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Review Article

Genetic and Metabolic Control of the Mitochondrial Transmembrane Potential and Reactive Oxygen Intermediate Production in HIV Disease

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ABSTRACT

Redox mechanims play important roles in replication of human immunodeficiency virus type 1 (HIV-1) and cellular susceptibility to apoptosis signals. Viral replication and accelerated turnover of $CD4^+$ T cells occur throughout a prolonged asymptomatic phase in patients infected by HIV-1. Disease development is associated with steady loss of $CD4^+$ T cells by apoptosis, increased rate of opportunistic infections and lymphoproliferative diseases, disruption of energy metabolism, and generalized wasting. Such pathological states are preceded by: (i) depletion of intracellular antioxidants, glutathione (GSH) and thioredoxin (TRX), (ii) increased reactive oxygen species (ROS) production, and (iii) changes in mitochondrial transmembrane potential ($\Delta\Psi_m$). Disruption of $\Delta\Psi_m$ appears to be the point of no return in the effector phase of apoptosis. Viral proteins Tat, Nef, Vpr, protease, and gp120, have been implicated in initiation and/or intensification of oxidative stress and disruption of $\Delta\Psi_m$. Redox-sensitive transcription factors, NF-κB, AP-1, and p53, support expression of viral genes and proinflammatory lymphokines. ROS regulate apoptosis signaling through Fas, tumor necrosis factor (TNF), and related cell death receptors, as well as the T-cell receptor. Oxidative stress in HIV-infected donors is accompanied by increased glucose utilization both on the cellular and organismal levels. Generation of GSH and TRX from their corresponding oxidized forms is dependent on NADPH provided through the pentose phosphate pathway of glucose metabolism. This article seeks to delineate the genetic and metabolic bases of HIV-induced oxidative stress. Such understanding should lead to development of effective antioxidant therapies in HIV disease. Antiox. Redox Signal. 2, 551–573.

INTRODUCTION

INFECTION BY THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) leads to a complex disease characterized by a variety of clinical symptoms, dysregulation of the immune system ranging from profound T-cell depletion to autoimmunity, opportunistic infections, systemic inflammatory responses, energy deficit, dementia, and increased incidence of cancers (Pantaleo *et al.*, 1993). Although the complexities of pathogenic mechanisms elicited by the virus are still being delineated, understanding the selective depletion of $CD4^+$ T cells remains a primary target of HIV research.

During the course of HIV infection, three major phases can be distinguished. Extensive viremia occurs within a few weeks after infection, giving rise to an acute mononucleosis-like syndrome. A second and relatively latent period represents an ongoing fierce battle between virus replication and replenishing of the

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$CD4^+$ T cell reservoirs. During latency, viral particles are constantly being produced while as much as 5% of $CD4^+$ T cells are killed and regenerated daily (Ho *et al.,* 1995; Wei *et al.,* 1995). On average, 10 years following infection, the delicate balance between viral replication and turnover of $CD4^+$ T cells becomes disturbed and a steady decline of $CD4^+$ T-cell counts ensues. Diminished $CD4^+$ T-cell function gives rise to opportunistic infections, lymphomagenesis, and autoimmune phenomena at the final stages of disease. Infection by HIV increases the levels of reactive oxygen intermediates (ROS) both within the infected cells and the plasma of asymptomatic patients early in the course of the disease (Table 1). Redox signaling plays an important role in gene transcription and replication of HIV-1 (Table 2), as well as regulation of cellular susceptibility to apoptosis signals (Hamilos and Wedner, 1985; Halliwell and Gutteridge, 1990; Lipton *et al.,* 1993; Buttke and Sandstrom, 1994; Stamler, 1994; Korsmeyer, 1995; Los *et al.,* 1995a; Banki *et al.,* 1996, 1998; Adler *et al.,* 1999). This review will assess redox checkpoints and underlying metabolic mechanisms regulating HIV replication and death of infected (Fig. 1) and uninfected cells (Fig. 2).

EVIDENCE FOR OXIDATIVE STRESS INDUCED BY HIV

Association of oxidative stress with HIV infection was originally implied from finding diminished levels of reduced glutathione (GSH) in the plasma and lymphocytes of HIV-infected individuals (Eck *et al.,* 1989). GSH, a cysteine-containing tripeptide, is the major source of intracellular free thiols and an important antioxidant (Meister and Anderson, 1983). GSH levels declined in the plasma, peripheral blood mononuclear cells (PBMC), monocytes (Eck *et al.,* 1989; de Quay *et al.,* 1992), and lung epithelial lining fluid of HIV-infected persons (Buhl *et al.,* 1989). Diminished intracellular GSH levels were reported in both $CD4^+$ and $CD8^+$ T cells on the basis of flow cytometry of glutathione-*S*-bimane fluorescence (Roederer *et al.,* 1991; Staal *et al.,* 1992). Cysteine deficiency of HIV-infected individuals served as a rationale for treatment with *N*-acetylcysteine (NAC) (Droge *et al.,* 1992). More recently, decreased GSH was correlated with increased levels of oxidized glutathione (GSSG) in HIV-infected $CD4^+$ T cells, suggesting that a lack of reducing equivalents rather than decreased GSSG synthesis was responsible for GSH deficiency

Table 1. Evidence for Oxidative Stress Induced by HIV

Evidence	*Body site/cell type*	*Reference*
GSH and cysteine depletion	Plasma of HIV-infected patients	(Buhl *et al.*, 1989; Eck *et al.*, 1989; Jacobson *et al.*, 1990; Droge *et al.*, 1992; Staal *et al.*, 1992; Fujita *et al.*, 1993)
GSH and NAC inhibited HIV replication	Monocytic cell line	(Kalebic *et al.*, 1991)
GSH elevation	$CD4^+$T cells	(van der Ven *et al.*, 1998)
GSH depletion/GSSG elevation	Peripheral blood T cells	(Aukrust *et al.*, 1995; Lopez Galera *et al.*, 1996; Staal, 1998)
TRX depletion	Lymph nodes of HIV-infected patients	(Masutani *et al.*, 1992)
TRX elevation	Plasma of HIV-infected patients	(Nakamura *et al.*, 1996)
Inhibition of HIV apoptosis by NAC	Peripheral blood T cells, monocytes	(Malorni *et al.*, 1993; Kinscherf *et al.*, 1994)
Increased ROS levels	Jurkat and H9 T cells	(Banki *et al.*, 1998)
Increased ROS levels	Peripheral blood monocytes	(Elbim *et al.*, 1999)
Vitamins E and C decrease ROS levels	Plasma of HIV-infected patients	(Allard *et al.*, 1998b)
Correlation of GSH levels and CD4 cell count	Peripheral blood $CD4^+$ T cells	(Herzenberg *et al.*, 1997)
Inverse correlation of ROS levels and CD4 cell count	Plasma of HIV-infected patients	(Malorni *et al.*, 1998)

TABLE 2. REDOX-REGULATED PROCESSES IN HIV-INFECTED CELLS

Effect	*Body site/cell type*	*Reference*
ROS enhance promoter activity of the HIV-1 LTR	Monocyte and T cell lines	(Kurata, 1996)
Tat-induced stimulation of Sp1-DNA interactions	HeLa cells	(Seve *et al.*, 1999)
Oxidative stress leads to caspase activation	HIV-infected T cell lines	(Banki *et al.*, 1998)
Tat induces oxidative stress and caspases	Brain cell cultures	(Kruman *et al.*, 1998)
Tat-induced Fas-ligand expression	Human peripheral blood T cells	(Ehret *et al.*, 1996)
Tat-induced stimulation of JNK and AP1	Human T cell lines	(Bofill *et al.*, 1995)
Tat-induced stimulation of NF-κB activity	HIV-infected peripheral blood T cells	(Westendorp *et al.*, 1995b)
Redox status directly affects NF-κB activity	Human T and monocytic cell lines	(Staal *et al.*, 1990; Israel *et al.*, 1992)

(Aukrust *et al.*, 1995; Lopez Galera *et al.*, 1996). The level of another thiol antioxidant, thioredoxin (TRX) is diminished in HIV-infected cells (Masutani *et al.*, 1992). There are several forms of TRX or adult T-cell leukemia-derived factor. Its 12-kD form is comprised of 105 amino acids with a redox-active dithiol site (Tagaya *et al.*, 1989; Nakamura *et al.*, 1997). Within the cell, TRX can translocate to the nucleus to regulate DNA-binding affinity of redox-sensitive transcription factors (Nakamura *et al.*, 1997). TRX is also secreted by lymphocytes in response to oxidative stress and functions as chemoattractant for neutrophils, monocytes, and T cells (Bertini *et al.*, 1999). Its 80- or 84-amino-acid-long amino-terminal fragment, termed eosinophil cytotoxicity-enhancing factor, stimulates HIV replication (Newman *et al.*, 1994). Thus, increased TRX levels in the plasma of HIV-infected individuals may contribute to the vicious cycle of viral replication (Nakamura *et al.*, 1996). Elevated serum levels of lipid peroxidation products, malondialdehyde (Sonnerberg *et al.*, 1988), and hydroperoxide (Revillard *et al.*, 1992; Allard *et al.*, 1998a) indicative of ongoing oxidative stress, were also found in HIV-infected patients compared to uninfected controls.

MITOCHONDRIA AND ROS

ROS modulate various signal-transduction pathways. ROS can elicit positive responses such as lymphocyte activation (Hamilos and Wedner, 1985; Hildeman *et al.*, 1999) and proliferation (Suthanthiran *et al.*, 1990), as well as negative responses such as apoptosis (Hockenberry *et al.*, 1993; Kane *et al.*, 1993; Lipton *et al.*, 1993; Buttke and Sandstrom, 1994). Although HIV can infect almost any human cell type, the major targets are $CD4^+$ T cells and monocytes. Upon infection by HIV, peripheral blood $CD4^+$ T cells rapidly die by apoptosis (Meyaard *et al.*, 1992). Infection of $CD4^+$ T cells leads to a several-fold increase in mitochondrial ROS levels, an initial increase of the mitochondrial transmembrane potential ($\Delta\Psi_m$) followed by a later fall in $\Delta\Psi_m$, caspase activation, and phosphatidylserine externalization (Banki *et al.*, 1998). Disruption of $\Delta\Psi_m$ is associated with release of cytochrome *c* from the mitochondria (Green and Reed, 1998). Cytochrome *c* activates apoptosis-activating factor 1 (Apaf-1), which in turn activates caspase 9 and caspase-3 (Li *et al.*, 1997). Sequential activation of effector/executioner caspases such as caspase-3, caspase-6, and caspase-7 leads to cleavage of key factors necessary for cell survival. Caspase-3, formerly called CPP-32 (Salvesen and Dixit, 1997), cleaves IκB-α between Asp-35 and Ser-36 (Barkett *et al.*, 1997). Cleavage by caspase-3 blocks the ability of IκB-α to undergo phosphorylation-induced degradation. Thus, accumulation of IκB-α would inhibit activation of NF-κB and their responsive genes (Barkett *et al.*, 1997). Substrates of caspases also include structural proteins such as lamins, actin, fodrin, and enzymes such as poly(ADP-ribose) polymerase (Salvesen and Dixit, 1997) and caspase-activated DNase (CAD). CAD degrades the ge-

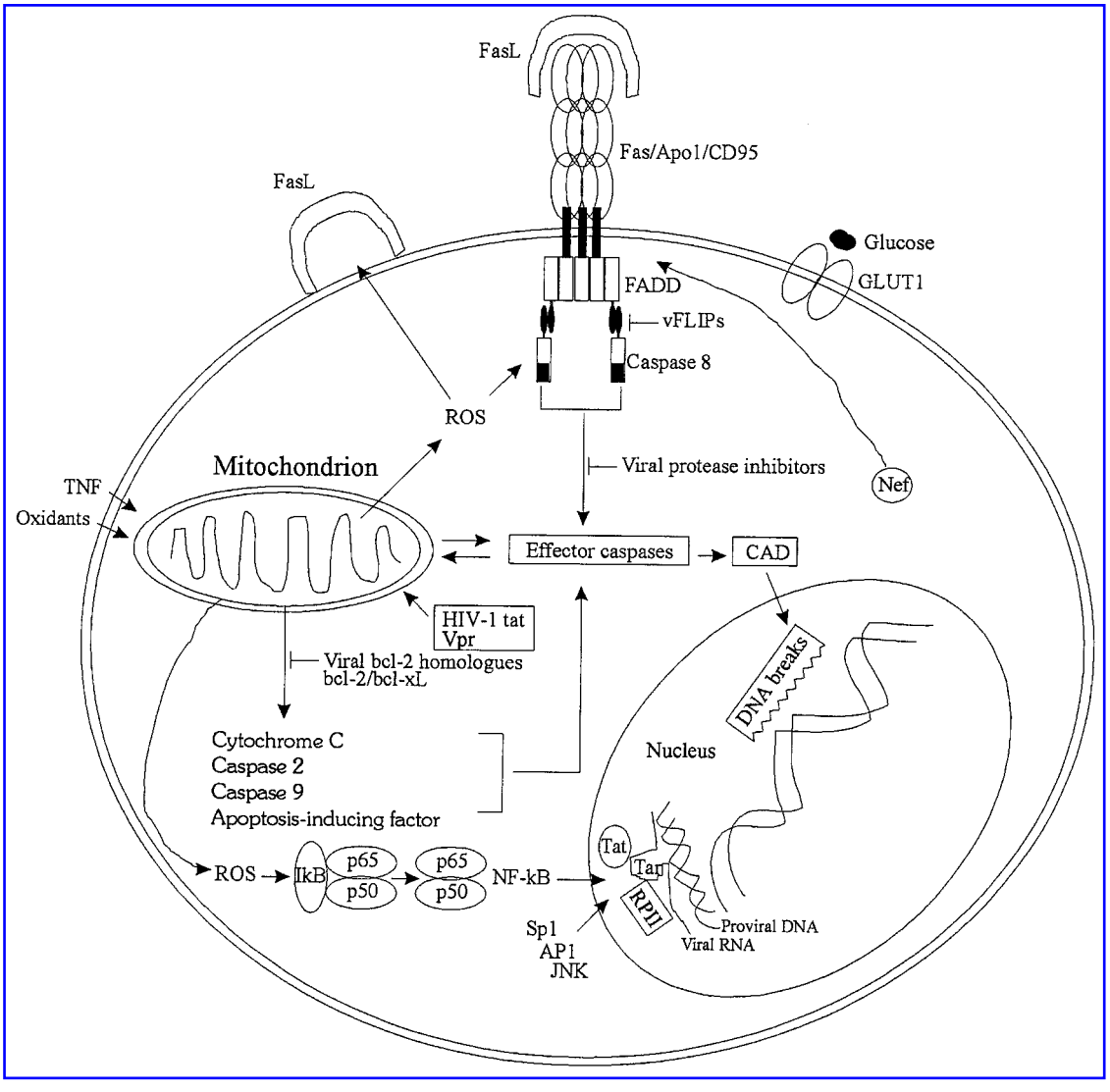

FIG. 1. Schematic overview of redox signaling and apoptosis pathways in HIV-infected cells. HIV-1 Tat promotes elongation of viral RNA by RNA polymerase II (RPII), enhances viral RNA transcription by redox-mediated stimulation of NF-κB, Sp1, Ap1, and JNK activities. Tat-induced ROS and Vpr-mediated changes in mitochondrial transmembrane potential increase susceptibility to apoptosis triggered by oxidants, TNF, and FasL. Expression of the Fas and TNF receptors is enhanced by the Nef protein. Activation of caspase-8 through Fas signaling and mitochondrial injury leads to the release of executioner caspase-activating factors. This process is inhibited by Bcl-2 and its viral homologues. The process is enhanced through cleavage of Bcl-2 by HIV-1 protease. Fas ligand (FasL) crosslinks the Fas receptor (Fas/Apo1/CD95), which recruits an adapter protein with a Fas-associated death domain (FADD). Viral FLIPs (vFLIPs) possess a death effector domain similar to those of FADD and caspase 8 and, thus, interrupt Fas signaling. While not shown, vFLIPs may also block TNF receptor-mediated signaling through FADD shared by both the Fas and TNF pathways. Upon recruitment of caspase 8, its oligomerization causes self-cleavage and activation of downstream effector caspases (Salvesen and Dixit, 1997). Caspase-3 activated DNase (CAD) causes host cellular DNA fragmentation (Enari *et al.*, 1998).

nomic DNA by cleavage at regular, 180- to 200-bp intervals (Salvesen and Dixit, 1997; Enari *et al.*, 1998).

The $\Delta\Psi_m$ is subject to regulation by an oxidation-reduction equilibrium of ROS, pyridine nucleotides (NADH/NAD + NADPH/NADP), and GSH levels (Constantini *et al.*, 1996). HIV-1 Tat inhibits expression of manganese superoxide dismutase which, in turn, may be responsible for elevation of ROS levels (Flores *et al.*, 1993). The Bcl-2 family of proteins play an important role in maintaining mitochondrial

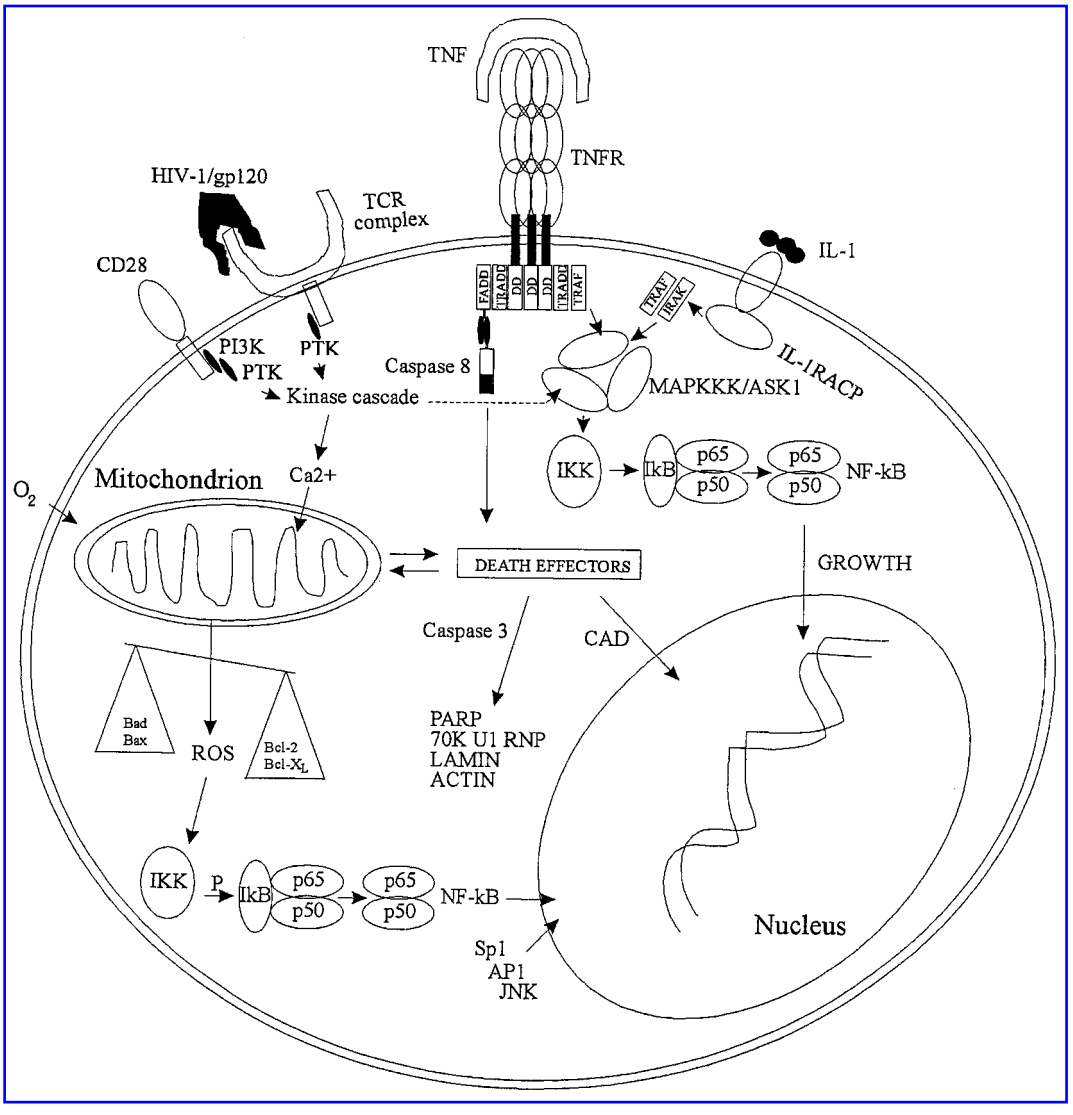

FIG. 2. Inflammatory cytokine and activation-induced oxidative stress and apoptosis pathways in T lymphocytes. Elevated serum levels of proinflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) signal through their specific receptors. Through death-domain (DD)-containing proteins, TNFR interacts with TRAF2, which, in turn, activates IKK via the MAPKKK/ASK1 cascade. ASK-1 is maintained in its inactive form when bound to TRX. ROS dissociate TRX from ASK-1 and promote multimerization required for kinase activity. Subsequently, IKK activation leads to phosphorylation-mediated degradation of I-κB and translocation of NF-κB and translocation of NF-κB into the nucleus. IL-1 also activates NF-κB through the TRAF pathway via the IL-1 receptor accessory protein (IL-1RACP) and IL-1 receptor-activated kinase (IRAK). Antigen, *e.g.*, HIV-1/gp120, binding-initiated signaling through the T-cell receptor complex/CD3 and the CD28 co-stimulatory molecule activate phosphatidylinositol 3-kinase (PI3K) and protein tyrosine kinases (PTK). This results in Ca flux into mitochondria followed by increased production of ROS and NF-κB activation (Green and Thompson, 1994; Los *et al.*, 1995b; Tatla *et al.*, 1999). Mitochondrial membrane integrity is maintained by a balance of membrane-stabilizing Bcl-2 and Bcl-X_L and pore-inducing Bax and Bad (Gross *et al.*, 1999) as well as the metabolic capacity to synthesize reducing equivalents (Fig. 4). Controlled increase of ROS levels activates NF-κB and promotes cell growth. Excess ROS production and disruption of $\Delta\Psi_m$ leads to activation-induced cell death executed by activation of caspase 3 (digesting vitally important proteins PARP, 70K U1RNP, lamin, and actin) and caspase 3-dependent DNase (CAD, causing nuclear DNA fragmentation).

membrane integrity (Green and Reed, 1998) (Fig. 1). This apoptosis-regulatory function may be related to their ability to form ion channels (Gross *et al.*, 1999). Enforced dimerization of proapoptotic Bax or Bad results in diminished membrane potential and increased production of ROS. By contrast, overexpression of Bcl-2 or Bcl-X_L will antagonize these effects by forming heterodimers with Bax or Bad. Bcl-2 overexpression also inhibits release of cy-

tochrome *c* from mitochondria (Kluck *et al.*, 1997; Yang *et al.*, 1997). Thus, cleavage of Bcl-2 by HIV-1 protease may contribute to elevated ROS levels and apoptosis (Strack *et al.*, 1996). Although persistent replication of HIV leads to apoptosis of $CD4^+$ T cells, monocytes and tissue macrophages actively produce virions without undergoing apoptosis. Viral replication in monocytes is associated with diminished Bcl-2 and TRX and augmented ROS levels (Elbim *et al.*, 1999). In macrophages, ROS enhance the production of proinflammatory lymphokines and chemokines (Chaudri and Clark, 1989). In turn, these chemokines stimulate respiratory burst by neutrophils, which are the major source of oxidant generation, and depletion of plasma antioxidants at all stages of the disease (Babior, 1984; Ryder *et al.*, 1988).

OXIDATIVE STRESS AND HIV GENE TRANSCRIPTION

The promoter of HIV is located in the 5′ long terminal repeat (LTR) region of the viral genome (Fig. 3). It contains recognition sites for several transcription factors, including Sp1, TBP, NF-κB, p53, and AP-1 (Jones and Peterlin, 1994; Pereira *et al.*, 2000). These cellular factors control the rate of transcription from the integrated provirus. Despite the importance of these factors, elongation of transcripts initiated at the HIV-1 promoter are rather inefficient in the absence of the Tat protein. Function of Tat is dependent on a bulged RNA stem–loop structure, TAR (Tat activation region), present at the 5′ end of the viral RNA. Tat binds a Tat-associated kinase (TAK), which phosphorylates the carboxy-terminal domain of RNA polymerase II (Herrmann and Rice, 1995), thus increasing the generation of full-length transcripts up to 100-fold (Emerman and Malim, 1998). Indirectly, Tat may also enhance the rate of transcription initiation through stimulation of activities of redox-sensitive transcription factors, NF-κB, p53, and AP-1 (Sen and Packer, 1996; Li and Karin, 1999). On one hand, HIV-1 Tat increases ROS levels by inhibiting expression of manganese superoxide dismutase (Flores *et al.*, 1993). On the other hand, via a TAR-like structure in the tumor necrosis factor (TNF) promoter, Tat increases expression of TNF-α and -β (Buonaguro *et al.*, 1994) which, in turn, further enhances oxidative stress. TNF-α increases transcription from the HIV-1 LTR *in trans* by stimulation of p53 binding to its recognition motif adjacent to the Sp1 sites (Gualberto and Baldwin, Jr., 1995). Thus, Tat enhances HIV-1 gene expression through both transcription initiation and elongation. Shifting of the redox equilibrium toward oxidation stimulates translocation of NF-κB into the nucleus and en-

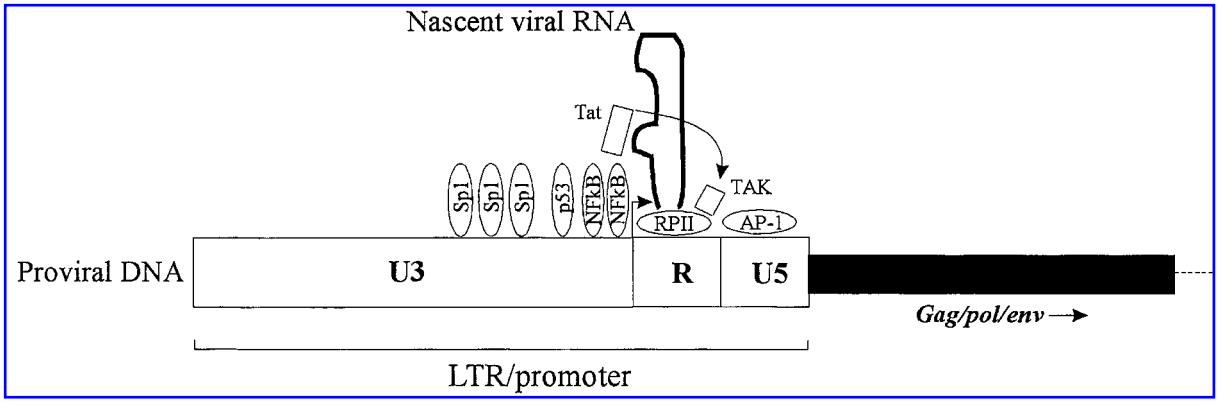

FIG. 3. Schematic organization of the HIV-1 promoter. U3, R, and U5 regions of the long terminal repeat (LTR) promoter and the 5′ coding region are shown. The rectangular arrow indicates transcription start site of viral RNA. Locations of three Sp1 core promoter binding sites and two NF-κB, p53, and Ap-1 motifs are shown. Sp1 recruits RNA polymerase II (RPII) to the TATA box located 23 nucleotides 5′ from the transcription start site. The nascent viral RNA loop is recognized by Tat. Tat recruits Tat-associated kinase (TAK), which phosphorylates the carboxyl-terminal domain of RPII, leading to stable elongation of viral RNA. Transcription of viral RNA is enhanced by redox-sensitive transcription factors NF-κB, p53, and AP-1.

hances DNA-binding by Sp1, allowing transcription from the HIV-1 LTR to occur (Staal *et al.*, 1990; Israel *et al.*, 1992). Activation of NF-κB is controlled by the IκB family of inhibitor proteins (Piette *et al.*, 1997). In the cytosol, IκB is bound to NF-κB, preventing its translocation to the nucleus. Redox-mediated phosphorylation and subsequent degradation of IκB releases NF-κB and allows its nuclear translocation (Schmidt *et al.*, 1995; Traenckner and Baeuerle, 1995; Imbert *et al.*, 1996; Li and Karin, 1999). Activation of NF-κB may also occur by a redox-dependent degradation of IκB without prior phosphorylation (Kretz-Remy *et al.*, 1998). Once NF-κB is translocated in the nucleus, its binding to the NF-κB motif may also be redox-controlled (Okamoto *et al.*, 1992; Hayashi *et al.*, 1993; Droge *et al.*, 1994; Okamoto *et al.*, 1997). The NF-κB/Rel protein family of transcription factors contains a cysteine residue within their arginine-rich DNA-binding domain (Droge *et al.*, 1994). TRX stimulates HIV LTR promoter activity and DNA-binding activity of NF-κB through reduction of a disulfide bond involving cysteine 62 of p50 subunit of NF-κB (Matthews *et al.*, 1992). Along the same line, elevated levels of GSSG inhibit DNA-binding activity and transcriptional activation by NF-κB (Galter *et al.*, 1994). Thus, TRX and GSSG have important regulatory roles in NF-κB-mediated HIV gene transcription (Fig. 4).

RELATIONSHIP OF OXIDATIVE STRESS, APOPTOSIS, AND VIRAL REPLICATION

In the early stages of infection, viral inhibitors of apoptosis allow for more extensive production of progeny (Table 4). At later stages, viral inducers of apoptosis facilitate spread of progeny to uninfected cells (Table 5). This general rule may also apply to HIV. Overexpression of the adenovirus E1B 19K, a protein functionally homologous to Bcl-2, inhibited HIV-induced apoptosis and enhanced viral replication and persistence in Jurkat cells (Antoni *et al.*, 1995). Along the same line, overexpression of Bcl-X_L inhibited apoptosis and viral replication in U937 monocytic cells (Marshall *et al.*, 1999). Activity of Bcl-2 may be regulated in a biphasic manner in HIV-infected cells. In the early stages of infection, Bcl-2 protein level is down-regulated by viral protease-mediated cleavage (Strack *et al.*, 1996; Aillet *et al.*, 1998). Initial down-regulation of Bcl-2 may facilitate opening of mitochondrial permeability transition pores (Gross *et al.*, 1999), increased oxidative stress (Hockenberry *et al.*, 1993; Kane *et al.*, 1993; Shimizu *et al.*, 1998), and higher rate of viral gene expression and replication. From day 5 post infection, viral replication is accompanied by increasing Bcl-2 transcription and protein levels which inhibit apoptosis and may contribute to viral persistence and $CD4^+$ T and monocytic cell lines (Aillet *et al.*, 1998).

Therefore, it follows that regulating the levels of oxidative stress is a key determinant of viral replication and latency as well as survival of HIV-infected cells. Balance of ROS production and antioxidant defenses is clearly critical in triggering caspase activation and apoptosis of HIV-infected cells (Banki *et al.*, 1998). The caspases themselves are cysteine-dependent enzymes, and as such appear to be redox sensitive (Hampton and Orrenius, 1998; Sen *et al.*, 1999). Prolonged and excessive oxidative stress may actually limit caspase activity in HIV-infected cells (Hampton and Orrenius, 1998). Death of virus-infected cells is known to occur independently from activation of the Fas pathway (Glynn *et al.*, 1996; Banki *et al.*, 1998; Gandhi *et al.*, 1998). HIV clearly has the ability to induce oxidative stress and kill virus-infected cells through apoptosis (Table 1). The loss of $CD4^+$ T cells is proportionate to the viral load in the plasma of HIV-infected individuals (Mellors *et al.*, 1996). However, there is compelling evidence that uninfected $CD4^+$ T cells also undergo apoptosis (Finkel *et al.*, 1995; Su *et al.*, 1995). Bystander apoptosis of uninfected T cells may be mediated through the Fas pathway. Expression of the Fas ligand is up-regulated on HIV-infected macrophages (Bradley *et al.*, 1996) and T cells (Xu *et al.*, 1997; Zauli *et al.*, 1999), which transduces death signal through the Fas receptor expressed on activated T cells (Alderson *et al.*, 1993). Up-regulation of the Fas ligand is mediated via the redox-sensitive transcription factor NF-κB (Kasibhatla *et al.*, 1999). Cell-surface expression of the Fas receptor is also redox-sensitive (Orlin-

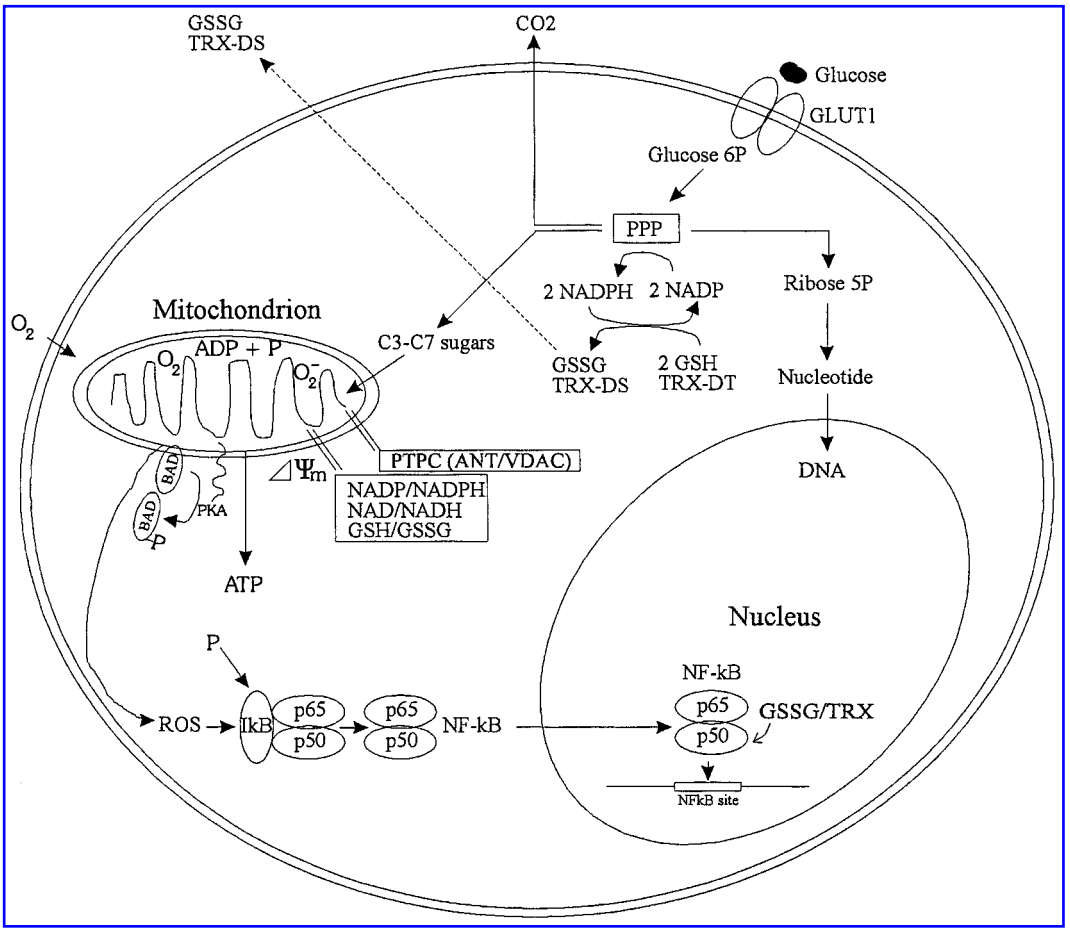

FIG. 4. Metabolic control of $\Delta\Psi_m$ and ROS levels. Intracellular antioxidants GSH and TRX-DT are regenerated at the expense of NADPH supplied primarily through metabolism of glucose via the PPP. Glutathione reductase and TRX reductase synthesizes GSH and TRX-DT at the expense of NADPH. Formulation of the PPP and its efficiency to provide NADPH is dependent on the expression of G6PD and TAL. $\Delta\Psi_m$ is controlled by intracellular GSH/NADH/NADPH levels, integrity of the permeability transition pore complex largely comprised of adenine nucleotide translocator (ANT, inner membrane), voltage-dependent anion channel (VDAC, outer membrane), and translocation and dimerization of pro- and anti-apoptotic Bcl-2 family members in the intermembrane space (Gross *et al.*, 1999). Phosphorylation of BAD by mitochondria-anchored PKA results in antiapoptotic sequestration of BAD into the cytosol. Secreted TRX functions as a chemoattractant for proinflammatory neutrophils and macrophages. Among PPP products, ribose 5-phosphate is required for nucleotide and DNA synthesis and support cell growth (Mayes, 1993), whereas C3–C7 influence mitochondrial function and ROS production (Barbieri *et al.*, 1994; Benov and Fridovich, 1998).

ick *et al.*, 1997; Li *et al.*, 1998). Export of the Fas receptor from the Golgi complex to the cell surface appears to be enhanced by p53 (Bennett *et al.*, 1998). Killing of uninfected $CD4^+$ T cells can be induced by HIV glycoprotein 120-expressing cells (Banda *et al.*, 1992), independently from Fas or TNF signaling (Ohnimus *et al.*, 1997). ROS also regulate T-cell receptor-mediated activation-induced apoptosis (Hildeman *et al.*, 1999). Therefore, a generalized oxidative stress is likely to promote cell death upon cognate interaction between the antigen and the corresponding T-cell receptor (Zauli *et al.*, 1996).

Disease progression and functional abnormalities of T and B cells have been correlated with an altered cytokine production profile, shift from T helper type 1 (Th1) to Th2-type cytokines (Clerici and Shearer, 1994). Secretion of Th1 cytokines, interleukin-2 (IL-2), interferon-γ (IFN-γ), and IL-12, necessary for maintenance of a classical T-cell-mediated immunity, is di-

Table 3. Metabolic Changes Associated with HIV Infection

Marker	*Body site/cell type*	*Reference*
Cachexia	Systemic	(Coodley *et al.*, 1994; Sharpstone *et al.*, 1996; Mulligan and Bloch, 1998)
Altered glucose metabolism	Brain, gut	(Rottenberg *et al.*, 1996; Lutz *et al.*, 1997)
Enhanced expression of glucose transporter	HIV-infected H9 cells, brain	(Sorbara *et al.*, 1996; Kovitz and Morgello, 1997)
Increased uptake of vitamin C	Monocytic and T cell lines	(Rivas *et al.*, 1997)
Decreased phosphatidylinositol metabolism	HIV-infected peripheral blood T cells	(Hofmann *et al.*, 1990)
Decreased *de novo* ribonucleotide synthesis	HIV-infected peripheral blood T cells	(Bofill *et al.*, 1995)
Decreased adenylate/guanylate ratio	HIV-infected peripheral blood T cells	(Tabucchi *et al.*, 1994)

minished while production of Th2 cytokines, in particular, IL-4, IL-5, IL-6, and IL-10, promoting B-cell function, is increased in HIV-infected patients (Clerici and Shearer, 1994). Interestingly, Th1-type cytokines protect against apoptosis, whereas Th2-type cytokines increase cell death (Clerici and Shearer, 1994). The *nef* (Collette *et al.*, 1996) and *tat* genes of HIV-1 are thought to mediate a Th1 to Th2 shift in cytokine production (Rubartelli *et al.*, 1998). Diminished IL-2 production may be responsible for descreased Bcl-2 and increased Fas expression and a generally augmented apoptosis sensitivity of T cells in HIV-infected patients (Lenardo *et al.*, 1999). Interestingly, expression of the receptor for IL-2 is dependent on activation of NF-κB (Ginn-Pease and Whisler, 1998). Balance of ROS production and endogenous synthesis of antioxidants, such as thioredoxin (Nakamura *et al.*, 1997), GSH, and NADPH are critical for controlling susceptibility to IL-2-dependent proliferative (Tian *et al.*, 1998) and cell death signals (Ursini *et al.*, 1997; Banki *et al.*, 1998).

OXIDATIVE STRESS AND CHANGES IN METABOLISM

Resting energy expenditure (REE) of HIV-infected subjects is elevated with disease pro-

Table 4. Viral Proteins Inhibing Apoptosis

Protein	*Virus*	*Pathway*	*Reference*
Large T	SV40	Inactivates p53	(Bargonetti *et al.*, 1992)
E1B 19K	Adenovirus	Bcl-2 homologue	(Rao *et al.*, 1992)
$\gamma_1$34.5	HSV	ND	(Chou and Roizman, 1992)
BHRF1	EBV	Bcl-2 homologue	(Henderson *et al.*, 1993)
HMW5-HL	ASFV	Bcl-2 homologue	(Neilan *et al.*, 1993)
pX	HBV	p53 antagonist	(Wang *et al.*, 1995)
E6	HPV	p53 antagonist	(Scheffner *et al.*, 1990)
p35, Iap	Baculovirus	Protease inhibitor	(Clem and Miller, 1994)
CrmaA	Cowpox	Protease inhibitor	(Ray *et al.*, 1992)
Vpr	HIV-1	Interference with TCR signaling	(Ayyavoo *et al.*, 1997)
Vpr	HIV-1	Inhibits bax/enhances bcl-2 expression	(Conti *et al.*, 1998)
Tax	HTLV-I	Fas, oxidative stress	(Masutani *et al.*, 1996; Kishi *et al.*, 1997)
Bcl-2	HIV-1	Oxidative stress	(Aillet *et al.*, 1998)
23K E8-FLIP	EHV-2	Fas, vFLIP	(Bertin, 1997; Thome *et al.*, 1997)
ORF159L-FLIP	MCV	Fas, vFLIP	(Bertin, 1997; Thome *et al.*, 1997)
ORF71-FLIP	HVS	Fas, vFLIP	(Bertin, 1997; Thome *et al.*, 1997)
ORF189-FLIP	HHV-8	Fas, vFLIP	(Bertin, 1997; Thome *et al.*, 1997)

ND, Not determined.

TABLE 5. VIRAL PROTEINS STIMULATING APOPTOSIS

Protein	*Virus*	*Pathway*	*Reference*
E1A	Adenovirus	Activates p53	(Whyte *et al.*, 1988; White *et al.*, 1991)
E7	HPV	Activates p53	(Pan and Gripe, 1995)
NS-1	Influenza	Fas, bcl-2 antagonism	(Hinshaw *et al.*, 1994)
ND	parvovirus B19	ND	(Morey *et al.*, 1993)
Tax	HTLV-I	Bcl-2	(Yamada *et al.*, 1994)
Nef	SIV-1	Increased Fas ligand expression	(Xu *et al.*, 1997)
gp120	HIV-1	CD4-crosslinking	(Banda *et al.*, 1992; Ohnimus *et al.*, 1997)
Tat	HIV-1	Oxidative stress	(Westendorp *et al.*, 1995a; Ehret *et al.*, 1996)
Tat	HIV-1	Increases bax/inhibits bcl-2 expression	(Sastry *et al.*, 1996)
Protease	HIV-1	Bcl-2 cleavage	(Strack *et al.*, 1996)
Nef	HIV-1	Increased Fas ligand expression	(Zauli *et al.*, 1999)
Nef	HIV-1	Interaction with TCRζ chain	(Xu *et al.*, 1999)
gp120	HIV-1	CXCR4-crosslinking	(Herbein *et al.*, 1998; Blanco *et al.*, 1999)
Vpr	HIV-1	G_2 arrest in dividing cells	(Bartz *et al.*, 1996; Planelles *et al.*, 1996; Yao *et al.*, 1998; Shostak *et al.*, 1999)

ND, Not determined.

gression (Grunfeld *et al.*, 1992). Multiple factors, including opportunistic infections, malabsorption, diarrhea, medications, and altered metabolism, contribute to a generalized wasting and loss of lean body and muscle mass (Kaminski, Jr. *et al.*, 1998). Elevated levels of TNFα, a "cachectic" lymphokine, oxidative stress, and reduced plasma levels of antioxidants correlate with the wasting syndrome (Walmsley *et al.*, 1997; Allard *et al.*, 1998a).

GSH is the most abundant intracellular reducing equivalent that protects the cell from damage by excess ROS (Mayes, 1993). *De novo* synthesis of GSH appears to be limited by cysteine deficiency in HIV-infected patients (Eck *et al.*, 1989; Droge *et al.*, 1994). Regeneration of GSH from its oxidized form, GSSG, depends on NADPH produced by the pentose phosphate pathway (PPP) (Mayes, 1993). Accordingly, increased oxidative stress in HIV-infected donors is accompanied by increased glucose utilization both on the cellular (Sorbara *et al.*, 1996) and organismal levels (Rottenberg *et al.*, 1996; Kovitz and Morgello, 1997; Lutz *et al.*, 1997) (Table 3). Increased glucose utilization is supported by enhanced expression of glucose transporter GLUT1 in HIV-infected H9 cells (Sorbara *et al.*, 1996) and in the HIV-infected brain (Kovitz and Morgello, 1997). Of note, oxidative stress results in elevated GLUT1 expression (Kozlovsky *et al.*, 1997). Increased expression of GLUT1 may be responsible for accumulation of vitamin C in HIV-infected cells (Rivas *et al.*, 1997). The oxidized form of vitamin C, dehydroascorbate, is transported into the cell via GLUT1 (Vera *et al.*, 1993). Intracellularly, dehydroascorbate is reduced back to vitamin C at the expense of GSH (Meister, 1994). Under physiological conditions, vitamin C has a predominantly antioxidant role (Carr and Frei, 1999). Pro-oxidant and pro-apoptotic effects of vitamin C may be related to hydroxylation (Udenfriend *et al.*, 1954) and/or formation of ascorbyl radicals (Sakagami and Satoh, 1997). In the event of oxidative stress, vitamin C can be further oxidized to ascorbate free radical (Munoz *et al.*, 1997), which may explain its toxicity for HIV-infected cells (Rivas *et al.*, 1997). Vitamin C, by itself, can enhance apoptosis of human lymphocytes and myelogenous leukemia cell lines (Amano *et al.*, 1998; Podmore *et al.*, 1998). These findings can explain the controversial impact of vitamin C treatment on survival of HIV-infected cells (Harakeh *et al.*, 1990; Aoki *et al.*, 1994; Rivas *et al.*, 1997). Additionally, metabolites of vitamin C, a 6-carbon sugar, may also contribute to cell death. Ribose 5-phosphate and other short-chain sugars are directly capable of inducing apoptosis (Barbieri *et al.*, 1994; Benov and Fridovich, 1998). High

glucose concentration can lead to oxidative stress (Efanova *et al.*, 1998; Fine *et al.*, 1999; Nomura *et al.*, 1999; Greene *et al.*, 1999), NF-κB activation, and cell death (Du *et al.*, 1999). Asymptomatic HIV-infected patients show diminished ribonucleotide synthesis (Bofill *et al.*, 1995), whereas symptomatic patients also exhibit diminished production of adenylate nucleotides (Tabucchi *et al.*, 1994; Bofill *et al.*, 1995). These observations may be related to depletion of NAD, an essential substrate of poly(ADP-ribose) polymerase activated by caspases during apoptotic signaling (Banki *et al.*, 1998). Phosphatidylinositol synthesis is also diminished in HIV-infected cells (Allard *et al.*, 1998a). CD3-mediated up-regulation of phosphatidylinositol 3′-kinase inhibits cleavage of caspase-8 and may be responsible for selective resistance of Th2 cells to Fas-induced apoptosis (Varadhachary *et al.*, 1999). Oxidative stress can trigger phosphatidylinositol 3′-kinase, which may trigger selective Fas-mediated killing of Th1 cells (Deora *et al.*, 1998).

REDOX SIGNALING AND THE MITOCHONDRIAL TRANSMEMBRANE POTENTIAL

Changes in mitochondrial membrane integrity leading to the release of cytochrome *c* and other caspase-activating factors (Salvesen and Dixit, 1997) appear to be the point of no return in the effector phase of apoptosis (Li *et al.*, 1997). Mitochondrial membrane permeability is subject to regulation by an oxidation-reduction equilibrium of ROS, pyridine nucleotides (NADH/NAD + NADPH/NADP), and GSH levels (Constantini *et al.*, 1996) (Fig. 4). Regeneration of GSH from its oxidized form, GSSG, depends on NADPH produced by the PPP (Mayes, 1993). In fact, a fundamental function of PPP is to maintain GSH in a reduced state and thereby protecting sulfhydryl groups and cellular integrity from emerging oxygen radicals. Regeneration of the dithiol form of another antioxidant, TRX, is mediated by TRX reductase at the expense of NADPH (Holmgren and Bjornstedt, 1995).

The PPP comprises two separate, oxidative and nonoxidative, phases. Reactions in the oxidative phase are irreversible, whereas all reactions of the nonoxidative phase are fully reversible. The two phases are functionally connected. The nonoxidative phase converts ribose 5-phosphate to glucose 6-phosphate for use by the oxidative phase and thus, indirectly, contributes to generation of NADPH. Different enzymes are rate limiting in the two phases. The oxidative phase primarily depends on glucose 6-phosphate dehydrogenase (G6PD) (Wood, 1985), whereas transaldolase (TAL) is the rate-limiting enzyme for the nonoxidative phase (Heinrich *et al.*, 1976). Formulation of the PPP and its efficiency to provide NADPH is dependent on the expression of G6PD (Salvemini *et al.*, 1999; Tian *et al.*, 1999) and TAL (Wood, 1974; McIntyre *et al.*, 1989; Banki *et al.*, 1996, 1998, 1999; Ni and Savageau, 1996). TAL overexpression lowers G6PD and 6-phosphogluconate dehydrogenase (6PGD) activities and NADPH and GSH levels and renders the cell highly susceptible to apoptosis induced by serum deprivation, hydrogen peroxide (H_2O_2), nitric oxide (NO), TNF-α, and Fas signaling. When TAL levels are reduced, G6PD and 6PGD activities and GSH levels are increased and apoptosis is inhibited. TAL activity profoundly impacts the balance between the two branches of PPP and the ultimate output of NADPH and GSH (Banki *et al.*, 1996). These findings are consistent with a dominant role for TAL within the metabolic network that controls the propagation of biochemical signals (Ni and Savageau, 1996).

Levels of TAL expression can determine the extent of mitochondrial ROS production, changes in the mitochondrial transmembrane potential ($\Delta\Psi_m$) and subsequent caspase activation, PS externalization, and cell death during HIV infection (Banki *et al.*, 1998). Overexpression of TAL accelerated HIV-induced oxidative stress, protease activation, PS externalization, and cell death in two human $CD4^+$ T cell lines. In contrast, suppression of TAL activity abrogated these effects and blocked HIV-induced cell death. Thus, increased mitochondrial ROS production is a defining step in HIV-induced apoptosis and may serve as a possible target in the development of new therapeutics against HIV disease.

Signaling through the APO-1/Fas/CD95 antigen (Westendorp *et al.*, 1995a) and the structurally related cell surface receptor for

TNF (Lahdevirta *et al.*, 1988; Malorni *et al.*, 1993) is accelerated in activated T cells. Elevation of $\Delta\Psi_m$ and ROS levels precede externalization of phosphatidylserine (PS), disruption of $\Delta\Psi_m$, and cell death, both in Jurkat human T cells and peripheral blood lymphocytes. Changes in $\Delta\Psi_m$ and ROS levels can be controlled by TAL through the supply of reducing equivalents from the PPP. Overexpression of TAL accelerated Fas-induced ROS production, $\Delta\Psi_m$ elevation, activation of caspase-8 and caspase-3, proteolysis of poly(ADP-ribose) polymerase, and PS externalization, while suppression of TAL diminished these activities. A series of caspase inhibitor peptides, DEVD-CHO, Z-VAD.fmk, and Boc-Asp.fmk, block Fas-induced PS externalization, disruption of $\Delta\Psi_m$, and cell death, showing that these changes are sequelae of caspase activation. By contrast, caspase inhibitors do not affect Fas-induced elevation of ROS levels and $\Delta\Psi_m$. Early increases in ROS levels and $\Delta\Psi_m$ as well as the dominant effect of TAL expression on activation of caspase-8/FLICE, the most upstream member of the caspase cascade, suggest a pivotal role for redox signaling at the initiation of Fas-mediated apoptosis.

Stimulation of the Fas or the RNF1 receptor can activate a second pathway, mediated by DAXX which can activate the Jun NH_2-terminal kinase (JNK) cascade through the redox-dependent apoptosis signal-regulating kinase 1, ASK1 (Chang *et al.*, 1998; Nishitoh *et al.*, 1998; Hoeflich *et al.*, 1999). ASK1 can undergo ROS-mediated multimerization in response to Fas or TNF stimulation (Gotoh and Cooper, 1998). In nonstressed cells, ASK1 is associated with TRX. The TRX-bound form of ASK1 cannot multimerize and remains inactive as a kinase (Saitoh *et al.*, 1998). Stimulation of the Fas or TNF receptor results in a ROS-dependent dissociation from TRX and multimerization of ASK1. Such activation of ASK1 can be prevented by antioxidants (Saitoh *et al.*, 1998). The precise mechanism by which Fas and TNF signaling leads to changes in $\Delta\Psi_m$ and ROS levels remains to be defined.

Alterations of $\Delta\Psi_m$ occur early in HIV-infected patients (Cossarizza *et al.*, 1997). Decrease of $\Delta\Psi_m$ was associated with elevated TNF-α or HIV Gag 24 levels and compensated by *in vitro* incubation with antioxidants (Cossarizza *et al.*, 1997). Mitochondrial damage was also shown in uninfected cells, possible explaining their increased susceptibility to cell death (Carbonari *et al.*, 1997). Indeed, activation of normal peripheral blood T cells with mitogenic stimulation alone elicited changes in $\Delta\Psi_m$ (Banki *et al.*, 1999) and increased susceptibility to Fas-induced apoptosis (Miyawaki *et al.*, 1992). Proapoptotic effects of the HIV-1 Vpr protein may also be mediated through disturbing $\Delta\Psi_m$, thus, causing dysfunction of the mitochondrial respiratory chain (Macreadie *et al.*, 1997). Vpr can form cation-selective ion channels across lipid bilayers and consequently perturb transmembrane potentials (Piller *et al.*, 1998). The carboxyl terminus of Vpr binds to the adenine nucleotide translocator in the permeability transition pore complex of the mitochondrial membrane (Figs. 1 and 4), thus causing rapid dissipation of $\Delta\Psi_m$ (Jacotot *et al.*, 2000). The HIV-1 Nef protein inhibits activity of Ca^{2+}-dependent K^+ channels in human glial (Kort and Jalonen, 1998) and T lymphoid cells (Zegarra-Moran *et al.*, 1999) and interferes with signaling through the TCR ζ chain (Xu *et al.*, 1999) and the Fas receptor (Xu *et al.*, 1997). It is presently unknown whether Nef can influence $\Delta\Psi_m$.

Proteins of the Bcl-2 family are localized to membranes of distinct organelles, including mitochondria (Korsmeyer, 1995; Green and Reed, 1998). They play key roles in maintenance of $\Delta\Psi_m$. Both the proapoptotic (Bax, Bad) and antiapoptotic members (Bcl-2, Bcl-X_L) of the family can form ion-conducting channels in lipid membranes (Gross *et al.*, 1999). Bax can create a channel in the outer mitochondrial membrane thus releasing cytochrome *c* and other caspase-activating moieties into the cytosol. Bcl-2 and Bcl-X_L inhibit this process through dimerization with Bad or Bax. Activity of Bad is controlled by a mitochondria-anchored cAMP-dependent protein kinase (PKA, Fig. 4) (Harada *et al.*, 1999). Cleavage of Bcl-2 by HIV-1 protease may contribute to increased ROS levels and apoptosis (Strack *et al.*, 1996) via disruption of $\Delta\Psi_m$.

HIV INFECTION AND ANTIOXIDANT SUPPLEMENTS

The ultimate goal of HIV research is development of an effective vaccine. While this goal

is being pursued, significant progress has been made in recent years by using highly active antiretroviral therapy (HAART) (Finzi and Siliciano, 1998). HAART can reduce the number of HIV-infected cells to $\sim 10^7$ $CD45RO^+$ resting/memory T cells in the entire body. Latent infection of these long-lived memory T cells may represent a major barrier of complete eradication of the virus. Survival of HIV-infected patients on HAART has dramatically improved. However, only about 10% of HIV-infected individuals can afford or tolerate combination therapy with reverse transcriptase and protease inhibitors (Kotler, 1998). Antioxidants are usually affordable and well tolerated. Nevertheless, the efficacy of current modalities remains questionable (Kotler, 1998). Decline of GSH levels in HIV-infected cells was associated with cysteine deficiency and served as a rationale for treatment with NAC (Droge *et al.*, 1992). In double-blind placebo-controlled studies, NAC administration resulted in: (i) a relative increase of the CD4/CD8 ratio and elevated serum selenium levels without effecting the viral load (Look *et al.*, 1998) and (ii) diminished serum TNF-α levels and a slower decline of the $CD4^+$ T cell count (Akerlund *et al.*, 1996). L-2-Oxothiazolidine 4-carboxylate (OTC), which is converted into cysteine by 5-oxoprolinase, significantly increased serum GSH levels without affecting GSH content of peripheral blood lymphocytes in a placebo-controlled study of asymptomatic HIV-infected patients (Barditch-Crovo *et al.*, 1998). Selenium and β-carotene supplementation were also reported to increase serum GSH levels in HIV-infected individuals (Delmas-Beauvieux *et al.*, 1996). Combined supplementation of vitamin E and C was found to diminish oxidative stress, serum lipid peroxides, and malondialdehyde in HIV-infected patients (Allard *et al.*, 1998b). Because vitamin C can increase death of HIV-infected cells (Aoki *et al.*, 1994; Rivas *et al.*, 1997), vitamin E alone may have had a more beneficial effect.

CONTROVERSIES

Both the significance and the source of HIV-induced oxidative stress have become controversial. Oxidative stress was originally implied from finding diminished GSH levels in the plasma, PBMC (Eck *et al.*, 1989; de Quay *et al.*, 1992), and lung epithelial lining fluid of HIV-infected persons (Buhl *et al.*, 1989), and cysteine deficiency of HIV-infected individuals served as a rationale for treatment with NAC (Droge *et al.*, 1992). Altered signaling through the redox-sensitive transcription factor NF-κB in AIDS was attributed to GSSG deficiency (Droge *et al.*, 1994). Another study failed to show GSH deficiency in PBMC of HIV-infected patients (Pirmohamed *et al.*, 1996). As raised by the respective authors themselves, differences in methodology may explain some of the discrepancies. Oral administration of NAC raised intracellular GSH levels, diminished the loss of $CD4^+$ T cells, and increased survival in a cohort of HIV-infected patients (Herzenberg *et al.*, 1997). Administration of GSH itself was found to inhibit development of a murine model of AIDS (Palamara *et al.*, 1996). In another report, NAC failed to increase GSH in lymphocytes and plasma of AIDS patients (Witschi *et al.*, 1995). Clearly, further double-blind placebo-controlled studies are needed to define the role of NAC in treatment of AIDS. More recently, decreased GSH was correlated with increased levels of GSSG in HIV-infected $CD4^+$ T cells, suggesting that a lack of reducing equivalents rather than decreased GSSG synthesis was responsible for GSH deficiency (Aukrust *et al.*, 1995; Lopez Galera *et al.*, 1996). Increased mitochondrial ROS production appears to precede decline of GSH levels (Banki *et al.*, 1998). Disruption of mitochondrial membrane integrity, a source of rapid ROS release, was recently associated with a direct pore-forming effect of Vpr (Jacotot *et al.*, 2000). Inversely, $\Delta\Psi_m$ is also subject to regulation by reducing equivalents.

Therefore, a dominant role for Vpr in ROS production requires confirmation using infectious HIV virions or DNA with targeted *vpr* mutations rather than *vpr* expression vectors and peptides alone (Jacotot *et al.*, 2000). The fundamental issue of whether HIV-1 predominantly kills infected cells or causes death of uninfected cells remains controversial. Evidence has been provided that HIV-1 directly kills $CD4^+$ T cells via a Fas-independent mechanism (Gandhi *et al.*, 1998). However, Fas signaling may play a role in apoptosis of uninfected cells

(Finkel *et al.,* 1995; Su *et al.,* 1995). These latter observations point out the significance of soluble mediators of oxidative stress, such as increased TRX and pro-apoptotic cytokines, TNF-α, and IL-1. Alternatively, deficiencies in cysteine/GSSG (Droge *et al.,* 1994), purine (Tabucchi *et al.,* 1994; Bofill *et al.,* 1995), and glucose metabolism (Rottenberg *et al.,* 1996; Sorbara *et al.,* 1996; Lutz *et al.,* 1997; Kovitz and Morgello, 1997) may also contribute to generalized oxidative stress and bystander cell death. Resolution of these controversial issues will help understand the pathogenesis of HIV disease.

CONCLUSIONS AND FUTURE DIRECTIONS

Oxidative stress and apoptosis commonly occur in virally infected cells (Schwarz, 1996) resulting in spread of virions, while ongoing virus replication within the host cell is supported by viral inhibitors of apoptosis (Table 5). Perhaps, it is a unique feature of HIV that oxidative stress plays an important role in viral replication and apoptosis of productively infected as well as noninfected but activated T cells. Several HIV-encoded proteins, Tat, gp120, Vpr, Nef, and protease, have already been identified as triggers of oxidative stress, disruption of $\Delta\Psi_m$, and apoptosis (Table 4). The precise sequence of activation and intricate networking of these pro-oxidant viral proteins and their cellular targets are largely unknown. While increased ROS levels are readily detectable by oxidation-sensitive fluorescent probes, the precise chemical composition of the ROS produced, their specific relationship to viral gene transcription, $\Delta\Psi_m$, and cell death also remain to be determined. Infection by HIV leads to diminished cysteine and GSH and increased TRX plasma levels. Increased glucose transporter expression and augmented glucose utilization may reflect increased metabolic demand needed to execute or combat oxidative stress in the infected cells. Therefore, short-chain sugars capable of altering susceptibility to apoptosis may become attractive targets for development of novel pharmacological agents aimed at controlling cell survival through metabolism. Additionally, patients harboring HIV are commonly infected by other lymphotropic viruses, such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus-8, or less commonly, by human T-cell lymphotropic virus type I (HTLV-1) or HTLV-II. Therefore, delineation of the interplay of pro- and antioxidant proteins from several viruses may be required for understanding the pathogenetic importance and therapeutic relevance of redox signaling in HIV disease.

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ABBREVIATIONS

Apaf-1, Apoptosis-activating factor 1; ASFV, African swine fever virus; CAD, caspase-activated DNase; CMV, cytomegalovirus; EBV, Epstein-Barr virus; EHV-2, equine herpesvirus-2; FLICE, Fas-associated death domain-like ICE; GLUT1, glucose transporter 1; GSH, reduced glutathione; GSSG, oxidized glutathione; G6PD, glucose 6-phosphate dehydrogenase; HAART, highly active antiretroviral therapy; HBV, hepatitis B virus; HHV-8, human herpesvirus-8; HIV-1, human immunodeficiency virus type 1; H_2O_2, hydrogen peroxide; HPV, human papilloma virus; HSV, herpes simplex virus; HTLV-I/II, human T-cell lymphotropic virus type I/II; ICE, IL-1β-converting enzyme; IFN-γ, interferon-r; IL-2, interleukin-2; JNK, Jun NH_2-terminal kinase; LTR, long terminal repeat; MCV, human molluscipoxvirus; NAC, *N*-acetylcysteine; NO, nitric oxide; OTC, L-2-oxothiazolidine 4-carboxylate; PBMC, peripheral blood mononuclear cells; 6PGD, 6-phosphogluconate dehydrogenase; PS, phosphatidylserine; PPP, pentose phosphate pathway; REE, resting energy expenditure; ROS, reactive oxygen species; SIV-1, simian immunodeficiency virus

type 1; SV40, simian virus 40; TAK, Tat-associated kinase; TAL, transaldolase; TAR, tat activation region; Tat, trans-activator of transcription; Th1, T helper type 1; TNF, tumor necrosis factor; TRX, thioredoxin; vFLIP, viral FLICE-inhibitory protein; $\Delta\Psi_m$, mitochondrial transmembrane potential.

REFERENCES

ADLER, V., YIN, Z., TEW, K.D., and RONAI, Z. (1999). Role of redox potential and reactive oxygen species in stress signaling. Oncogene **18,** 6104–6111.

AILLET, F., MASUTANI, H., ELBIM, C., RAOUL, H., CHENE, L., NUGEYRE, M.T., PAYA, C., GOUGEROT-POCIDALO, M.A., and ISRAEL, N. (1998). Human immunodeficiency virus induces a dual regulation of Bcl-2, resulting in persistent infection of CD4(+)T- or monocytic cel lines. J. Virol. **72,** 9698–9705.

AKERLUND, B., JARSTRAND, C., LINDEKE, B., SONNERBORG, A., AKERBLAD, A.C., and RASOOL, O. (1996). Effect of N-acetylcysteine (NAC) treatment on HIV-1 infection: a double-blind placebo-controlled trial. Eur. J. Clin. Pharmacol. **50,** 457–461.

ALERSON, M.K., ARMITAGE, R.J., MARASKOVSKY, E., TOUGH, T.W., ROUX, E., SCHOOLEY, K., RAMSDELL, F., and LYNCH, D.H. (1993). Fas transduces activation signals in normal human T lymphocytes. J. Exp. Med. **178,** 2231–2235.

ALLARD, J.P., AGHDASSI, E., CHAU, J., SALIT, L., and WALMSLEY, S. (1998a). Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection. Am. J. Clin. Nutr. **67,** 143–147.

ALLARD, J.P., AGHDASSI, E., CHAU, J., TAM, C., KOVACS, C.M., SALIT, I.E., and WALMSLEY, S.L. (1998b). Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. AIDS **12,** 1653–1659.

AMANO, Y., SAKAGAMI, H., TANAKA, T., YAMANAKA, Y., NISHIMOTO, Y., and YAMAGUCHI, M. (1998). Uncoupling of incorporation of ascorbic acid and apoptosis induction. Anticancer Res. **18,** 2503–2506.

ANTONI, B.A., SABBATINI, P., RABSON, A.B., and WHITE, E. (1995). Inhibition of apoptosis in human immunodeficiency virus-infected cells enhances virus production and facilitates persistent infection. J. Virol. **69,** 2384–2392.

AOKI, K., NAKASHIMA, H., HATTORI, T., SHIOKAWA, D., NI-IMI, E., TANIMOTO, Y., UCHIUMI, F., KOCHI, M., YAMAMOTO, N., *ET AL.* (1994). Sodium benzylideneascorbate induces apoptosis in HIV-replicating U1 cells. FEBS Lett. **351,** 105–108.

AUKRUST, P., SVARDAL, A.M., MULLER, F., LUNDEN, B., BERGE, R.K., UELAND, P.M., and FROLAND, S.S. (1995). Increased levels of oxidized glutathione in $CD4^+$ lymphocytes associated with disturbed intracellular redox balance in human immunodeficiency virus type 1 infection. Blood **86,** 258–267.

AYYAVOO, V., MAHROUBI, A., MAHALINGAM, S., RAMALINGAM, R., KUDCHODKAR, S., WILLIAMS, W.V., GREEN, D.R., and WEINER, D.B. (1997). HIV-1 vpr suppresses immune activation and apoptosis through regulation of nuclear factor κB. Nature Med. **3,** 1117–1123.

BABIOR, B.M. (1984). Oxidants from phagocytes: agents of defense and destruction. Blood **64,** 959–966.

BANDA, N.K., BERNIER, J., KURAHARA, D.K., KURRLE, R., HAIGWOOD, N., SEKALY, R.P., and FINKEL, T.H. (1992). Cross-linking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. J. Exp. Med. **176,** 1099–1106.

BANKI, K., HUTTER, E., COLOMBO, E., GONCHOROOFF, N.J., and PERL, A. (1996). Glutathione levels and sensitivity to apoptosis are regulated by changes in transaldolase expression. J. Biol. Chem. **271,** 32994–33001.

BANKI, K., HUTTER, E., GONCHOROFF, N.J., and PERL, A. (1998). Molecular ordering in HIV-induced apoptosis: oxidative stress, activation of caspases, and cell survival are regulated by transaldolase. J. Biol. Chem. **273,** 11944–11953.

BANKI, K., HUTTER, E., GONCHOROFF, N., and PERL, A. (1999). Elevation of mitochondrial transmembrane potential and reactive oxygen intermediate levels are early events and occur independently from activation of caspases in Fas signaling. J. Immunol. **162,** 1466–1479.

BARBIERI, D., GRASSILLI, E., MONTI, D., SALVIOLI, S., FRANCESCHINI, M.G., FRANCHINI, A., BELLESIA, E., SALOMONI, P., NEGRO, P., CAPRI, M., TROIANO, L., COSSARIZZA, A., and FRANCESCHI, C. (1994). D-ribose and deoxy D-ribose induce apoptosis in human quiescent peripheral blood mononuclear cells. Biochem. Biophys. Res. Commun. **201,** 1109–1116.

BARDITCH-CROVO, P., NOE, D., SKOWRON, G., LEDERMAN, M., KALAYJIAN, R.C., BORUM, P., ROWE, W.B., GOLDBERG, D., and LIETMAN, P. (1998). A phase I/II evaluation of oral L-2-oxothiazolidine-4-carboxylic acid in asymptomatic patients infected with human immunodeficiency virus. J. Clin. Pharmacol. **38,** 357–363.

BARGONETTI, J., REYNISDOTTIR, I., FRIEDMAN, P.N., and PRIVES, C. (1992). Site-specific binding of wild-type p53 to cellular DNA is inhibited by SV40 T antigen and mutant p53. Genes Dev. **6,** 1886–1898.

BARKETT, M., XUE, D., HORVITZ, H.R., and GILMORE, T.D. (1997). Phosphorylation of IkB-α inhibits its cleavage by caspase CPP32 in vitro. J. Biol. Chem. **272,** 29419–29422.

BARTZ, S.R., ROGEL, M.E., and EMERMAN, M. (1996). Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G_2 accumulation by a mechanism which differs from DNA damage checkpoint control. J. Virol. **70,** 2324–2331.

BENNETT, M., MACDONALD, K., CHAN, S., LUZIO, J.P., SIMARI, R., and WEISSBERG, P. (1998). Cell sur-

face trafficking of Fas: A rapid mechanism of p53-induced apoptosis. Science **282,** 290–293.

BENOV, L., and FRIDOVICH, I. (1998). Superoxide dependence of the toxicity of short chain sugars. J. Biol. Chem. **273,** 25741–25744.

BERTIN, J. (1997). Death effector domain-containing herpesvirus and poxvirus proteins inhibit Fas- and TNFR1-induced apoptosis. Proc. Natl. Acad. Sci. USA **94,** 1172–1176.

BERTINI, R., HOWARD, O.M., DONG, H.F., OPPENHEIM, J.J., BIZZARRI, C., SERGI, R., PAGLIEI, S., ROMINES, B., WILSHIRE, J.A., MENGOZZI, M., NAKAMURA, H., PEKKARI, K., GURUNATH, R., HOLMGREN, A., HERZENBERG, L.A., HERZENBERG, L.A., and GHEZZI, P. (1999). Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes, and T cells. J. Exp. Med. **189,** 1783–1789.

BLANCO, J., JACOTOT, E., CABRERA, C., CARDONA, A., CLOTET, B., DE CLERCQ, E., and ESTEJA. (1999). The implication of the chemokine receptor CXCR4 in HIV-1 envelope protein-induced apoptosis is independent of the G protein-mediated signalling. AIDS **13,** 909–917.

BOFILL, M., FAIRBANKS, L.D., RUCKEMENANN, K., LIPMAN, M., and SIMMONDS, H.A. (1995). T lymphocytes from AIDS patients are unable to synthesize ribonucleotides *de novo* in response to mitogenic stimulation. J. Biol. Chem. **270,** 29690–29697.

BRADLEY, A.D., McELHINNY, J.A., LEIBSON, P.L., LYNCH, D.H., ALDERSON, M.R., and PAYA, C.V. (1996). Upregulation of Fas ligand expression by human immunodefiociency virus in human macrophages mediates apoptosis of uninfected T lymphocytes. J. Virol. **70,** 199–206.

BUHL, R., HOLROYD, K.J., MASTRANGELI, A., CANTIN, A.M., JAFFE, H.A., WELLS, F.B., SALTINI, C., and CRYSTAL, R.G. (1989). Systemic glutathione deficiency in symptom-free HIV seropositive individuals. Lancet **II,** 1294–1298.

BUONAGURO, L., BUONAGURO, F.M., GIRALDO, G., and ENSOLI, B. (1994). The human immunodeficiency virus type i Tat protein transactivates tumor necrosis factor beta gene expression through a TAR-like structure. J. Virol. **68,** 2677–2682.

BUTTKE, T.M., and SANDSTROM, P.A. (1994). Oxidative stress as a mediator or apoptosis. Immunol. Today **15,** 7–10.

CARBONARI, M., PESCE, A.M., CIBATI, M., MODICA, A., DELL'ANNA, L., D'OFFIZI, G., UCCINI, S., MODESTI, A., and FIORILLI, M. (1997). Death of bystander cells by a novel pathway involving early mitochondrial damage in human immunodeficiency virus-related lymphadenopathy. Blood **90,** 209–216.

CARR, A., and FREI, B. (1999). Does vitamin C act as a pro-oxidant under physiological conditions? FASEB. J. **13,** 1007–1024.

CHANG, H.Y., NISHITOH, H., YANG, X., ICHIJO, H., and BALTIMORE, D. (1998). Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx. Science **281,** 1860–1863.

CHAUDRI, G., and CLARK, I.A. (1989). Reactive oxygen species facilitate the in vitro and in vivo lipopolysaccharide-induced release of tumor necrosis factor. J. Immunol. **143,** 1290–1297.

CHOU, J., and ROIZMAN, B. (1992). The gamma$_1$ 34.5 gene of herpes simplex virus 1 precludes neuroblastoma cells from triggering total shutoff of protein synthesis characteristic of programmed cell death in neuronal cells. Proc. Natl. Acad. Sci. USA **89,** 3266–3270.

CLEM, R.J., and MILLER, L.K. (1994). Control of programmed cell death by the baculovirus genes p35 and iap. Mol. Cell. Biol. **14,** 5212–5222.

CLERICI, M., and SHEARER, G.M. (1994). The Th1-Th2 hypothesis of HIV infection: new insights. Immunol. Today **15,** 575–581.

COLLETTE, Y., CHANG, H., CERDAN, C., CHAMBOST, H., ALGARTE, M., MAWAS, C., IMBERT, J., BURNY, A., and OLIVE, D. (1996). Specific Th1 cytokine down-regulation associated with primary clinically derived human immunodeficiency virus type 1 *nef* gene-induced expression. J. Immunol. **156,** 360–370.

CONSTANTINI, P., CHERNYAK, B.V., PETRONILLI, V., and BERNARDI, P. (1996). Modulation of the mitochondrial permeability transition pore by pyridine nucleotides and dithiol oxidation at two separate sites. J. Biol. Chem. **271,** 6746–6751.

CONTI, L., RAINALDI, G., MATARRESE, P., VARANO, B., RIVABENE, R., COLUMBA, S., SATOA., BELARDELLI, F., MALORNI, W., and GESSANI, S. (1998). The HIV-1 vpr protein acts as a negative regulator of apoptosis in a human lymphoblastoid T cell line: possible implications for the pathogenesis of AIDS. J. Exp. Med. **187,** 403–413.

COODLEY, G.O., LOVELESS, M.O., and MERRILL, T.M. (1994). The HIV wasting syndrome. J. AIDS **7,** 681–694.

COSSARIZZA, A., MUSSINI, C., MONGIARDO, N., BORGHI, V., SABBATINI, A., DE RIENZOB., and FRANCESCHI, C. (1997). Mitochondria alterations and dramatic tendency to undergo apoptosis in peripheral blood lymphocytes during acute HIV syndrome. AIDS **11,** 19–26.

DE QUAY, B., MALINVERNI, R., and LAUTERBURG, B.H. (1992). Glutathione depletion in HIV-infected patients: role of cysteine deficiency and effect of oral N-acetylcysteine. AIDS **6,** 815–819.

DELMAS-BEAUVIEUX, M., PEUCHANT, E., COUCHOURON, A., CONSTANS, J., SERGEANT, C., SIMONOFF, M., PELLEGRIN, J., LENG, B., CONRI, C., and CLERC, M. (1996). The enzymatic antioxidant system in blood an glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium or β-carotene. Am. J. Clin. Nutr. **64,** 101–107.

DEORA, A.A., WIN, T., VANHAESEBROECK, B., and LANDER, H.M. (1998). A redox-triggered ras-effector interaction. Recruitment of phosphatidylinositol 3′-kinase to Ras by redox stress. J. Biol. Chem. **273,** 29923–29928.

DROGE, W., ECK, H., and MIHM, S. (1992). HIV-induced cysteine deficiency and T-cell dysfunction—a rationale for treatment with N-acetylcysteine. Immunol. Today **13,** 211–214.

DROGE, W., SCHULZE-OSTHOFF, K., MIHM, S., GALTER, D., SCHENK, H., ECK, H.P., and ROTH, S.G. (1994). Functions of glutathione and glutathione disulfide in immunology and immunopathology. FASEB J. **8,** 1131–1138.

DU, X., STOCKLAUSER-FARBER, K., and ROSEN, P. (1999). Generation of reactive oxygen intermediates, activation of NF-kappaB, and induction of apoptosis in human endothelial cells by glucose: role of nitric oxide synthase? Free Rad. Biol. Med. **27,** 752–763.

ECK, H.P., GMUNDER, H., HARTMANN, M., PETZOLDT, D., and DANIEL, V. (1989). Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol. Chem. Hoppe-Seyler **370,** 101–108.

EFANOVA, I.B., ZAITSEV, S.V., ZHIVOTOVSKY, B., KOHLER, M., EFENDIC, S., and ORRENIUS, S. (1998). Glucose and tolbutamide induce apoptosis in pancreatic beta-cells. A process dependent on intracellular Ca2+ concentration. J. Biol. Chem. **273,** 33501–33507.

EHRET, A., WESTENDORP, M.O., HERR, I., DEBATIN, K., HEENEY, J.L., FRANK, R., and KRAMMER, P.H. (1996). Resistance of chimpanzee T cells to human immunodeficiency virus type I tat-enhanced oxidative stress and apoptosis. J. Virol. **70,** 6502–6507.

ELBIM, C., PILLET, S., PREVOST, M.H., PREIRA, A., GIRARD, P.M., ROGINE, N., MATUSANIH., HAKIM, J., ISRAEL, N., and GOUGEROT-POCIDALO, M.A. (1999). Redox and activation status of monocytes from human immunodeficiency virus-infected patients: relationship with viral load. J. Virol. **73,** 4561–4566.

EMERMAN, M., and MALIM, M.H. (1998). HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. Science **280,** 1880–1884.

ENARI, M., SAKAHIRA, H., YOKOYAMA, H., OKAWA, K., IWAMATSU, A., and NAGATA, S. (1998). A capase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature **391,** 43–50.

FINE, E.L., HORAL, M., CHANG, T.I., FORTIN, G., and LEOKEN, M.R. (1999). Evidence that elevated glucose causes altered gene expression, apoptosis, and neural tube defects in a mouse model of diabetic pregnancy. Diabetes **48,** 2454–2462.

FINKEL, T.H., TUDOR-WILLIAMS, G., BANDA, N.K., COTTON, M.F., CURIEL, T., MONKS, C., BABA, T.W., RUORECHT, R.M., and KUPFER, A. (1995). Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nature Med. **1,** 129–134.

FINZI, D., and SILICIANO, R.F. (1998). Viral dynamics in HIV-1 infection. Cell **93,** 665–671.

FLORES, S.C., MARECKI, J.C., HARPER, K.P., BOSE, S.K., NELSON, S.K., and MCCORD, J.M. (1993). Tat protein of human immunodeficiency virus type 1 represses expression of manganese superoxide dismutase in HeLa cells. Proc. Natl. Acad. Sci. USA **90,** 7632–7636.

FUJITA, K., MIKI, N., MOJICA, M.P., TAKAO, S., PHUCHAREON, J., NISHIKAWA, S., and TOKUHISA, T. (1993). B cell development is perturbed in bone marrow from c-fos/v-jun doubly transgenic mice. Internat. Immunol. **5,** 227–230.

GALTER, D., MIHM, S., and DROGE, W. (1994). Distinct effects of glutathione disulphide on the transcription factors NFκB and AP-1. Eur. J. Biochem. **221,** 639–648.

GANDHI, R.T., CHEN, B.K., STRAUS, S.E., DALE, J.K., LENARDO, M.J., and BALTIMORE, D. (1998). HIV-1 directly kills $CD4^+$ T cells by a Fas-independent mechanism. J. Exp. Med. **187,** 1113–1122.

GINN-PEASE, M.E., and WHISLER, R.L. (1998). Redox signals and NF-kappaB activation in T cells. Free Rad. Biol. Med. **25,** 346–361.

GLYNN, J.M., McELLIGOTT, M., and MOSIER, D.E. (1996). Apoptosis induced by HIV infection in H9 T cells is blocked by ICE-family protease inhibition but not by a Fas(CD95) antagonist. J. Immunol. **157,** 2754–2758.

GOTOH, Y., and COOPER, J.A. (1998). Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. J. Biol. Chem. **273,** 17477–17482.

GREEN, D.R., and REED, J.C. (1998). Mitochondria and apoptosis. Science **281,** 1309–1312.

GREEN, J.M., and THOMPSON, C.B. (1994). Modulation of T cell proliferative response by accessory cell interactions. Immunol. Res. **13,** 234–243.

GREENE, D.A., STEVENS, M.J., OBROSOVA, I., and FELDMAN, E.L. (1999). Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. Eur. J. Pharmacol. **375,** 217–223.

GROSS, A., McDONNELL, J.M., and KORSMEYER, S.J. (1999). BCL-2 family members and the mitochondria in apoptosis. Genes Dev. **13,** 1899–1911.

GRUNFELD, C., PANG, M., SHIMIZU, L., SHIGENAGA, J.K., JENSEN, P., and FEINGOLD, K.R. (1992). Resting energy expenditure, caloric intake, and short-term weight change in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. Am. J. Clin. Nutr. **55,** 455–460.

GUALBERTO, A., and BALDWIN, A.S., JR. (1995). p53 and Sp1 interact and cooperate in the tumor necrosis factor-induced transcriptional activation of the HIV-1 long terminal repeat. J. Biol. Chem. **270,** 19680–19683.

HALLIWELL, B., and GUTTERIDGE, J.M. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. **186,** 1–85.

HAMILOS, D.L., and WEDNER, H.J. (1985). The role of glutathione in lymphocyte activation. I. Comparison of inhibitory effects of buthionine sulfoximine and 2-cyclohexene-1-one by nuclear size transformation. J. Immunol. **135,** 2740–2747.

HAMPTON, M.B., and ORRENIUS, S. (1998). Redox regulation of apoptotic cell death in the immune system. Toxicology Lett. **102–103,** 355–358.

HARADA, H., BECKNELL, B., WILM, M., MANN, M., HUANG, L.J., TAYLOR, S.S., and SCOTT, J.D. (1999). Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A. Mol. Cell **3,** 413–422.

HARAKEH, S., JARIWALLA, R.J., and PAULING, L. (1990). Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. Proc. Natl. Acad. Sci. USA **87,** 7245–7249.

HAYASHI, T., UENO, Y., and OKAMOTO, T. (1993). Oxidoreductive regulation of nuclear factor kappa B. Involvement of a cellular reducing catalyst thioredoxin. J. Biol. Chem. **268,** 11380–11388.

HEINRICH, P.C., MORRIS, H.P., and WEBER, G. (1976). Behavior of transaldolase (EC 2.2.1.2.) and transketolase (EC 2.2.1.1.) in normal neoplastic, differentiating, and regenerating liver. Cancer Res. **36,** 3189–3197.

HENDERSON, S., HUEN, D., ROWE, M., DAWSON, C., JOHNSON, G., and RICKINSON, A. (1993). Epstein-Barr virus-coded BHRF1 protein, a viral homologue of bcl-2, protects human B cells from programmed cell death. Proc. Natl. Acad. Sci. USA **90,** 8479–8483.

HERBEIN, G., MAHLKNECHT, U., BATLIVALLA, F., GREGERSEN, P., PAPPAS, T., BUTLER, J., O'BRIEN, W.A., and VERDIN, E. (1998). Apoptosis of $CD8^+$ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. Nature **395,** 189–194.

HERRMANN, C.H., and RICE, A.P. (1995). Lentivirus Tat proteins specifically associate with a cellular protein kinase, TAK, that hyperphosphorylates the carboxy-terminal domain of the large subunit of RNA polymerase II: candidate for a Tat cofactor. J. Virol. **69,** 1612–1620.

HERZENBERG, L.A., DE ROSA, S.C., DUBS, J.G., ROEDERER, M., ANDERSON, M.T., ELA, S.W., and DERESINSKI, S.C. (1997). Glutathione deficiency is associated with impaired survival in HIV disease. Proc. Natl. Acad. Sci. USA **94,** 1967–1972.

HILDEMAN, D.A., MITCHELL, T., TEAGUE, T.K., HENSON, P., DAY, B.J., and KAPPLER, J. (1999). Reactive oxygen species regulate activation-induced T cell apoptosis. Immunity **10,** 735–744.

HINSHAW, V.S., OLSEN, C.W., DYBDAHL-SISSOKO, N., and EVANS, D. (1994). Apoptosis: a mechanism of cell killing by influenza A and B viruses. J. Virol. **68,** 3667–3673.

HO, D.D., NEUMANN, A.U., PERELSON, A.S., CHEN, W., LEONARD, J.M., and MARKOWITZ, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature **373,** 123–126.

HOCKENBERRY, D.M., OLTVAI, Z.N., YIN, X., MILLIMAN, C.L., and KORSMEYER, S.J. (1993). Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell **75,** 241–251.

HOEFLICH, K.P., YEH, W.C., YAO, Z., MAK, T.W., and WOODGETT, J.R. (1999). Mediation of TNF receptor-associated factor effector functions by apoptosis signal-regulating kinase-1 (ASK1). Oncogene **18,** 5814–5820.

HOFMANN, B., NISHANIAN, P., BALDWIN, R.L., INSIXIENGMAY, P., NEL, A., and FAHEY, J.L. (1990). HIV inhibits the early steps of lymphocyte activation, including initiation of inositol phospholipid metabolism. J. Immunol. **145,** 3699–3705.

HOLMGREN, A., and BJORNSTEDT, M. (1995). Thioredoxin and thioredoxin reductase. Methods Enzymol. **252,** 199–208.

IMBERT, V., RUPEC, R.A., LIVOLSI, A., PAHL, H.L., TRAENCKNER, E.B., MUELLER-DIECKMANNC., FARAHIFAR, D., ROSSI, B., AUBERGER, P., and BAEUERLE, P.A. (1996). Tyrosine phosphorylation of I kappa B-alpha activates NF-kappa B without proteolytic degradation of I kappa B-alpha. Cell **86,** 787–798.

ISRAEL, N., GOUGEROT-POCIDALO, M., AILLET, F., and VIRELIZIER, J. (1992). Redox status of cells influences constitutive or induced NF-κB translocation and HIV long terminal repeat activity in human T and monocytic cell lines. J. Immunol. **149,** 3386–3393.

JACOBSON, S., SHIDA, H., MCFARLIN, D.E., FAUCI, A.S., and KOENIG, S. (1990). Circulating $CD8^+$ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-1 associated neurological disease. Nature **348,** 245–248.

JACOTOT, E., RAVAGNAN, L., LOEFFLER, M., FERRI, K.F., VIEIRA, H.L.A., ZAMZANI, N., COSTANTINI, P., DRUILLENNEC, S., HOEBEKE, J., BRIAND, J.P., IRINOPOULOU, T., DAUGAS, E., SUSIN, S.A., COINTE, D., XIE, Z.H., REED, J.C., ROQUES, B.P., and KROEMER, G. (2000). The HIV-1 viral protein r induces apoptosis via a direct effect on the mitochondrial permeability transition pore. J. Exp. Med. **191,** 33–45.

JONES, K.A., and PETERLIN, B.M. (1994). Control of RNA initiation and elongation at the HIV-1 promoter. Annu. Rev. Biochem. **63,** 717–743.

KALEBIC, T., KINTER, A., POLI, G., ANDERSON, M.E., and MEISTER, A. (1991). Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and N-acetylcysteine. Proc. Natl. Acad. Sci. USA **88,** 986–990.

KAMINSKI, M., JR., WEIL, S., BLAND, J., and JAN, P. (1998). AIDS wasting syndrome as an enterometabolic disorder: the gut hypothesis. Altern. Med. Rev. **3,** 40–53.

KANE, D.J., SARAFIAN, T.A., ANTON, R., HAHN, H., GRALLA, E.B., VALENTINE, J.S., ORD, T., and BREDESEN, D.E. (1993). Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. Science **262,** 1274–1277.

KASIBHATLA, S., GENESTIER, L., and GREEN, D.R. (1999). Regulation of Fas ligand expression during activation-induced cell death in T lymphocytes. J. Biol. Chem. **274,** 987–992.

KINSCHERF, R., FISCHBACH, T., MIHM, S., ROTH, S., HOHENHAUS-SIEVERT, E., WEISS, C., EDLER, L., BARTSCH, P., and DROGE, W. (1994). Effect of glutathione depletion and oral N-acetyl-cystein treatment on $CD4^+$ and $CD8^+$ cells. FASEB J. **8,** 448–451.

KISHI, S., SAIJYO, S., ARAI, M., KARASAWA, S., UEDA, S., KANNAGI, M., IWAKURA, Y., FUJII, M., and YONEHARA, S. (1997). Resistance to Fas-mediated

apoptosis of peripheral T cells in human T lymphocyte virus type I (HTLV-I) transgenic mice with autoimmune arthropathy. J. Exp. Med. **186,** 57–64.

KLUCK, R.M., BOSSY-WETZEL, E., GREEN, D.R., and NEWMEYER, D.D. (1997). The release of cytochrome c from mitochondria: a primary site for bcl-2 regulation of apoptosis. Science **275,** 1132–1136.

KORSMEYER, S.J. (1995). Regulators of cell death. Trends Genet. **11,** 101–105.

KORT, J.J., and JALONEN, T.O. (1998). The nef protein of the human immunodeficiency virus type 1 (HIV-1) inhibits a large-conductance potassium channel in human glial cells. Neurosci. Lett. **251,** 1–4.

KOTLER, D.P. (1998). Antioxidant therapy and HIV infection: 1998. Am. J. Clin. Nutr. **67,** 7–9.

KOVITZ, C.A., and MORGELLO, S. (1997). Cerebral glucose transporter expression in HIV infection. Acta Neuropathol. **94,** 140–145.

KOZLOVSKY, N., RUDICH, A., POTASHNIK, R., EBINA, Y., MURAKAMI, T., and BASHAN, N. (1997). Transcriptional activation of the Glut1 gene in response to oxidative stress in L6 myotubes. J. Biol. Chem. **272,** 33367–33372.

KRETZ-REMY, C., BATES, E.E., and ARRIGO, A.P. (1998). Amino acid analogs activate NF-kappaB through redox-dependent IkappaB-alpha degradation by the proteasome without apparent IkappaB-alpha phosphorylation. Consequence on HIV-1 long terminal repeat activation. J. Biol. Chem. **273,** 3180–3191.

KRUMAN, I.I., NATH, A., and MATTSON, M.P. (1998). HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress. Exp. Neurol. **154,** 276–288.

KURATA, S.I. (1996). Sensitization of the HIV-1-LTR upon long term low dose oxidative stress. J. Biol. Chem. **271,** 21798–21802.

LAHDEVIRTA, J., MAURY, C.P., TEPPO, A.M., and REPO, H. (1988). Elevated levels of circulating cachectins/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am. J. Med. **85,** 289–291.

LENARDO, M., CHAN, K.M., HORNUNG, F., MCFARLAND, H., SIEGEL, R., WANG, J., and ZHENG, L. (1999). Mature T lymphocyte apoptosis-immune regulation in a dynamic and unpredictable antigenic environment. Annu. Rev. Immunol. **17,** 221–253.

LI, D., YANG, B., and MEHTA, J.L. (1998). Ox-LDL induces apoptosis in human coronary artery endothelial cells: role of PKC, PTK, bcl-2, and Fas. Am. J. Physiol. **275,** H568–H576.

LI, N., and KARIN, N. (1999). Is NF-κB the sensor of oxidative stress? FASEB J. **13,** 1137–1143.

LI, P., NIJHAWAN, D., BUDIHARDJO, I., SRINIVASULA, S.M., AHMAD, M., ALNEMRI, E.S., and WANG, X. (1997). Cytochrome c and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates and apoptotic protease cascade. Cell **91,** 479–489.

LIPTON, S.A., CHOI, Y., PAN, Z., LEI, S.Z., CHEN, H.V., SUCHER, N.J., LOSCALZO, J., SINGEL, D.J., and STAMLER, J.S. (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature **364,** 626–631.

LOOK, M.P., ROCKSTROH, J.K., RAO, G.S., BARTON, S., LEMOCH, H, KAISER, R., KUPFER, B., SPENGLER, U., and SAUERBRUCH, T. (1998). Sodium selenite and N-acetylcysteine in antiretroviral-naive HIV-1-infected patients: a randomized, controlled pilot study. Eur. J. Clin. Invest. **28,** 389–397.

LOPEZ GALERA, R.M., JUAREZ GIMENEZ, J.C., MONTORO RONSANO, J.B., SEGURA CARDONA, R.M., ARBOS VIA, M.A., ALTISENT ROCA, C., and TUSELL PUIGBERT, J.M. (1996). Glutathione and cysteine in HIV-infected hemophiliacs. Clin. Chim. Acta **254,** 63–72.

LOS, M., DROGE, W., STRICKER, K., BAUERLE, P.A., and SCHULZE-OSTHOFF, K. (1995a). Hydrogen peroxide as a potent activator of T lymphocyte functions. Eur. J. Immunol. **25,** 159–165.

LOS, M., SCHENK, H., HEXEL, K., BAEUERLE, P.A., DROGE, W., and SCHULZE-OSTHOFF, K. (1995b). IL-2 gene expression and NF-kappaB activation through CD28 requires reactive oxygen production by 5-lipoxygenase. EMBO J. **14,** 3731–3740.

LUTZ, N.W., YAHI, N., FANTINI, J., and COZZONE, P.J. (1997). Perturbations of glucose metabolism associated with HIV infection in human intestinal epithelial cells: a multinuclear magnetic resonance spectroscopy study. AIDS **11,** 147–155.

MACREADIE, I.G., THORBURN, D.R., KIRBY, D.M., CASTELLI, L.A., DE ROZARIO, N.L., and AZADAA. (1997). HIV-1 protein Vpr causes gross mitochondrial dysfunction in the yeast Saccharomyces cerevisiae. FEBS Lett. **410,** 145–149.

MALORNI, W., RIVABENE, R., SANTINI, M.T., and DONELLI, G. (1993). N-acetylcysteine inhibits apoptosis and decreases viral particles in HIV-chronically infected U937 cells. FEBS Lett. **327,** 75–78.

MALORNI, W., RIVABENE, R., LUCIA, B.M., FERRARA, R., MAZZONE, A.M., and CAUDA, R. (1998). The role of oxidative imbalance in progression to AIDS: effect of the thiol supplier N-acetylcysteine. AIDS Res. Hum. Retrovirus. **14,** 1589–1596.

MARSHALL, W.L., DATTA, R., HANIFY, K., TENG, E., and FINBERG, R.W. (1999). U937 cells overexpressing bcl-xl are resistant to human immunodeficiency virus-1-induced apoptosis and human immunodeficiency virus-1 replication. Virology **256,** 1–7.

MASUTANI, H., NAITO, M., TAKAHASHI, K., HATTORI, T., KOITO, A., TAKATSUKI, K., GOT, NAKAMURA, H., FUJII, S., YOSHIDA, Y., and ET AL. (1992). Dysregulation of adult T-cell leukemia-derived factor (ADF)/thioredoxin in HIV infection: loss of ADF high-producer cells in lymphoid tissues of AIDS patients. AIDS Res. Hum. Retrovirus. **8,** 1707–1715.

MASUTANI, H., HIROTA, K., SASADA, T., UEDA-TANIGUCHI, Y., TANIGUCHI, Y., SONO, H., and YODOI, J. (1996). Transactivation of an inducible anti-ox-

idative stress protein, human thioredoxin by HTLV-I tax. Immunol. Lett. **54,** 67–71.

MATTHEWS, J.R., WAKASUGI, N., VIRELIZIER, J.L., YODOI, J., and HAY, R.T. (1992). Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulphide bond involving cysteine 62. Nucleic Acids Res. **20,** 3821–3830.

MAYES, P. (1993). The pentose phosphate pathway & other pathways of hexose metabolism. In: *Harper's Biochemistry.* R. Murray, D. Granner, P. Mayes, and V. Rodwell, eds. (Appleton & Lange, Norwalk, CT) pp. 201–211.

McINTYRE, L.M., THORBURN, D.R., BUBB, W.A., and KUCHEL, P.W. (1989). Comparison of computer simulations of the F-type and L-type non-oxidative hexose monophosphate shunts with ^{31}P-NMR experimental data from human erythrocytes. Eur. J. Biochem. **180,** 399–420.

MEISTER, A. (1994). Glutathione-ascorbic acid antioxidant system in animals. J. Biol. Chem. **269,** 9397–9400.

MEISTER, A., and ANDERSON, M.E. (1983). Glutathione. Annu. Rev. Biochem. **52,** 711–760.

MELLORS, J.W., RINALDO, C., GUPTA, P., WHITE, R.M., TODD, J.A., and KINGSLEY, L.A. (1996). Prognosis of HIV infection predicted by the quantity of virus in plasma. Science **272,** 1167–1170.

MEYAARD, L., OTTO, S.A., JONKER, R.R., MIJNSTER, M.J., KEET, R.P.M., and MIEDEMA, F. (1992). Programmed death of T cells in HIV infection. Science **257,** 217–219.

MIYAWAKI, T., UEHARA, T., NIBU, R., TSUJI, T., YACHIE, A., YONEHARA, S., and TANIGUCHI, N. (1992). Differential expression of apoptosis related Fas antigen on lymphocyte subpopulations in human peripheral blood. J. Immunol. **149,** 3753–3758.

MOREY, A.L., FERGUSON, D.J., and FLEMING, K.A. (1993). Ultrastructural features of fetal erythroid precursors infected with parvovirus B19 in vitro: evidence for cell death by apoptosis. J. Pathol. **169,** 213–220.

MULLIGAN, K., and BLOCH, A.S. (1998). Energy expenditure and protein metabolism in human immunodeficiency virus infection and cancer cachexia. Sem. Oncol. **25,** 82–91.

MUNOZ, E., BLAZQUEZ, M.V., ORTIZ, C., GOMEZ-DIAZ, C., and NAVAS, P. (1997). Role of ascorbate in the activation of NF-kappaB by tumour necrosis factor-alpha in T-cells. Biochem. J. **325,** 23–28.

NAKAMURA, H., DE ROSA, S., ROEDERER, M., ANDERSON, M.T., DUBS, J.G., YODOI, J., and HERZENBERG, L.A. (1996). Elevation of plasma thioredoxin levels in HIV-infected individuals. Internat. Immunol. **8,** 603–611.

NAKAMURA, H., NAKAMURA, K., and YODOI, J. (1997). Redox regulation of cellular activation. Annu. Rev. Immunol. **15,** 351–369.

NEILAN, J.G., LU, Z., AFONZO, C.L., KUTISH, G.F., SUSSMAN, M.D., and ROCK, D.L. (1993). An African swine fever virus gene with similarity to the proto-oncogene bcl-2 and the Epstein-Barr virus gene BHRF1. J. Virol. **67,** 4391–4394.

NEWMAN, G.W., BALCEWICZ-SABLINSKA, M.K., GUARNACCIA, J.R., REMOLD, H.G., and SILBERSTEINDS. (1994). Opposing regulatory effects of thioredoxin and eosinophil cytotoxicity-enhancing factor on the development of human immunodeficiency virus 1. J. Exp. Med. **180,** 359–363.

NI, T., and SAVAGEAU, M.A. (1996). Application of biochemical systems theory to metabolism in human red blood cells. Signal Propagation and accuracy of representation. J. Biol. Chem. **271,** 7927–7941.

NISHITOH, H., SAITOH, M., MOCHIDA, Y., TAKEDA, K., NAKANO, H., ROTHE, M., MIYAZONOK., and ICHIJO, H. (1998). ASK1 is essential for JNK/SAPK activation by TRAF2. Mol. Cell **2,** 389–395.

NOMURA, K., IMAI, H., KOUMURA, T., ARAI, M., and NAKAGAWA, Y. (1999). Mitochondrial phospholipid hydroperoxide glutathione peroxidase suppresses apoptosis mediated by a mitochondrial death pathway. J. Biol. Chem. **274,** 29294–29302.

OHNIMUS, H., HEINKELEIN, M., and JASSOY, C. (1997). Apoptotic cell death upon contact of $CD4^+$ T lymphocytes with HIV glycoprotein-expressing cells is mediated by caspases but bypasses CD95 (Fas/Apo-1) and TNF receptor 1. J. Immunol. **159,** 5246–5252.

OKAMOTO, T., OGIWARA, H., HAYASHI, T., MITSUI, A., KAWABE, T., and YODOI, J. (1992). Human thioredoxin/adult T cell leukemia-derived factor activates the enhancer binding protein of human immunodeficiency virus type 1 by thiol redox control mechanism. Int. Immunol. **4,** 811–819.

OKAMOTO, T., SAKURADA, S., YANG, J.P., and MERIN, J.P. (1997). Regulation of NF-kappa B and disease control: identification of a novel serine kinase and thioredoxin as effectors for signal transduction pathway for NF-kappa B activation. Curr. Topics Cell. Regul. **35,** 149–161.

ORLINICK, J.R., VAISHNAW, A., ELKON, K.B., and CHAO, M.V. (1997). Requirement of cysteine-rich repeats of the Fas receptor for binding of the Fas ligand. J. Biol. Chem. **272,** 28889–28894.

PALAMARA, A.T., GARACI, E., ROTILIO, G., CIRIOLO, M.R., CASABIANCA, A., FRATERNALEA, ROSSI, L., SCHIAVANO, G.F., CHIARANTINI, L., and MAGNANI, M. (1996). Inhibition of murine AIDS by reduced glutathione. AIDS Res. Hum. Retrovirus. **12,** 1373–1381.

PAN, H., and GRIEP, A.E. (1995). Temporally distinct patterns of p53-dependent and p53-independent apoptosis apoptosis during mouse lens development. Genes Dev. **9,** 2157–2169.

PANTALEO, G., GRAZIOSI, C., and FAUCI, A.S. (1993). The immunopathogenesis of human immunodeficiency virus infection. N. Engl. J. Med. **328,** 327–335.

PEREIRA, L.A., BENTLEY, K., PEETERS, A., CHURCHILL, M.J., and DEACON, N.J. (2000). A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. Nucleic Acids Res. **28,** 663–668.

PIETTE, J., PIRET, B., BONIZZI, G., SCHOONBROODT, S., MERVILLEMP., LEGRAND-POELS, S., and BOURS, V. (1997). Multiple redox regulation in NF-kappaB

transcription factor activation. Biol. Chem. **378,** 1237–1245.

PILLER, S.C., JANS, P., GAGE, P.W., and JANS, D.A. (1998). Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultured hippocampal neurons: implications for AIDS pathology. Proc. Natl. Acad. Sci. USA **95,** 4595–4600.

PIRMOHAMED, M., WILLIAMS, D., TINGLE, M.D., BARRY, M., KHOO, S.H., O'MAHONEY, C., WILKINS, E.G.L., BRECKENRIDGE, A.M., and PARK, B.K. (1996). Intracellular glutathione in the peripheral blood cells of HIV-infected patients: failure to show a deficiency. AIDS **10,** 501–507.

PLANELLES, V., JOWETT, J.B.M., LI, Q., XIE, Y, HAHN, B., and CHEN, I.S.Y. (1996). Vpr-induced cell cycle arrest is conserved among primate lentiviruses. J. Virol. **70,** 2516–2524.

PODMORE, I.D., GRIFFITHS, H.R., HERBERT, K.E., MISTRY, N., MISTRY, P., and LUNEC, J. (1998). Vitamin C exhibits pro-oxidant properties. Nature **392,** 559.

RAO, L., DEBBAS, M., SABBATINI, P., HOCKENBERRY, D., KORSMEYER, S., and WHITE, E. (1992). The adenovirus E1A proteins induce apoptosis which is inhibited by the E1B 19K and bcl-2 proteins. Proc. Natl. Acad. Sci. USA **89,** 7742–7746.

RAY, C.A., BLACK, R.A., KRONHEIM, S.R., GREENSTREET, T.A., SLEATH, P.R., SALVESEN, G.S., and PICKUP, D.J. (1992). Viral inhibition of inflammation; Cowpox virus encodes an inhibitor of the interleukin-1β converting enzyme. Cell **69,** 597–604.

REVILLARD, J., VINCENT, C.M.A., FAVIER, A.E., RICHARD, M., ZITTOUN, M., and KAZATCHKIN, M.D. (1992). Lipid peroxidation in human immunodeficiency virus infection. J. AIDS. **5,** 637–638.

RIVAS, C.I., VERA, J.C., GUAIQUIL, V.H., VELASQUEZ, F.V., BORQUEZ-OJEDA, O.A., CARCAMOJG., CONCHA, I.I., and GOLDE, D.W. (1997). Increased uptake and accumulation of vitamin C in human immunodeficiency virus 1-infected hematopoietic cell lines. J. Biol. Chem. **272,** 5814–5820.

ROEDERER, M., STAAL, F.J., OSADA, H., HERZENBERG, L.A., and HERZENBERG, L.A. (1991). CD4 and CD8 T cells with high intracellular glutathione levels are selectively lost as the HIV infection progresses. Internat. Immunol. **3,** 933–937.

ROTTENBERG, D.A., SIDTIS, J.J., STROTHER, S.C., SCHAPER, K.A., ANDERSON, J.R., NELSON, M.J., and PRICE, R.W. (1996). Abnormal cerebral glucose metabolism in HIV-1 seropositive subjects with and without dementia. J. Nuclear Med. **37,** 1133–1141.

RUBARTELLI, A., POGGI, A., SITIA, R., and ZOCCHI, M.R. (1998). HIV-1 tat: a polypeptide for all seasons. Immunol. Today **19,** 543–545.

RYDER, M.I., WINKLER, J.R., and WEINREB, R.N. (1988). Elevated phagocytosis, oxidative burst, and F-actin formation in PMNs from individuals with intraoral manifestations of HIV infection. J. AIDS. **1,** 346–353.

SAITOH, M., NISHITOH, H., FUJII, M., TAKEDA, K., TOBIUME, K., SAWADA, Y., KAWABATAM, MIYAZONO, K., and ICHIJO, H. (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J. **17,** 2596–2606.

SAKAGAMI, H., and SATOH, K. (1997). Modulating factors of radical intensity and cytotoxic activity of ascorbate. Anticancer Res. **17,** 3513–3520.

SALVEMINI, F., FRANZE, A., IERVOLINO, A., FILOSA, S., SALZANO, S., and URSINI, M.V. (1999). Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. J. Biol. Chem. **274,** 2750–2757.

SALVESEN, G.S., and DIXIT, V.M. (1997). Caspases: Intracellular signaling by proteolysis. Cell **91,** 443–446.

SARAFIAN, T.A., and BREDESEN, D.E. (1994). Is apoptosis mediated by reactive oxygen species? Free Rad. Res. **21,** 1–8.

SASTRY, K.J., MARIN, M.C., NEHETE, P.N., MCCONNELL, K., EL-NAGGAR, A.K., and McDONNELL, T.J. (1996). Expression of human immunodeficiency virus type I tat results in down-regulation of bcl-2 and induction of apoptosis in hematopoietic cells. Oncogene **13,** 487–493.

SCHEFFNER, M., WERNESS, B.A., HUIBREGTSE, J.M., LEVINE, A.J., and HOWLEY, P.M. (1990). The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell **63,** 1129–1136.

SCHMIDT, K.N., AMSTAD, P., CERUTTI, P., and BAEUERLE, P.A. (1995). The roles of hydrogen peroxide and superoxide as messengers in the activation of transcription factor NF-kappa B. Chem. Biol. **2,** 13–22.

SCHWARZ, K.B. (1996). Oxidative stress during viral infection: a review. Free Rad. Biol. Med. **21,** 641–649.

SEN, C.K., and PACKER, L. (1996). Antioxidant and redox regulation of gene transcription. FASEB J. **10,** 709–720.

SEN, C.K., SASHWATI, R., and PACKER, L. (1999). Fas mediated apoptosis of human Jurkat T-cells: intracellular events and potentiation by redox-active alpha-lipoic acid. Cell Death Different. **6,** 481–491.

SEVE, M., FAVIER, A., OSMAN, M., HERNANDEZ, D., VAITAITIS, G., FLORES, N.C., McCORDJM., and FLORES, S.C. (1999). The human immunodeficiency virus-1 Tat protein increases cell proliferation, alters sensitivity to zinc chelator-induced apoptosis, and changes Sp1 DNA binding in HeLa cells. Arch. Biochem. Biophys. **361,** 165–172.

SHARPSTONE, D.R., MURRAY, C.P., ROSS, H.M., HANCOCK, M.R., PHELAN, M.S., CRANE, R.C., MENZIES, I.S., REAVELEY, D.A., LEPRI, A.C., NELSON, M.R., and GAZZARD, B.G. (1996). Energy balance in asymptomatic HIV infection. AIDS **10,** 1377–1384.

SHIMIZU, S., EGUCHI, Y., KAMIIKI, W., FUNAHASHI, Y., MIGNON, A., LACRONIQUE, V., MATSUDA, H., and TSUJIMOTO, Y. (1998). Bcl-2 prevents apoptotic mitochondrial dysfunction by regulating proton flux. Proc. Natl. Acad. Sci. USA **95,** 1455–1459.

SHOSTAK, L.D., LUDLOW, J., FISK, J., PURSELL, S., RIMEL, B.J., NGUYEN, D., ROSENBLATTJD., and PLANELLES, V. (1999). Roles of p53 and caspases in the induction of cell cycle arrest and apoptosis by HIV-1 vpr. Exp. Cell Res. **251,** 156–165.

SONNERBERG, A., CARLIN, G., AKERLUND, B., and JARSTRAND, C. (1988). Increased production of malondialdehyde in patients with HIV infection. Scand. J. Infect. Dis. **20,** 287–290.

SORBARA, L.R., MALDARELLI, F., CHAMOUN, G., SCHILLING, B., CHOKEKUCAHI, S., STAUDT, L., MITSUYA, H., SIMPSON, I.A., and ZEICHNER, S.L. (1996). Human immunodeficiency virus type 1 infection of H9 cells induces increased glucose transporter expression. J. Virol. **70,** 7275–7279.

STAAL, F.J. (1998). Glutathione and HIV infection: reduced reduced, or increased oxidized? Eur. J. Clin. Invest. **28,** 194–196.

STAAL, F.J., ROEDERER, M., ISRAELSKI, D.M., BUBP, J., MOLE, L.A., DERESINSKI, S.C., ROSS, W., SUSSMAN, H., RAJU, P.A., *ET AL.* (1992). Intracellular glutathione levels in T cell subsets decrease in HIV-infected individuals. AIDS Res. Hum. Retrovirus. **8,** 305–311.

STAAL, F.J.T., ROEDERER, M., and HERZENBERG, L.A. (1990). Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **87,** 9943–9947.

STAAL, F.J.T., ELA, S.W., ROEDERER, M., ANDERSON, M.T., and HERZENBERG, L.A. (1992). Glutathione deficiency and human immunodeficiency virus infection. Lancet **339,** 909–912.

STAMLER, J.S. (1994). Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell **78,** 931–936.

STRACK, P.R., FREY, M.W., RIZZO, C.J., CORDOVA, B., GEORGE, H.J., MEADE, R., HO, S.P., CORMAN, J., TRITCH, R., and KORANT, B.D. (1996). Apoptosis mediated by HIV protease is preceeded by cleavage of bcl-2. Proc. Natl. Acad. Sci. USA **93,** 9571–9576.

SU, L., KANESIMA, H., BONYHADI, M., SALIMI, S., KRAFT, D., RABIN, L., and MCCUNE, J.M. (1995). HIV-1-induced thymocyte depletion is associated with indirect cytopathicity and infection of progenitor cells in vivo. Immunity **2,** 25–36.

SUTHANTHIRAN, M., ANDERSON, M.E., SHARMA, V.K., and MEISTER, A. (1990). Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. Proc. Natl. Acad. Sci. USA **87,** 3343–3347.

TABUCCHI, A., CARLUCCI, F., CONSOLMAGNO, E., MONARI, P., RE, M.C., MARINELLO, E., PIZZICHINI, M., PAGANI, R., and LAI, C.Y. (1994). Changes in purine nucleotide content in the lymphocyte subpopulations of patients infected with HIV. Clin. Chim. Acta **225,** 147–153.

TAGAYA, Y., MAEDA, Y., MITSUI, A., KONDO, N., MATSUI, H., HAMUROJ., BROWN, N., ARAI, K., YOKOTA, T., WAKASUGI, H., *ET AL.* (1989). ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction [published erratum appears in EMBO J 1994 May 1;13(9):2244]. EMBO J. **8,** 757–764.

TATLA, S., WOODHEAD, V., FOREMAN, J.C., and CHAIN, B.M. (1999). The role of reactive oxygen species in triggering proliferation and IL-2 secretion in T cells. Free Radic. Biol. Med. **26,** 14–24.

THOME, M., SCHNEIDER, P., HOFMAN, K., FICKENSCHER, H., MEINL, E., NEIPEL, F., MATTMAN, C., BURNS, K., BODMER, J., SCHROTER, M., SCAFFIDI, C., KRAMMER, P.H., PETER, M.E., and TSCHOPP, J. (1997). Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature **386,** 517–521.

TIAN, W.N., BRAUNSTEIN, L.D., PANG, J., STUHLMEIER, K.M., XI, Q.C., and STANTON, R.C. (1998). Importance of glucose-6-phosphate dehydrogenase activity for cell growth. J. Biol. Chem. **273,** 10609–10617.

TIAN, W.N., BRAUNSTEIN, L.D., APSE, K., PANG, J., ROSE, M., TIAN, X., and STANTON, R.C. (1999). Importance of glucose-6-phosphate dehydrogenase activity in cell death. Am. J. Physiol. **276,** C1121–C1131.

TRAENCKNER, E.B., and BAEUERLE, P.A. (1995). Appearance of apparently ubiquitin-conjugated I kappa B-alpha during its phosphorylation-induced degradation in intact cells. J. Cell Sci. **19,** (Suppl.) 79–84.

UDENFRIEND, S., CLARK, C.T., AXELROD, J., and BRODIE, B.B. (1954). Ascorbic acid in aromatic hyroxylation. I. A model system for aromatic hydroxylation. J. Biol. Chem. **208,** 731–739.

URSINI, M.V., PARRELLA, A., ROSA, G., SALZANO, S., and MARTINI, G. (1997). Enhanced expression of glucose-6-phosphate dehydrogenase in human cells sustaining oxidative stress. Biochem. J. **323,** 801–806.

VAN DER VEN, A.J., BLOM, H.J., PETERS, W., JACOBS, L.E., VERVER, T.J., KOOPMANS, P.P., and VAN DER MEER, J.W. (1998). Glutathione homeostasis is disturbed in CD4-positive lymphocytes of HIV-seropositive individuals. Eur. J. Clin. Invest. **28,** 187–193.

VARADHACHARY, A.S., PETER, M.E., PERDOW, S.N., KRAMMER, P.H., and SALGAME, P. (1999). Selective up-regulation of phosphatidylinositol 3′-kinase activity in Th2 cells inhibits caspase-8 cleavage at the death-inducing complex: a mechanism for Th2 resistance from Fas-mediated apoptosis. J. Immunol. **163,** 4772–4779.

VERA, J.C., RIVAS, C.I., FISCHBARG, J., and GOLDE, D.W. (1993). Mammalian facilitative hexose transporters mediate the transport of dehyrdoascorbic acid. Nature **364,** 79–82.

WALMSLEY, S.L., WINN, L.M., HARRISON, M.L., UETRECHT, J.P., and WELLS, P.G. (1997). Oxidative stress and thiol depletion in plasma and peripheral blood lymphocytes from HIV-infected patients: toxicological and pathological implications. AIDS **11,** 1689–1697.

WANG, X.W., GIBSON, M.K., VERMEULEN, W., YEH, H., FORRESTER, K., STURZBECHER, H.W., HOEIJMAKERS, J.H., and HARRIS, C.C. (1995). Abrogation of p53-induced apoptosis by the hepatitis B virus X gene. Cancer Res. **55,** 6012–6016.

WEI, X., GHOSH, S.K., TAYLOR, M.E., JOHNSON, V.A., EMINI, E.A., DEUTSCH, P., LIFSON, J.D., BONHOEF-

FER, S., NOWAK, M.A., HAHN, B.H., SAAG, M.S., and SHAW, G.M. (1995). Viral dynamics in human immunodeficiency virus type 1 infection. Nature **373,** 117–122.

WESTENDORP, M.O., FRANK, R., OCHSENBAUER, C., STRICKER, K., DHEIN, J., WALCZAK, H., DEBATIN, K., and KRAMMER, P.H. (1995a). Sensitization of T cells to CD95-mediated apoptosis by HIV-1 tat and gp120. Nature **375,** 497–500.

WESTENDORP, M.O., SHATROV, V.A., SCHULZE-OSTHOFF, K., FRANK, R., KRAFT, M., LOS, M., KRAMMER, P.H., DROGE, W., and LEHMANN, V. (1995b). HIV-1 tat potentiates TNF-induced NF-κB activation and cytotoxicity by altering the cellular redox state. EMBO J. **14,** 546–554.

WHITE, E., CIPRIANI, R., SABBATINI, P., and DENTON, A. (1991). The adenovirus E1B 19-kilodalton protein overcomes the cytotoxicity of E1A proteins. J. Virol. **65,** 2968–2978.

WHYTE, P., BUCHKOVICH, K., HOROWITZ, J.M., FRIEND, S.H., RAYBUCK, M., WEINBERG, R.A., and HARLOW, E. (1988). Association between and oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature **334,** 124–129.

WITSCHI, A., JUNKER, E., SCHRANZ, C., SPECK, R.F., and LAUTENBURG, B.H. (1995). Supplementation of N-acetylcysteine fails to increase glutathione in lymphocytes and plasma of patients with AIDS. AIDS Res. Hum. Retrovirus. **11,** 141–143.

WOOD, T. (1974). Catalysis of pentose phosphate pathway reactions by cytoplasmic fractions from muscle, uterus and liver of the rat, and the presence of a reduced nicotinamide-adenine dinucleotide phosphate-triose phosphate oxidoreductase in rat muscle. Biochem. J. **138,** 71–76.

WOOD, T. (1985). *The Pentose Phosphate Pathway.* (Academic Press, New York).

XU, X., SCREATON, G.R., GOTCH, F.M., DONG, T., TAN, R., ALMOND, N., WALKER, B., STEBBINGS, R., KENT, K., NAGATA, S., STOTT, J.E., and McMICHAEL, A.J. (1997). Evasion of cytotoxic T lymphocyte (CTL) response by *Nef*-dependent induction of Fas ligand (CD95L) expression on simian immunodeficiency virus-infected cells. J. Exp. Med. **186,** 7–16.

XU, X., LAFFERT, B., SCREATON, G.R., KRAFT, M., WOLF, D., and KOLANUS, W. (1999). Induction of Fas ligand expression by HIV involves the interaction of Nef with the T cell receptor zeta chain. J. Exp. Med. **189,** 1489–1496.

YAMADA, T., YAMAOKA, S., GOTO, T., NAKAI, M., TSUJIMOTO, Y., and HATANAKA, M. (1994). The human T-cell leukemia virus type I tax protein induces apoptosis which is blocked by the bcl-2 protein. J. Virol. **68,** 3374–3379.

YANG, J., LIU, X., BHALLA, K., KIM, C.N., IBRADO, A.M., CAI, J., PENG, T., JONES, D.P., and WANG, X. (1997). Prevention of apoptosis by bcl-2: release of cytochrome c from mitochondria blocked. Science **275,** 1129–1132.

YAO, X., MOULAND, A.J., SUBBRAMANIAN, R.A., FORGET, J., ROUGEAU, N., BERGERON, D., and COHEN, E.A. (1998). Vpr stimulates viral expression and induces cell killing in human immunodeficiency virus type 1-infected dividing Jurkat T cells. J. Virol. **72,** 4688–4693.

ZAULI, G., GIBELLINI, D., CELEGHINI, C., MISCHIATI, C., BASSINIA, LA PLACA, M., and CAPITANI, S. (1996). Pleiotropic effects of immobilized versus soluble recombinant HIV-1 Tat protein on CD3-mediated activation, induction of apoptosis, and HIV-1 long terminal repeat transactivation in purified $CD4^+$ T lymphocytes. J. Immunol. **157,** 2216–2224.

ZAULI, G., GIBELLINI, D., SECCHIERO, P., DUTARTRE, H., OLIVE, D., CAPITANI, S., and COLETTE, Y. (1999). Human immunodeficiency virus type 1 nef protein sensitizes $CD4^+$ T lymphoid cells to apoptosis via functional upregulation of the CD95/CD95 ligand pathway. Blood **93,** 1000–1010.

ZEGARRA-MORAN, O., RASOLA, A., RUGOLO, M., PORCELLI, A.M., ROSSI, B., and GALIETTA, L.J. (1999). HIV-1 nef expression inhibits the activity of a $Ca2^+$-dependent K+ channel involved in the control of the resting potential in CEM lymphocytes. J. Immunol. **162,** 5359–5366.

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2. Ying Zhang, Linsheng Song, Jianmin Zhao, Lingling Wang, Pengfei Kong, Lin Liu, Mengqiang Wang, Limei Qiu. 2010. Protective immunity induced by CpG ODNs against white spot syndrome virus (WSSV) via intermediation of virus replication indirectly in Litopenaeus vannamei. *Developmental & Comparative Immunology* **34**:4, 418-424. [CrossRef]
3. Timea Stromájer-Rácz, Zoltán Gazdag, József Belágyi, Csaba Vágvölgyi, Richard Y. Zhao, Miklós Pesti. 2010. Oxidative stress induced by HIV-1 F34IVpr in Schizosaccharomyces pombe is one of its multiple functions. *Experimental and Molecular Pathology* **88**:1, 38-44. [CrossRef]
4. Virginia L. Scofield, Mingshan Yan, Xianghong Kuang, Soo-Jin Kim, Derek Crunk, Paul K.Y. Wong. 2009. The drug monosodium luminol (GVT®) preserves thymic epithelial cell cytoarchitecture and allows thymocyte survival in mice infected with the T cell-tropic, cytopathic retrovirus ts1. *Immunology Letters* **122**:2, 159-169. [CrossRef]
5. Alessandra Rosati, Kamel Khalili, Satish L. Deshmane, Sujatha Radhakrishnan, Maria Pascale, M. Caterina Turco, Liberato Marzullo. 2009. BAG3 protein regulates caspase-3 activation in HIV-1-infected human primary microglial cells. *Journal of Cellular Physiology* **218**:2, 264-267. [CrossRef]
6. X Yu, K Bauer, P Wernhoff, S M Ibrahim. 2007. Using an advanced intercross line to identify quantitative trait loci controlling immune response during collagen-induced arthritis. *Genes and Immunity* **8**:4, 296-301. [CrossRef]
7. J. Antal, M. Pesti. 2006. The dose-dependent H2O2 stress response promotes increased survival forSchizosaccharomyces pombe cells expressing HIV-1 Vpr. *Folia Microbiologica* **51**:5, 406-412. [CrossRef]
8. David R. Wallace. 2006. HIV Neurotoxicity: Potential Therapeutic Interventions. *Journal of Biomedicine and Biotechnology* **2006**, 1-11. [CrossRef]
9. J S Ladha, M K Tripathy, D Mitra. 2005. Mitochondrial complex I activity is impaired during HIV-1-induced T-cell apoptosis. *Cell Death and Differentiation* **12**:11, 1417-1428. [CrossRef]
10. Hong Pu, Jing Tian, Ibolya E Andras, Kentaro Hayashi, Govinder Flora, Bernhard Hennig, Michal Toborek. 2005. HIV-1 Tat protein-induced alterations of ZO-1 expression are mediated by redox-regulated ERK1/2 activation. *Journal of Cerebral Blood Flow & Metabolism* **25**:10, 1325-1335. [CrossRef]
11. Mehdi Shakibaei , Gundula Schulze-Tanzil , Yasunari Takada , Bharat B. Aggarwal . 2005. Redox Regulation of Apoptosis by Members of the TNF Superfamily. *Antioxidants & Redox Signaling* **7**:3-4, 482-496. [Abstract] [Full Text PDF] [Full Text PDF with Links]
12. ANDRAS PERL, PETER GERGELY, KATALIN BANKI. 2004. MITOCHONDRIAL DYSFUNCTION IN T CELLS OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS. *International Reviews of Immunology* **23**:3-4, 293-313. [CrossRef]
13. Risa Kurozumi, Sachiko Tokuzumi, Shuji Kojima. 2003. Does Peroxynitrite Involve in the Elevation of Cellular Glutathione Induced by Sodium Nitroprusside (SNP) in RAW 264.7 Cells?. *Biological & Pharmaceutical Bulletin* **26**:8, 1070-1075. [CrossRef]
14. Ralf Geiben-Lynn . 2002. Anti-Human Immunodeficiency Virus Noncytolytic CD8+ T-Cell Response: A Review. *AIDS Patient Care and STDs* **16**:10, 471-477. [Abstract] [Full Text PDF] [Full Text PDF with Links]
15. Thomas F. Kresina , Charles W. Flexner , Jacqueline Sinclair , Maria Almira Correia , Jack T. Stapleton , Samuel Adeniyi-Jones , Victoria Cargill , Laura W. Cheever . 2002. Alcohol Use and HIV

Pharmacotherapy. *AIDS Research and Human Retroviruses* **18**:11, 757-770. [Abstract] [Full Text PDF] [Full Text PDF with Links]

16. Andras Perl , Peter Gergely Jr , Ferenc Puskas , Katalin Banki . 2002. Metabolic Switches of T-Cell Activation and Apoptosis. *Antioxidants & Redox Signaling* **4**:3, 427-443. [Abstract] [Full Text PDF] [Full Text PDF with Links]
17. Ferenc Puskas , Peter Gergely , Brian Niland , Katalin Banki , Andras Perl . 2002. Differential Regulation of Hydrogen Peroxide and Fas-Dependent Apoptosis Pathways by Dehydroascorbate, the Oxidized Form of Vitamin C. *Antioxidants & Redox Signaling* **4**:3, 357-369. [Abstract] [Full Text PDF] [Full Text PDF with Links]
18. Sheila M. Brooke, John R. McLaughlin, Karen M. Cortopassi, Robert M. Sapolsky. 2002. Effect of GP120 on glutathione peroxidase activity in cortical cultures and the interaction with steroid hormones. *Journal of Neurochemistry* **81**:2, 277-284. [CrossRef]