, HVS ORF71 and HHSV8 ORF K13 proteins have been shown to bind to DEDs of caspase-8 or FADD and to block the cell death mediated by the death domain-containing receptors (Thome *et al.*, 1997).

The oncogenic potential of gammaherpesviruses may in part result from their ability to regulate cell cycle by means of D-type cyclins. Animal D-type cyclins (a) bind to cyclin-dependent kinases 4 and 6, (b) are required for the activation as well as the specificity of the kinase complex, and (c) are essential for cell cycle progression through G1 phase (Ajchenbaum *et al.*, 1993). Although EBV does not encode a cyclin homologue(s), EBV infection (or expression of EBV LMP-1) up-regulates expression of D2 cyclin (Arvanitakis *et al.*, 1995). In contrast, HHV-8, HVS, and MHV-68 contain ORFs predicted to encode proteins with high level of homology to mammalian D-type cyclins. The MHV-68 cyclin is homologous in 25% to the HVS cyclin. Viral cyclin (v-cyclin) is able to promote cell cycle progression in primary culture of T lymphocytes. This effect is mediated by expression of v-cyclin at a level comparable to that seen in lytically infected cells (Van Dyk *et al.*, 1999). Functional studies of HHV-8 D-cyclin demonstrated that KSHV cyclin can form a complex with CDK6 and induce cell proliferation when co-transfected with wild-type RB1. Unlike cellular D cyclins, KSHV cyclin can also control phosphorylation of histone H1 (Ojala *et al.*, 1999; Li *et al.*, 1997).

Human immunodeficiency viruses

HIV-1 and HIV-2 (HIV) represent threat of a global pandemic with enormous public health implications and are thought to be the etiologic agents of AIDS. HIV cause a long lasting, asymptomatic infection characterized by normal to elevated numbers of circulating $CD8^+$ T lymphocytes accompanied by progressive decline in $CD4^+$ cell number, high levels of cell-associated and plasma viremia, p24 antigenemia and proviral burden followed by multisystem dysfunction (Ameisen and Capron, 1991; Gougeon *et al.*, 1993; Skowron *et al.*, 1997). Although HIV cause devastating chronic infection, it is also responsible for an acute viral infection. In 1991, several groups of scientists proposed that HIV can modulate apoptosis (Laurent-Crawford *et al.*, 1991). The gradual depletion of $CD4^+$ T cells during AIDS develops by apoptosis secondary to inappropriate induction of cell death instead of activation pathways (Famularo *et al.*, 1997; Gougeon *et al.*, 1998). It is clear that HIV influence the apoptosis in a complex and heterogenous manner. Some of the molecular and cellular mechanisms that mediate the various effects have been investigated and pleiomorphic effects of specific HIV-1 proteins on apoptosis have been found. E. g., although HIV-1 Tat can induce apoptosis (Bartz and Emerman, 1999; Li *et al.*, 1995; Purvis *et al.*, 1995), it has also capacity to protect cells from dying (Zauli *et al.*, 1993). Tat protein up-regulates FasL, which in turn activates Fas-R (Kaplan and Sieg, 1998). Over-expression and release of FasL leads to apoptosis in infected and uninfected $CD4^+$ T cells (Finkel *et al.*, 1995; Gougeon, 1997). Tat has also been shown to down-regulate Bcl-2 expression, causing an up-regulation of Bax expression in various hematopoetic cell lines (Zauli *et al.*, 1993).

Recently, HIV Vpr has been shown to induce apoptosis of non-activated T lymphocytes, while in activated cells it was inhibited by Vpr *via* IκB-mediated suppression of NF-κB (Ayyavoo *et al.*, 1997; Copeland and Heeney, 1996; Stewart *et al.*, 1999; Conti *et al.*, 1999). HIV-1 structural glycoprotein 120 (gpl20) also acts as proapoptotic factor which mediates the entry of HIV-1 into susceptible cells by interaction with CD4 molecules and any of several chemokine receptors (CCR or CXCR4) on cell surface (Herbein *et al.*, 1998). It has frequently been observed that infected cells display resistance to apoptotic stimuli. However, uninfected "bystander" cells undergo severe apoptosis. Some studies have shown that gp120 can induce morphological changes associated with apoptosis in uninfected primary $CD4^+$ cells (Finkel *et al.*, 1995). Experimental findings have also indicated that gp120 binding to either the CD4 or CXCR4 receptors is sufficient signal to induce apoptosis (Wang *et al.*, 1999). Induction of apoptosis due to the virus-binding receptor is dependent on the presence of the cytoplasmatic tail of CD4 molecule, indicating the need for signal transduction (Kaplan and Sieg, 1998). Once the cell is infected, the expression of viral genes, such as *tat*, may provide protection. However, the apoptotic program is unavoidable for uninfected cells, leading to gradual depletion of lymphoid and neuronal cell populations. Synthetic peptides that mimic the CD4-binding-epitope can antagonize the gpl20-induced apoptosis (Kaplan and Sieg, 1998). Exposure of cell surface death receptors, such as Fas and TNF-R also plays a role during HIV-1 infection, although the process is more intricate. Blood cells freshly isolated from HIV-1-infected patients have a deficiency in Fas-L cell surface expression and activity in comparison to those from uninfected, healthy volunteers (Copeland and Heeney, 1996; Gougeon, 1998; Kaplan and Sieg, 1998). It has been reported that soluble gp120 released from infected cells can induce TNF synthesis in macrophages and can also upregulate the TNF-R II expression on CD8 cells provided its binding to CXCR4 receptors (Skowron *et al.*, 1997). This mechanism of the T cell death pathway initiation was unpredictable, because reports on apoptosis through TNF-RII signaling were rare (Teodoro and Branton, 1997). Another report has shown that CD4 cross-linking sensitized cells to Fas triggering due to simultaneous expression of both Fas and FasL (Zauli *et al.*, 1993). At the same time cells became sensitive to the TNF-mediated death by an unknown mechanism (Kolesnitchenko *et al.*, 1997). It seems that HIV-1 regulates the apoptosis pathway in AIDS patients. HIV-1 infection of $CD4^+$ cell leads to significant decrease of cellular antioxidants including superoxide dismutase and also Bcl-2. If cells were supplemented with exogenous antioxidants they presented a substantial reduction of apoptosis. It supports hypothesis on the role of oxidative stress in $CD4^+$ T cell death during the course of HIV-1 infection.

Early after systemic infection, HIV-1 is detectable in the CNS and recent results show that comparable numbers of astrocytes and macrophages/microglia cells are infected with HIV-1. For astrocytes it was proposed that Nef induces apoptosis by a mechanism involving a Nef-mediated transactivation of the c-kit promoter (Kohleisen *et al.*, 1999). On the other hand, the establishment of astrocytic cell lines stably expressing Nef has been described, suggesting that synthesis of Nef does not invariably lead to cell death in astrocytes (Kohleisen *et al.*, 1999). As for lymphocytes, Nef functions as an activating viral factor and an inducer of apoptosis in T cells. Cell surface expression of a hybrid Nef-CD8 molecule has also induced activation of T cell followed by apoptosis (Baur *et al.*, 1994).

It seems that HIV-1 regulates apoptosis in a way that is beneficial for its own replication and provides optimal conditions for viral progeny production and for cell-to-cell transmission of the virus. This viral pressure *in vivo* is very complex, since it involves both the inhibition and induction

of apoptosis. It may depend on different mechanisms with respect to the progress of HIV infection so that newly infected cells are prevented from dying and with respect to the cell type so that certain uninfected cells, perhaps those able to mount an effective antiviral immune response are driven to apoptotic death.

Note of the Editor-in-Chief. The nomenclature of some viruses used in this article (e.g. BHV-4, EHV-2, HHV-8 (KHSV), and HVS) is not conform with that presently valid according to the International Committee on Taxonomy of Viruses (Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarwis, A.W., Martelli, G.P., Mayo, M.A., Summers, M.D. (Eds): *Virus taxonomy. Classification and Nomenclature of Viruses. Sixth Report of the International Committee on Taxonomy of Viruses.* Wien–New York, Springer Verlag, 1995.

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