

Modulation of Nuclear Factor- κ B by Human T Cell Leukemia Virus Type 1 Tax Protein

Implications for Oncogenesis and Inflammation

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Abstract

Activation of the nuclear factor kappa B (NF- κ B) transcription factor family by different stimuli, such as inflammatory cytokines, stress inducers, or pathogens, results in innate and adaptive immunity. While the main function of NF- κ B is to promote the host's immune response, the NF- κ B pathway is frequently dysregulated by invading viral pathogens. Human T cell leukemia virus type 1 (HTLV-1) is the causative agent of a fatal malignancy known as adult T cell leukemia (ATL) and an inflammatory disease named tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM). HTLV-1 encodes an oncoprotein, Tax, which plays a significant role in the initiation of cellular transformation and the elicitation of the host's inflammatory responses. Here, we review current thinking on how Tax may affect both diseases through activation of NF- κ B signaling.

Key Words

Human T cell leukemia virus
(HTLV-1)
Adult T cell leukemia (ATL)
HTLV-1 Tax
NF- κ B
IKK
NIK
Inflammation

Introduction

Many of the molecular alterations associated with carcinogenesis occur in cell signaling pathways that regulate cell proliferation and differentiation. HTLV-1 is the etiological agent for ATL, an aggressive human T-cell malignancy, and for inflammatory pathologies variously named as HTLV-1 associated myelopathy or tropical spastic paraparesis (HAM or TSP)

(1–4). ATL develops in 2–5% of HTLV-1 infected individuals after a prolonged disease-free period (5,6). This long latency for ATL suggests that multiple discrete events in a normal cell are subverted by HTLV-1 in the virus process of cellular transformation. How HTLV-1 transforms T cells remains incompletely understood. However, the expression of virus-encoded protein early in infection likely plays an important role (7–9).

HTLV-1 encodes several regulatory proteins in its pX region located between Env and the 3' LTR; these proteins are translated from four partially overlapping open reading frames (ORF) including p12^I (ORF-1), p13^{II} (ORF-2), p30^{II} (ORF-3), Rex (ORF-3), and Tax (ORF-4) (10). Among these proteins, Tax is a potent transcriptional activator that drives the transcription of all HTLV-1 transcripts from the viral LTR. Tax activates LTR-directed transcription by recruiting members of the CRE-binding/activating transcription factors (CREB/ATF) family to the viral promoter (11). In addition, Tax activates other cellular transcription factors such as nuclear factor kappa B (NF- κ B) and activator protein-1 (AP-1), promoting accelerated cell proliferation and cell survival (12).

There is conclusive evidence including results from transgenic mice that the 40-kDa nuclear HTLV-1 Tax protein is the entity responsible for cellular transformation (8,13,14). When expressed singularly, Tax is sufficient to immortalize primary human T lymphocytes and to transform rodent cells (9,15). In part, cellular transformation by HTLV-1 is explained by Tax's ability to deregulate cellular signaling pathways and perturb cellular gene expression (16). Tax can also override normal cell cycle controls via multiple means including transcriptional activation and direct binding to cell cycle regulators (17). Specifically, Tax upregulates the expression of several cyclins (cyclin C, D2, and E) and CDK inhibitors such as CDK2, CDK4, and p21^{Waf1/Cip1} (8). In addition, HTLV-1 infected cells avoid growth arrest and/or apoptosis because Tax inhibits the functions of the p53 tumor suppressor (18). *En toto*, Tax utilizes multiple mechanisms from activation of pro-proliferation genes to dysregulation of checkpoints and DNA damage repair pathways to influence genomic instability, which may form the basis for transformation (13). In

this review, we limit our focus to the role of Tax-activation of NF- κ B and discuss, in a non-exhaustive manner, how this activity relates to oncogenesis and inflammation.

The NF- κ B Pathways

First described over 20 yr ago, the nuclear factor kappa B (NF- κ B) family of transcriptional factors comprises a group of important regulators of innate and adaptive immune response (19,20). NF- κ B promotes the expression of well over 100 target genes, including cytokines and chemokines, receptors for immune recognition, and proteins involved in antigen presentation (13,21,22). NF- κ B is viewed as a central mediator of immune responses (20,23). Gene knockout studies have established roles for NF- κ B in the ontogeny of the immune system and at multiple steps during normal and abnormal cellular proliferation as well as in cell survival and programmed cell death (24–28). Perhaps because it sits at the nexus of multiple pathways, NF- κ B is frequently targeted by viral pathogens that infect cells. Indeed, many viruses exploit NF- κ B in order to enhance viral replication, host cell survival, and evasion of immune responses.

The NF- κ B family contains five members: NF- κ B₁ (p105/p50), NF- κ B₂ (p100/p52), RelA (p65), RelB, and cRel (Fig. 1) (19,29,30). RelA, RelB, and cRel are expressed as transcriptionally active proteins, whereas NF- κ B₁ and NF- κ B₂ are synthesized as precursor proteins that are processed to counterpart smaller, transcriptionally active subunits, p50 and p52, respectively (19,29,30). NF- κ B members have an approx 300-amino-acid Rel-homology domain (RHD) at their amino-termini that contain sequences used for dimerization, DNA binding, nuclear transport (NLS), and interaction with the cytoplasmic inhibitory I κ B proteins (Fig. 1) (19,30,31). With the exception of

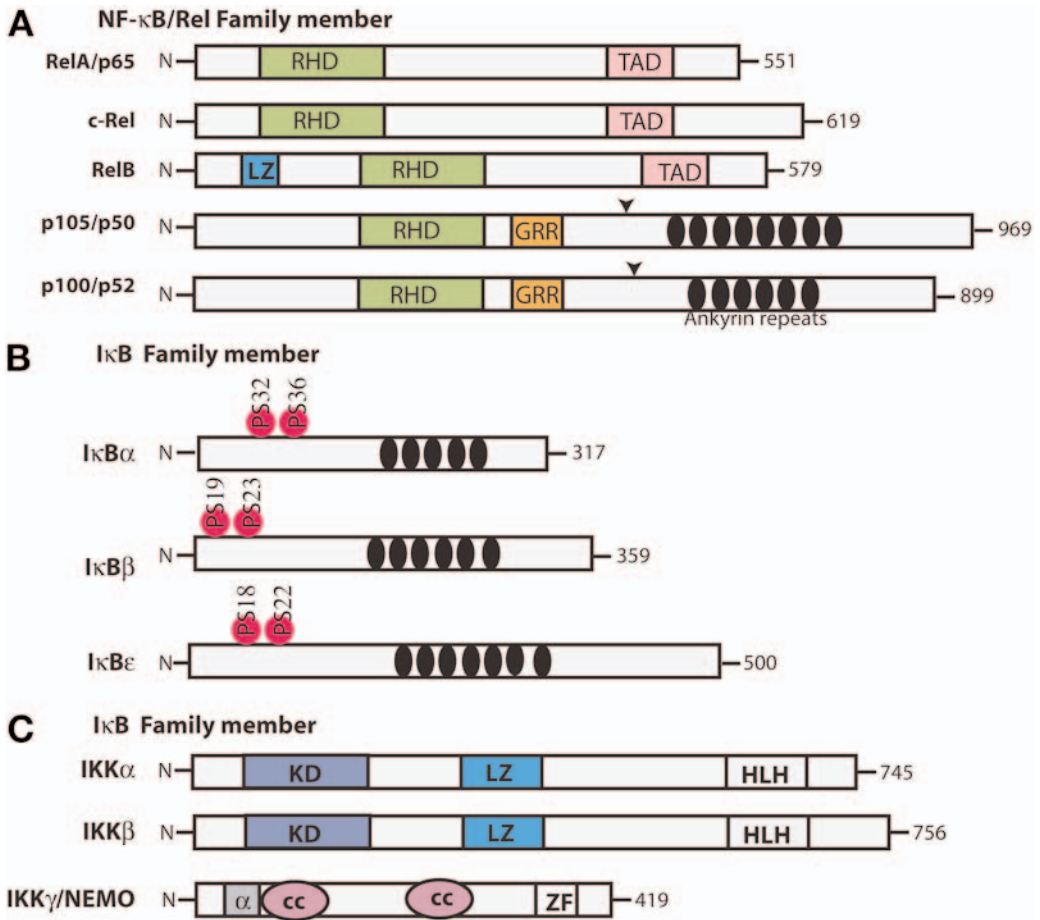


Fig. 1. Schematic representations of NF-κB and IκB family members. (A) NF-κB family members are characterized by a Rel Homology Domain (RHD) important for DNA binding, dimerization, and nuclear localization. p105 and p100 are processed by the proteasome (processing site is indicated by arrow) to give rise to the NF-κB proteins p50 and p52 respectively. (B) IκB family contains a series of ankyrin repeats that allow for interaction with the RHD of NF-κB. (C) IKKα (IKK1) and IKKβ (IKK2) contain N-terminal kinase domains, central leucine zippers, and a C-terminal helix-loop-helix domain. IKKγ/NEMO contains an N-terminal α-helix (α), two coiled-coil domains, and a C-terminal zinc finger. Transactivation domain (TAD), leucine zipper (LZ), glycine rich region (GRR), leucine zipper (LZ), helix-loop-helix (HLH), coiled-coil (CC), and zinc finger (ZF).

RelB, NF-κB proteins form homo- and heterodimers when activated. Examples include a p50/52 heterodimer, which lacks transcriptional activation domains, and a p50/p65 heterodimer, which is transcriptionally competent.

NF-κB is inactive in the cytoplasm of latent or unstimulated cells because of its

association with inhibitors of kappa B (IκB) proteins (Fig. 2) (21,29,32). IκB proteins include IκBα, IκBβ, IκBε, Bcl-3, and C-terminal subunits of p105 (NF-κB₁) and p100 (NF-κB₂) (32,33). IκB proteins conserve ankyrin-like repeat domains, which regulate the subcellular localization of Rel-NF-κB

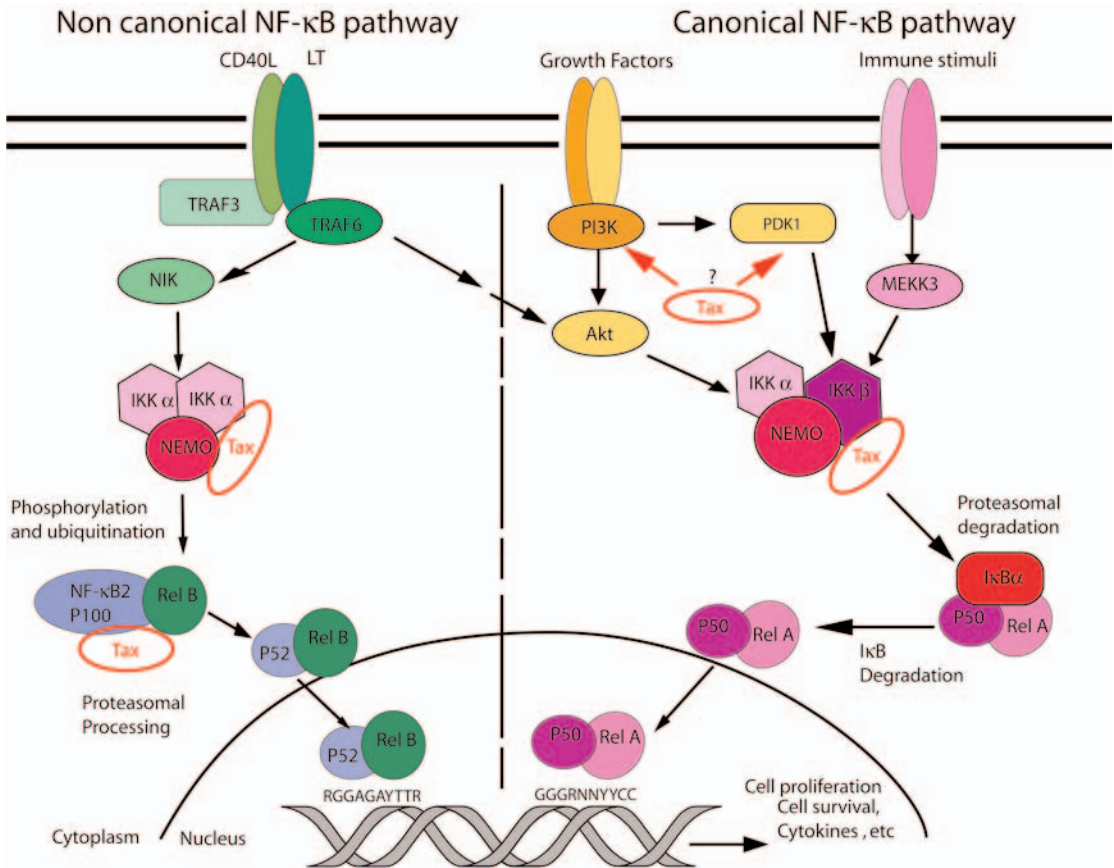


Fig. 2. Mechanisms of Tax activation of the canonical and non-canonical NF- κ B pathways. The canonical pathway (right) consists of IKK-mediated phosphorylation, ubiquitination, and degradation of I κ B proteins leading to ubiquitination and degradation by the proteasome, and the nuclear translocation of RelA and c-Rel-containing heterodimers. Tax activates the canonical pathway through its interaction with IKK γ . Tax may recruit upstream activators to trigger IKK activation such as PI3K. The non-canonical pathway (left panel) consists of NIK and IKK α -mediated p100 processing and nuclear mobilization of RelB/p52 heterodimers. Tax triggers activation of this NIK-downstream pathway by activating and recruiting IKK α to p100 stimulating phosphorylation, ubiquitination, and processing to p52.

proteins by masking the NLS, located in the latter's RHD (34–37). The I κ B proteins show different affinity and specificity for NF- κ B dimers. For example, I κ B β inhibits the p50/p65 heterodimer more efficiently than the p50/RelB or p50/cRel complexes. By contrast, I κ B α recognizes all three complexes similarly.

Cytoplasmic NF- κ B complexes can be activated by a variety of stimuli, including

viral and bacterial pathogens, cytokines, and stress-inducing agents, through two discrete signaling routes: the canonical and non-canonical pathways (Fig. 2) (21,29,32). The canonical pathway is governed by an I κ B kinase (IKK) complex that phosphorylates I κ B molecules (32,38). The IKK complex consists of three subunits: the enzymatic IKK α , IKK β , and the regulatory IKK γ (also termed the NF- κ B essential modulator

[NEMO]) subunit (39–44). IKK phosphorylates specific serines (S32 and S36) in the N-terminus of I κ B, targeting the phosphorylated moieties for ubiquitination and proteasomal degradation and compelling I κ B molecules to release otherwise sequestered NF- κ B proteins. After being activated, NF- κ B translocates to the nucleus, where it stimulates transcription of genes containing the consensus sequence 5'-GGGRNNYYCC-3' (R is a purine, Y is a pyrimidine, and N is any nucleic acid) (Fig. 2) (45).

The non-canonical pathway does not require the complete IKK complex but does utilize IKK α and the NF- κ B inducing kinase (NIK) (46,47). In this pathway, unprocessed full-length NF- κ B2 (p100) can dimerize with RelB and act as a cytoplasmic I κ B molecule preventing the nuclear translocation of RelB. In this setting, proteolytic processing that cleaves the ankyrin repeats removes p100's I κ B function while generating an active RelB/p52 complex, which can then translocate into the nucleus (Fig. 2) (46,47). RelB/p52 dimers recognize a novel NF- κ B sequence (5'-RGGAGAYTTR-3', where R is a purine and Y is a pyrimidine) (48). In general, the canonical pathway is considered to be important for the generation of inflammatory and adaptative immune responses, whereas the non-canonical NF- κ B pathway largely serves specific cell types such as B lymphoid stromal cells and contributes to B-cell maturation.

HTLV-1 Co-opts the Cell's Canonical NF- κ B Pathway

Canonical NF- κ B activation in T cells is generally transient. Brief NF- κ B activation required for proliferation and survival of stimulated T-cells is achieved through strictly controlled feedback regulation. Hence, upon stimulation by antigen, the T-cell receptor

(TCR) and its proximal signaling molecules are downregulated to prevent sustained signaling through the cell surface receptor. Furthermore, activation of NF- κ B induces the expression and *de novo* synthesis of I κ B α which can enter the nucleus and halt NF- κ B's transcriptional function. In distinction to its strict physiologically regulated control, the NF- κ B family of transcription factors are known to be constitutively activated in various human malignancies, including leukemias (49), lymphomas (50,51), and solid tumors (52). Of relevance to this review, HTLV-1 transformed T cell lines and freshly isolated ATL cells both show persistently unchecked activation of NF- κ B (53,54). In transformed cell lines, Tax is the intracellular NF- κ B inducer. However, in cells from ATL patients, NF- κ B remains activated despite the eventual shut-down of Tax expression, suggesting that Tax may be needed to initiate but not to maintain NF- κ B activation (55). In a recent study, Higuchi et al. (56) proposed that CD30 can play a role in Tax-independent activation of NF- κ B. CD30 is a member of the tumor necrosis factor (TNF) receptor superfamily and is used as a marker of malignancy in Hodgkin's lymphoma (HL) (57). Overexpression of CD30 in HL cell contributes to constitutive NF- κ B activation in these cells. Similar to HL cells, ATL cells line and fresh ATL cells also show elevated expression of CD30 (56).

Different models have been proposed to explain how Tax activates NF- κ B. Initially, it was thought that Tax directly interacted with a latent NF- κ B/I κ B α complex to trigger activation (58). Following the identification of IKK-mediated phosphorylation of I κ B α , it was reconsidered that Tax might activate NF- κ B by signaling through the IKK complex (58). Indeed, inside cells, expression of Tax is closely followed first by the phosphorylation and then the degradation of I κ B α and I κ B β

(58). A clue to how Tax interdigitates with the IKK complex came from the unexpected finding that this viral oncoprotein failed to activate NF- κ B in either IKK γ ^{-/-} knocked-out mouse embryonic fibroblasts or in mutant Jurkat T-cells inactivated for IKK γ (59). A mechanistic explanation was offered by the discovery that Tax physically associates with IKK γ in mammalian cells (58,60), and that Tax/IKK association causally activated NF- κ B (61).

Extensive mutagenesis studies have defined several Tax point mutants that cannot activate NF- κ B (62,63). When investigated further, it was verified that Tax point mutants inactive for NF- κ B were also unable to bind IKK γ (9,64). Tax was found to associate directly with the 201–250 region in IKK γ (60,61). Interestingly, while IKK γ 's function is also triggered by proinflammatory cytokines (60,61), such as TNF α , this latter activity maps to a C-terminal zinc finger domain in IKK γ separate from its Tax-relevant site. Thus, signaling through IKK γ by viral oncoprotein or cytokine appears to be discrete and independent (60).

Recent studies by Jeong et al. (65) and Tanaka et al. (66) provide insights into the mechanism employed by Tax to activate NF- κ B using kinase(s) upstream of IKK. In this regard, Tax appears to interact with an upstream kinase in the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. The PI3K/Akt signaling pathway is activated by numerous growth-factor and immune receptors and regulates fundamental cellular functions such as transcription, translation, proliferation, growth, and survival (67,68). Akt, also called protein kinase B (PKB), is a Ser/Thr kinase bearing some homology with protein kinase C (PKC) and protein kinase A (PKA). Akt is the cellular homolog of the viral oncoprotein *v*-Akt, which is responsible for a subpopulation of leukemias in mice (69). Activated Akt modulates the function of

numerous substrates related to cell proliferation such as glycogen synthase kinase-3 (GSK-3), cyclin-dependent kinase inhibitors P21^{Waf1/Cip1} and P27^{Kip2}, and mammalian target of rapamycin (mTOR) (67,68). Akt also phosphorylates and activates IKK α , which, in turn, phosphorylates I κ B α , targeting it for degradation (70). Perturbation of the PI3k/Akt pathway has been associated with the development of diseases such as cancer, diabetes, and autoimmunity (67,71,72). Recently, Jeong et al. (65) suggested that Akt is activated in HTLV-1-transformed cells and that NF- κ B activation is linked to Tax activation of Akt. Interestingly, inhibition of endogenous Akt with the PI3K/Akt inhibitor LY294002 or anti-Akt siRNA in Tax-expressing cells not only prevented NF- κ B activation but also impaired p53 regulation. These findings suggest that Akt plays a role in the activation of pro-survival pathways in HTLV-1-infected cells, possibly through NF- κ B activation and inhibition of p53. Interestingly, Tanaka et al. (66) showed that independent of Akt activation, the 3-phosphoinositide-dependent protein kinase-1 (PDK1) directly phosphorylates IKK β at Ser 181 to induce NF- κ B activation.

Direct phosphorylation of p65 in response to proinflammatory cytokines and Tax provides another way for NF- κ B activation. O'Mahony et al. (73) showed that IKK phosphorylates p65 on S529 and S536 in response to TNF α treatment. Tax expression also enhances IKK α -mediated p65 phosphorylation. Phosphorylation of Ser536 is required for a complete NF- κ B-response to Tax, whereas phosphorylation of Ser529 appears to be less important for Tax responsiveness (73). Thus, Tax can not only modulates upstream degradation of I κ B in the cytoplasm, but can also facilitate transcriptionally proximal nuclear modifications of p65 to maximize the activation of NF- κ B target genes.

Tax and the Non-canonical NF- κ B Pathway

Unlike the canonical pathway, non-canonical NF κ B activation occurs in B cells and lymphoid stromal cells. Until now, a very limited number of p100 processing inducers [lymphotoxin beta (LT- β) (47,74), B-cell activating factor (BAFF) (75,76), and CD40 ligand (77)], tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) (78) and receptor activator of NF kappa B ligand (RANKL) (79) have been identified. Ligation of these inducers to their cognate receptors activates NF κ B-inducing kinase (NIK). Activated NIK triggers IKK α kinase activity by phosphorylating the latter's activation loop (80). In addition, NIK also functions as an adaptor molecule that enhances the binding of IKK α and p100 (80). Once bound to p100, activated IKK γ phosphorylates S99, S108, S115, S123, and S872 of p100 facilitating β -transducin repeat-containing protein (β -TrCP)-mediated ubiquitination and proteasome-mediated degradation (81). The newly formed p52/RelB dimer then translocates into the nucleus and activates expression of target genes such as chemokines BLC, ELC, and SDF-1 (48).

Under physiological conditions, processing of p100 is tightly regulated. This is reflected by the low ratio of p52 to p100 in resting cells. Dysregulated expression of p52 leads to severe abnormalities in lymphoid development (75–77). Moreover, constitutive processing of p100 has been linked to various lymphomas and leukemia (82–85), including ATL (86,87). It has been suggested that T-cell transformation by HTLV-1 may correlate with inappropriate induction of p100 processing by Tax (86). Tax physically interacts with two short amino-terminal helices (α A and α B) in p100 (86,88). Like NIK, Tax is thought to act as an adaptor protein that assembles an IKK α /p100 complex (80). Either NIK- or

Tax-mediated IKK α binding to p100 requires a recognition site formed by the latter's serine 866 and 870. Subsequent to protein-complex assembly, phosphorylation of p100 by IKK α triggers the ubiquitination and processing of p100 by the 26S proteasome (80). Of note, IKK γ is also required for Tax-induced p100 processing. It is hypothesized that IKK γ acts as an adaptor for Tax to recruit IKK α to p100 (Fig. 2) (61,64,89–91). Hence, in the absence of IKK γ , neither Tax's activation of the canonical NF- κ B pathway nor Tax's induction of non-canonical p100 processing (86) occurs.

While NIK-induced p100 processing depends absolutely on β -TrCP, this mechanism only partially explains Tax-induced p100 processing (92). It is noteworthy that increasing p100 nuclear translocation also enhances its processing. This suggests that p100 could be processed in the nucleus through a yet identified mechanism (93). Since Tax is predominantly a nuclear protein that binds p100 (86,88), the above finding suggests that Tax might ferry p100 into the nucleus facilitating a nuclear β -TrCP-independent p100 processing.

HTLV-1 and Chronic Inflammation

HTLV-1 infection is associated with ATL (6,94) and a chronic inflammatory disease variously manifested as HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), HTLV-1 uveitis (HAU), HTLV-1 associated arthropathy (HAAP), rheumatoid arthritis, and dermatitis (4,95,96). While it is not fully understood how HTLV-1 infection causes chronic inflammation, it is believed that the efficiency of the host's CD8⁺ cytotoxic T cell (CTLs) response to HTLV-1 plays an important role in determining the proviral load of HTLV-1 and a consequential chronic inflammatory outcome (97). Interestingly, most of the CTL response in an infected individual is directed to the Tax protein (97,98).

The main roles of CD8⁺ cytotoxic T cells during viral infection are to eliminate infected cells and to suppress viral replication by producing inflammatory cytokines such as interferon- γ (IFN γ) or tumor necrosis factor- α (TNF α). Infiltrations of CD8⁺ T cells have been found in inflammatory lesions in patients with HAM/TSP (99). It is widely assumed that tissue damage observed in the HTLV-1 associated inflammatory diseases such as HAM/TSP is due to bystander damage caused by the infiltrating lymphocytes. In their model, Asquith and Bangham (100), proposed that in patients with HAM/TSP, Tax expressing CD4⁺ T cells infiltrate the CNS and attract pro-inflammatory CD8⁺ T cells. Because of the high viral load and the presence of abundant Tax antigen, CTLs can simultaneously kill the target cells and produce inflammatory cytokines, resulting in collateral damage to the CNS tissue (98,100). A recent transgenic mouse study by Kwon et al. (101) provides strong support for this model. Kwon et al. (101) generated mice conditionally expressing two different Tax mutant, Tax M22 and Tax M47, which have NF- κ B⁻/CREB⁺ and NF- κ B⁺/CREB⁻ functional phenotypes, respectively. They observed that Tax and Tax M47 but not Tax M22 transgenic mice developed a progressive dermatitis. The skin lesions were characterized by the infiltration of T cells and the local increase of inflammatory cytokines such as TNF α , IL-6, IL-1 α , IL-1 β , lymphotoxin- β and IFN γ . Of note, many of those genes are NF- α B inducible chemokines. Inter-

estingly, similar inflammatory changes have been observed in mice with up-regulated NF- κ B signaling (102).

Conclusion

Since the HTLV-1's discovery 25 yr ago (6,94), significant progress has been made toward understanding the virus' oncogenic and inflammatory properties. Activation of NF- κ B plays an important role in the both oncogenesis and inflammation. Through Tax, HTLV-1 has devised multiple strategies to activate efficiently both canonical and non-canonical NF- κ B pathways. Despite important findings such as the identification of IKK as a cellular target of Tax and an essential component in Tax-stimulated NF- κ B signaling, some unanswered questions remain. How does physical interaction of Tax with IKK trigger activation of this kinase complex? What are the role(s) of kinase(s) upstream of IKK such as Akt or PDK1 in Tax-mediated NF- κ B activation and in ATL progression? What are the NF- κ B downstream events that contribute to Tax-related inflammation? These and other questions will keep researchers busy for the next 25 yr and beyond.

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