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Redox Events in HTLV-1 Tax-Induced Apoptotic T-Cell Death

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ABSTRACT

A number of studies implicate reactive oxygen intermediates in the induction of DNA damage and apoptosis. Recent studies suggest that the human T-cell leukemia virus type 1 (HTLV-1) Tax protein induces oxidative stress and apoptotic T-cell death. Activation of the T-cell receptor/CD3 pathway enhances the Tax-mediated oxidative and apoptotic effects. Tax-mediated apoptosis and oxidative stress as well as activation of nuclear factor-κB can be potently suppressed by antioxidants. This review focuses on Tax-dependent changes in the intracellular redox status and their role in Tax-mediated DNA damage and apoptosis. The relevance of these observations to HTLV-1 virus-mediated T-cell transformation and leukemogenesis are discussed. *Antioxid. Redox Signal.* 4, 471–477.

INTRODUCTION

THE HUMAN T-CELL LEUKEMIA VIRUS TYPE 1 (HTLV-1) is the etiologic agent of adult T-cell leukemia (ATL), a highly aggressive T-cell malignancy (55, 75), and has been linked to the neurodegenerative disorder known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (19, 52), as well as other T-cell associated diseases (for review, see 72). Both ATL and HAM/TSP have their etiologies in unregulated activation and proliferation of CD4-positive T cells.

Only a small percentage of HTLV-1-infected individuals (3–5%) actually develop HTLV-1-related diseases after periods of clinical latency following viral infection. HTLV-1 does not encode any viral oncogenes with known homology to cellular genes. However, the 3′ terminal region of the HTLV-1 genome, the so called pX region, codes for several regulatory proteins, including Tax, Rex, and others. Tax, a 40-kDa protein mainly localized in the nucleus, has been shown to be essential for the replication of HTLV-1, because of its capacity to transactivate the proviral transcription from the 5′ long terminal repeat. Importantly, this trans-acting viral factor also acts as a transcriptional regulator of cellular gene expression. Such a function confers a pivotal role to Tax in the viral pathogenicity (56). Tax is able to transcriptionally activate the expression of cellular genes involved in growth and proliferation, and to repress the expression of tumor suppressor genes (66, 74). Tax exerts these effects by interacting with cellular transcription factors, such as cyclic AMP responsive element-binding protein (CREB), nuclear factor-κB (NF-κB), and serum responsive element-binding factor, and with the transcriptional coactivators CBP (CREB-binding protein)/p300 (66, 73, 74). Finally, Tax promotes cell-cycle progression in infected cells by interacting with negative regulators of cyclin-dependent kinases, inhibitors of CDK4 (p16INK4a and p15INK4b) (62, 74). The pleiotropic properties of Tax allow this viral protein to favor immortalization/transformation of infected cells, and cause the onset of the HTLV-1-associated diseases (18, 56, 74).

Several studies have shown that persistent Tax expression is associated with apoptosis (7, 10, 11, 21, 35, 40, 50, 57, 71). As such, Tax resembles functionally other viral and cellular oncogenes, which aside from their inherent transforming properties have the ability to induce programmed cell death. Apopto-

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sis-promoting properties were reported for other viral oncogenes, like adenovirus E1A, simian virus 40 large tumor antigen, and the human papilloma virus E7 proteins (36, 64, 69). This paradoxical observation is consistent with the hypothesis that genetic defects are required to support tumor growth.

Apoptosis is an active physiological process that plays an essential role during tissue development, and may be important for the elimination of virus-infected or potentially cancerous cells. Accumulating evidence indicates that the process of oncogenesis in part reflects the outcome of an imbalanced cell death and proliferation. In this context, the elucidation of mechanisms underlying oncogene-associated cell death may help to identify the genetic events that are required for the transformation process.

The present review summarizes reports concerning the apoptotic action, as well as potential antiapoptotic effects of Tax. These observations will be discussed in the general context of the contribution of Tax to HTLV-1-mediated T-cell immortalization and ATL.

OXIDATIVE STRESS AS A MEDIATOR OF TAX-INDUCED APOPTOSIS

Oxidative stress is the result of a disturbance in the balance between prooxidants and antioxidants in favor of the prooxidants, namely reactive oxygen intermediates (ROIs) and free radicals. Excessive formation of ROIs, as well as the depletion of cellular antioxidants, can result in apoptosis (6, 22). Cell death is caused by the detrimental effects of ROIs, which lead to lipid peroxidation, protein denaturation, and DNA damage. ROIs, however, may not always induce apoptotic cell death. Several lines of evidence indicate that intracellular ROIs, which are insufficient to induce cellular damage, may play a physiological role as second messengers by regulating gene expression through activation of important immunoregulatory transcription factors (2). A subset of such genes may be involved in promoting active cell death, whereas others may be involved in normal functions of the cell. Two well characterized transcriptional complexes that are regulated by redox-dependent processes are NF-κB and activator protein-1 (2, 29, 48).

Tax is a very potent transcriptional activator of NF-κB, a prooxidant inducible nuclear transcription factor. The use of antioxidants provided the first indication for involvement of oxygen radicals in HTLV-1 Tax-induced effects. The radical scavenger pyrrolidine dithiocarbamate (PDTC) strongly suppressed the Tax-induced activation of the DNA-binding activity of NF-κB in transient transfection assays in Jurkat cells (60). Moreover, PDTC and other antioxidants strongly interfered with transactivation of the long terminal repeat of the human immunodeficiency virus type 1 (HIV-1) by Tax (60).

Direct evidence for the production of ROIs by Tax was provided in Jurkat cells expressing a conditional version of Tax, which was generated by the fusion of the HTLV-1 Tax protein and the hormone-binding domain of the estrogen receptor (ERTax). Using this posttranslationally inducible ERTax system for hormone-dependent activation of Tax, it was shown that induction of oxidative stress was an immediate effect of Tax function (40). Activation of the ERTax fusion protein resulted in a rapid (15-min postinduction) drop in the intracellular amount of glutathione (GSH) and in considerably reduced levels within 2 h after Tax activation. A slow recovery of the GSH levels approaching the levels seen for control cells was seen at 5 days after the beginning of hormone treatment. ERTax cells cultured in the presence of hormone for a prolonged period of time (1 month) showed normal levels of GSH (40). In addition, the intracellular hydrogen peroxide levels were measured in this study to confirm the above observations. Hormone-treatment of ERTax Jurkat cells for 2 h led to a marked increase in the intracellular levels of hydrogen peroxide and a concomitant decrease of GSH. Tax and CD3/T-cell receptor (TCR)-mediated signals synergized in inducing a prooxidant state. Treatment of the cells with anti-CD3 monoclonal antibody and hormone led to an additive enhancement of intracellular hydrogen peroxide levels in ERTax Jurkat cells. The combination of both signals resulted in sustained and long-lasting (for several days) prooxidant effects (40).

Various defense mechanisms have evolved to protect cells against the harmful effects of ROIs. Intracellular redox homeostasis is regulated by thiol-containing molecules, such as GSH and adult T-cell leukemia-derived factor/thioredoxin (ADF/TRX). GSH and ADF/TRX systems work in concert and primarily contribute to create an intra- and extracellular reducing environment. Physiologically, thioredoxin has cytoprotective effects against oxidative stress (63). GSH, a cysteine-containing tripeptide, is the major source for intracellular free thiols and an important antioxidant (15, 44). The Tax-induced prooxidant state was inhibited by pretreating the cells with the physiologic antioxidative protein ADF/TRX or with PDTC (40).

Interestingly, Tax induces the transcription and synthesis of ADF/TRX (42). ADF/TRX is a potent antioxidative protein, first derived from ATL cells and shown to be homologous to human thioredoxin. ADF/TRX is a stress-inducible protein that is secreted from cells. This protein plays an essential role in cellular protection against oxidative stress and cell death, and is itself induced by various oxidative agents (28, 49, 63). ADF/TRX is particularly important for gene expression as it facilitates protein–nucleic acid interactions by reducing cysteine in the DNA-binding loop of several transcription factors (43, 51). Depending on the cell type, ADF/TRX can either activate or inhibit NF-κB (25, 43, 59). Recent studies have shown that ADF/TRX up-regulates DNA binding of activated NF-κB *in vitro* and enhanced κB-dependent gene expression *in vivo* (24, 51). In the cytoplasm, ADF/TRX interferes with the signals to IκB kinases and blocks the degradation of IκB. In the nucleus, however, ADF/TRX enhances NF-κB transcriptional activities by enhancing its ability to bind DNA (25).

Apoptotic T-cell death was the consequence of these early Tax-mediated prooxidant effects. Oxidative stress plays a crucial role in the induction of T-cell apoptosis by Tax, because Tax-induced apoptosis could be inhibited by pretreating these cells with antioxidants (40). ADF/TRX could block the Tax-induced apoptotic effect. To what extent induction of ADF/TRX is a prerequisite in the transformation process of HTLV-1 by inhibiting the Tax-mediated apoptotic effects needs to be determined. Further investigations are necessary to clarify the actual role of this reducing catalyst in the leukemogenesis process.

The induction of oxidative stress is a common effect shared by other apoptosis-inducing viral proteins, like HIV-1 Tat (16, 68). Tat, like Tax, is a potent inducer of CD95 ligand (CD95L) (67) and has been reported to induce oxidative stress and subsequent NF-κB activation through the down-regulation of the mitochondrial antioxidant enzyme manganese superoxide dismutase (Mn-SOD). Decreased Mn-SOD expression was associated with decreased levels of GSH (16, 68).

Nitric oxide is a highly reactive radical, known to function as a signaling mediator. Interestingly, Tax induced activation of the human inducible nitric oxide synthase (hiNOS), and expression of hiNOS was observed in HTLV-1-infected T-cell lines, as well as primary ATL cells (47). The potential role of such radicals in the pathogenesis of HTLV-1-associated diseases is not known and would need further investigations.

TAX-INDUCED APOPTOTIC CELL DEATH

Tax-mediated induction of apoptotic cell death was first observed in studies using inducible forms of Tax. Experiments with Jurkat T cells stably expressing hormone-inducible forms of Tax (ERTax) showed estradiol-inducible Tax activity upon short-term exposure to hormone. Hormone-dependent activation of Tax resulted in an inhibition of proliferation and in an induction of apoptotic cell death (activation-induced apoptotic T-cell death) besides the promotion of T-cell activation events (10). The antiproliferative effects were dependent upon the duration of ERTax activity and were significantly enhanced when the CD3/TCR complex was simultaneously activated (10). The Tax-induced proapoptotic effects in Jurkat cells were confirmed using another inducible system where *tax* expression was up-regulated by heavy metal ions (7). Moreover, retrovirus-mediated expression of Tax in Jurkat cells resulted in massive cell death within a few days in culture (57).

Induction of programmed cell death by Tax was also shown in nonlymphoid cells. Apoptosis was observed in Tax-transformed Rat-1 fibroblasts after serum starvation, and was blocked by Bcl-2 (71). Tax was shown to activate NF-κB and subsequently tumor necrosis factor-α (TNF-α) production, leading to apoptosis of murine clonal osteoblasts (35). Extracellular Tax induced human neuronal cells to produce TNF-α *in vitro* (13). Moreover, Tax induced programmed cell death in HeLa cells. Expression of the CBP/p300-binding domain of Tax in these cells triggered an apoptotic death-inducing signal, which was blocked by Bcl-2 or by ectopic expression of the p300 coactivator (50).

Support for the Tax-induced proapoptotic effects *in vivo* also came from the finding that Tax expression was closely associated with apoptosis in tumors of HTLV-1 Tax transgenic mice (21). Moreover, thymuses from mature outbred rabbits lethally inoculated with an HTLV-1-infected T-cell line showed morphological and biochemical evidence of apoptosis (37).

Recent studies showed that cysteine proteases that serve as common intracellular mediators of apoptosis are mediating the Tax-induced cell death. Indeed, Tax-induced apoptosis requires caspase activation and is prevented by Bcl-2 expression (7, 11, 50, 57, 71). Noteworthy, Bcl-2 acts in an antioxidant way to prevent apoptosis (26). Tax activity induced TNF-α, CD95L, and TNF-related apoptosis-inducing ligand (TRAIL) gene expression in Jurkat cells (7, 11, 57). All these ligands are known to induce cell death by binding to their respective death receptors on target cells. Recent evidence suggests that Tax-induced T-cell death is largely mediated by TRAIL (57). Moreover, Tax activity induced TR3/Nur77 nuclear transcription factor gene expression in Jurkat cells, and transcripts of this gene are highly expressed in HTLV-1-infected cell lines (8). This molecule is also involved in the induction of apoptotic T-cell death (9, 39, 70). In response to apoptotic stimuli, TR3/Nur77 translocates from the nucleus to mitochondria to induce cytochrome *c* release and apoptosis. Mitochondrial targeting of this factor is essential for its proapoptotic effect (38). To what extent the TR3/Nur77 pathway represents another apoptotic mechanism triggered by Tax needs to be determined.

A number of studies point to a direct association between Tax activity and the accumulation of DNA damage that results in apoptotic death (41, 45, 56). Tax-expressing cells displayed reduced DNA repair activity and showed increased DNA damage followed by apoptotic cell death (32, 33). Further evidence includes induction of micronuclei in Tax-transfected cells (41, 58) and enhanced mutation frequency of the cellular genome in Tax-expressing cells (45). Tax activity repressed the expression of the human β-polymerase gene, an important cellular DNA repair enzyme (30). In addition, a direct effect of Tax on DNA repair was the Tax-mediated suppression of cellular nucleotide excision repair and base excision repair (32, 54). This inability to repair DNA damage correlated with apoptotic cell death. Tax-expressing cells displayed increased cell death following DNA damage (33). A functional p53 signaling pathway could rescue Tax-mediated suppression of DNA repair (34). These reports are consistent with the observations that HTLV-1-transformed cells exhibit chromosomal abnormalities (17, 31). The DNA damaging and mutagenic properties of Tax may favor the induction of genetic changes that are required for tumor initiation and progression. Remarkably, ADF/TRX prevented the DNA damaging effect of ultraviolet A radiation (14). Further studies should address the role of ADF/TRX in prevention of the DNA damage induced by ROIs.

TAX-INDUCED NF-KB ACTIVATION AND APOPTOSIS

NF-κB is a key regulator of Tax-mediated effects. Observations made by several groups point to the importance of Tax-mediated NF-κB responses for the induction of apoptosis (7, 11, 40, 50, 57). The aberrant, persistent activation of NF-κB transcription by Tax exerts a proapoptotic function. Indeed, Tax mutants defective in NF-κB activation exhibited reduced apoptosis-inducing activities, and inhibition of Tax-mediated NF-κB transactivation partially inhibited cell death (40, 50, 57). NF-κB plays either an antiapoptotic or a proapoptotic role, depending upon the cell type, nature of stimulation, the Rel-related subunit composition, and the du-

ration of the NF-κB response (3, 4). Tax expression is known to result in prolonged induction of NF-κB-dependent transcription through the targeted activation of IκB-kinase complex components and constitutive phosphorylation or degradation of IκBα and IκBβ (61). Noteworthy, long-term expression of Tax in Jurkat cells (>30 days) has led to selection of clones that could no longer respond to NF-κB-inducing signals. This apparent unresponsiveness was due to selective expansion, by virtue of their growth advantage, of cells that have lost Tax activity. No obvious changes in the inherent properties of the cells could be detected, as a new transfection with ERTax was able to reestablish hormone-dependent Tax-induced NF-κB activation (40). It is assumed that T cells may not be able to tolerate the intracellular constitutive expression of Tax for prolonged time periods. Whether this intolerance is related to the apparent shutdown of Tax expression in HTLV-1-infected leukemic cells needs to be determined.

Interestingly, constitutive activation of NF-κB was detected in primary ATL cells despite the lack of detectable Tax expression (46). It has been suggested that Tax-independent mechanisms operate in ATL cells to promote constitutive NF-κB activation and overexpression of cellular genes (46). However, transient Tax activity in earlier phases might be important for the constitutive activation of NF-κB in ATL cells. With regards to the sensitivity of Jurkat cells and resistance of ATL cells to apoptosis, it is not known how the activated NF-κB subunits in the two situations may resemble or differ from one another. Two possibilities to consider are: (a) the activated NF-κB complex in ATL cells and Jurkat cells may be composed of different subunits, and therefore have different effects on cell survival; and (b) additional genetic alteration in ATL cells may render these cells resistant to the apoptotic consequences of the constitutively active NF-κB.

Figure 1 shows all the known pathways involved in HTLV-1 Tax-induced apoptosis.

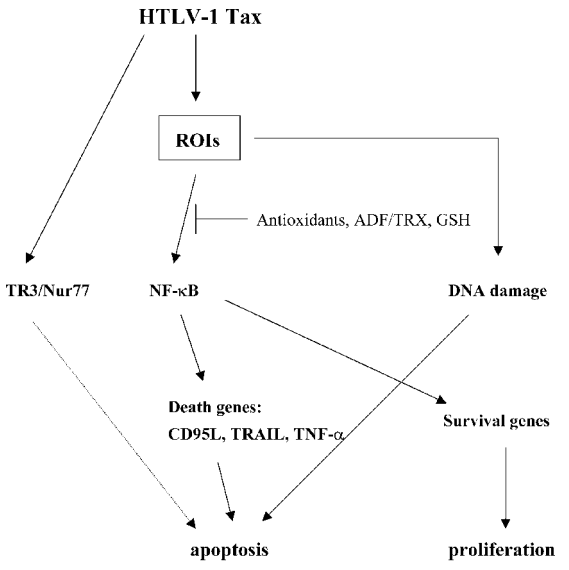

FIG. 1. Schematic representation of the pathways involved in HTLV-1 Tax-induced apoptosis (ROIs, reactive oxygen intermediates).

OXIDATIVE STRESS, APOPTOSIS, AND LEUKEMOGENESIS

Expression of Tax has been suggested to be sufficient for immortalizing human T-lymphocytes *in vitro*. Direct evidence for this notion comes from lines of studies. Defective Herpesvirus saimiri recombinants encoding Tax were used to immortalize primary human $CD4^+$ cord blood lymphocytes in culture (20). A single report documented transduction and immortalization of human peripheral blood lymphocytes with retroviral vectors expressing HTLV-1 Tax alone (1). The ability of Tax to immortalize primary T cells may suggest a potential antiapoptotic effect of Tax, which would be in agreement with other reports (5, 12, 65). Tax is able to prevent apoptosis through activation of NF-κB and induction of Bcl-x(L) expression, or repression of Bax gene expression. However, it cannot be excluded that immortalization of primary or cord blood T cells with *tax* using retroviral or Herpesvirus saimiri vectors involved further viral or cellular genes. The failure of many attempts to immortalize human lymphocytes through the expression of *tax* and the difficulties to achieve stable expression of this oncoprotein pointed to the antiproliferative effects of this molecule. Several studies have demonstrated over the last years a potent apoptosis-inducing activity of HTLV-1 Tax (7, 10, 11, 21, 35, 40, 50, 57, 71).

Abundant evidence suggests that Tax expression may not be necessary for the maintenance of the ATL neoplastic state, even though infection of the target cell by HTLV-1 is a prerequisite for the leukemia. How are the Tax-induced redox events linked to the HTLV-1-induced leukemogenesis? Can these events initiate changes that eventually transform T cells and promote ATL?

A striking feature of HTLV-1-infected lymphocytes *in vivo* is the absence of detectable viral gene expression. Tax can be detected only by PCR in <5% of ATL cells. This may be related to the antiproliferative properties of Tax, as well as the highly immunogenic nature of the protein (23; for review, see 53). The Tax protein is important for the T-cell immortalizing properties of the virus *in vitro* and is likely to be responsible for the early stages of leukemogenesis in infected hosts. Increases in Tax protein levels in HTLV-1-infected cells may occur in response to T-cell activation. This can lead to bursts of viral gene expression, followed by apoptotic cell death or clearance by immune responses. Selective pressures for reduced levels of Tax expression may contribute to viral latency, allowing infected cells to evade host immune surveillance mechanisms.

The late onset of many cancers, including ATL, may be due to the requirement for a genetic selection process, whereby neoplasia is initiated in a clonal or oligoclonal manner. Changes in the intracellular redox status may be a critical factor in Tax-mediated DNA damage in HTLV-1-infected cells. Oxidative stress and DNA damage can contribute to the initiation of the transformation process by subjecting infected T cells to appropriate genetic changes that eventually promote the neoplastic state. Apoptosis and immune surveillance would then exert the necessary selection pressure for eliminating genetically healthy cells that would otherwise proliferate and expand by virtue of viral infection. The immune and apoptosis escape variants would constitute a subpopulation of

genetically altered cells, prone to neoplasia. In this context, one could postulate that the antiapoptotic effects of ADF/TRX may act to delay the total elimination of the infected cells, fine tuning the balance of survival in favor of selection for transformation. More studies need to elucidate the role of ADF/TRX in the later stages of leukemia.

CONCLUDING REMARK

Recent investigations implicate important roles for oxidative stress and DNA damage in the HTLV-1 Tax-induced apoptotic T-cell death. Induction of oxidative stress is one of the first events of Tax action. These findings envisage new possibilities for the contribution of Tax to the mechanism of leukemia and transformation. Tax leads to T-cell activation and activation-induced apoptotic T-cell death. Tax induces DNA damage and enhances mutation frequency. At the same time, Tax is highly immunogenic, and therefore Tax-expressing cells are kept under control by the immune system. Dividing cells are in any case more prone to replication errors. There may be a specific selection for dividing cells that no longer express Tax, but have undergone cellular genetic events that promote leukemia. Thus, Tax function seems to be necessary for the initiation of the transformation events, establishing a state that predisposes cells to somatic mutations associated with the neoplastic state.

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ABBREVIATIONS

ADF/TRX, adult T-cell leukemia-derived factor/thioredoxin; ATL, adult T-cell leukemia; CBP, CREB-binding protein; CD95L, CD95 ligand; CREB, cyclic AMP responsive element-binding protein; ERTax, fusion of HTLV-1 Tax protein and hormone-binding domain of estrogen receptor; GSH, glutathione; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; hiNOS, human inducible nitric oxide synthase; HIV-1, human immunodeficiency virus type 1; HTLV-1, human T-cell leukemia virus type 1; Mn-SOD, manganese superoxide dismutase; NF-κB, nuclear transcription factor-κB; PDTC, pyrrolidine dithiocarbamate; ROIs, reactive oxygen intermediates; TCR, T-cell receptor; TNF-α, tumor necrosis factor α; TRAIL, TNF-related apoptosis-inducing ligand.

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