JOURNAL OF VIROLOGY, Nov. 1990, p. 5412–5419
0022-538X/90/115412-08$02.00/0

Vol. 64, No. 11

Lymphotoxin Activation by Human T-Cell Leukemia Virus Type I-Infected Cell Lines: Role for NF-κB

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Received 12 February 1990/Accepted 30 July 1990

Human T-cell leukemia virus type I (HTLV-I)-infected T-cell lines constitutively produce high levels of biologically active lymphotoxin (LT; tumor necrosis factor-beta) protein and LT mRNA. To understand the regulation of LT transcription by HTLV-I, we analyzed the ability of a series of deletions of the LT promoter to drive the chloramphenicol acetyltransferase (CAT) reporter gene in HTLV-I-positive MT-2 cells. The smallest LT promoter fragment (−140 to +77) that was able to drive CAT activity contained a site that was similar to the immunoglobulin κ-chain NF-κB-binding site. Since the HTLV-I *tax* gene activates the nuclear form of NF-κB, this finding suggested a possible means of HTLV-I activation of LT production. We found that the LT κB-like site specifically formed a complex with NF-κB-containing nuclear extract from MT-2, C81-66-45, and other activated T cells. Mutation of the LT κB site in the context of the LT promoter (−293 to +77) (mutant M1) reduced the ability of the promoter to drive the CAT gene in HTLV-I-infected and noninfected human T-cell lines. These data suggest a general role for NF-κB activation in the induction of LT gene transcription. Activation of LT in HTLV-I-infected cells may explain the pathology associated with HTLV-I infection, including the hypercalcemia that is prevalent in adult T-cell leukemia.

To understand the mechanism of the T-cell dysfunction and other pathogenic effects caused by human T-cell leukemia virus type I (HTLV-I), we studied production of the multipotent lymphotoxin (LT; tumor necrosis factor-beta [TNF-β]) molecule by HTLV-I-infected T-cell lines. Infection with the HTLV-I retrovirus is etiologically associated with two diseases, adult T-cell leukemia (ATL; 49, 70) and tropical spastic paraparesis (TSP; 2, 26). ATL is a terminal malignancy that is frequently associated with hypercalcemia (5). TSP is a slowly progressing myelopathy whose lesions show some similarities with those of multiple sclerosis (26). It is not yet clear how HTLV-I can cause such distinct clinical outcomes. The isolation of HTLV-I from the T lymphocytes of both ATL and TSP patients suggests the common feature of a T-lymphocyte-mediated pathogenesis. Some CD4+ T lymphocytes are immortalized after exposure to ATL-derived HTLV-I (69). HTLV-I infection also causes deregulation of T-cell function and alteration of cytokine production (50, 56). The activation of interleukin-2 (IL-2) and IL-2 receptor expression as a consequence of HTLV-I infection is suggested to be a possible mechanism of ATL leukemogenesis (8, 42, 61). Here, we consider the role of LT in other manifestations of HTLV-I infection.

In a previous study of induction of cytotoxic factors by human immunodeficiency virus (HIV), we found that HTLV-I-infected T cells constitutively produced a factor(s) that killed L929 cells (53). Hinuma et al. (24) also demonstrated such biological activity in the supernatants from HTLV-I-infected T cells. LT and the related TNF-α molecule are assayed by their ability to kill L929 or WEHI-164 cells. The cloning and genetic analysis of LT and TNF-α have indicated that these are two distinct but homologous genes (45) that can both be transcribed by T and B lymphocytes (10, 27, 59, 63). In the studies presented here and in previous reports (29, 30, 67), LT is demonstrated to be the predominant component of the cytotoxic activity produced by HTLV-I-infected cells.

LT has several biological activities in addition to its originally defined cytotoxic effects (48). These include its ability to act as an osteoclast-activating factor and cause stimulation of bone resorption (66). LT has been demonstrated to act as a B-cell growth factor (28), and it activates the expression of class I and class II major histocompatibility complex (MHC) (31, 52). Many of these phenomena are also associated with HTLV-I infection. Hypercalcemia is a frequent concomitant of ATL and has been proposed to be caused by an osteoclast-activating factor (5). Infection of helper T cells with HTLV-I causes their indiscriminate activation of B cells (50). Increased expression of MHC class I and class II has also been demonstrated to result from HTLV-I infection (13, 40, 71). It is thus possible that LT activation during HTLV-I infection contributes to these phenomena.

Studies on the mechanism of HTLV-I pathogenesis have indicated a role for the virus in transcriptional activation of cellular genes. The HTLV-I genome contains two *trans*-acting regulatory genes, *tax* and *rex*, in addition to the structural *gag*, *pol*, and *env* genes (23). Tax-I activates the transcription factor NF-κB, which is a pleiotropic mediator of gene induction in many cell types (34). Tax-I induction of the IL-2 receptor α chain, IL-2, granulocyte-macrophage colony-stimulating factor, and HIV is mediated at least in part by NF-κB activation (1, 3, 9, 36, 54, 57, 60). Other cellular factors are implicated in Tax-I activation of its own long terminal repeat (41, 65) and the cellular c-*fos* (16) and granulocyte-macrophage colony-stimulating factor (43) genes.

In this report, we demonstrate a high level of production of LT mRNA and functional protein by HTLV-I-infected CD4+ T cells. We have also explored the mechanism for elevated LT mRNA in HTLV-I-infected cells. We found that

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TABLE 1. Lymphotoxin biological activity in human T-cell lines

Cell line[a]	LT cytotoxic activity (U/ml)	
	Untreated	After addition of anti-LT antibody
MT-2	262,144	0
HUT-102	2,048	0
C81-66-45	4,096	0
H9	16	0
H9 + PHA	512	0
H9 + anti-CD3	640	0
Jurkat E6.1	0	0
Jurkat E6.1 + PHA + PMA	0	0
U937	0	NT[b]
Recombinant TNF-α	2,048	2,048

[a] Cell supernatants from HTLV-I-transformed cells were collected when cells were in late log phase. H9 cells at a concentration of 10^6/ml were stimulated with 1 μg of PHA per ml for 10 h or with anti-CD3 (2.5 μg/ml absorbed to the tissue culture dish) for 8 h. Jurkat cells were stimulated at 10^6/ml with 1 μg of PHA and 50 ng of PMA per ml for 24 h. The U937 supernatant was tested on L929 cells; all other cell supernatants were tested on WEHI-164 indicator cells. Recombinant human TNF-α was diluted and tested at 2,048 U/ml.

[b] NT, Not tested.

a small but highly active portion of the LT promoter contains a binding site for the nuclear regulatory protein NF-κB. We demonstrate that this LT κB site strongly contributes to the activation of the LT promoter in either HTLV-I-infected or noninfected T cell lines that produce LT. An understanding of the mechanism of LT activation in HTLV-I-associated pathology will aid in design of agents to treat and prevent these diseases.

(This work was performed by N.L.P. in partial fulfillment of the requirements for the Ph.D. degree from Yale University).

MATERIALS AND METHODS

Cell lines. HUT-102 (49) and MT-2 (44) are CD4+ T cells that produce complete HTLV-I particles. C81-66-45 is a CD4+ T-cell line that produces the *tax*-I gene product yet does not produce mature HTLV-I virions (32, 55). H9 (51) and Jurkat E6-1 (68) are human CD4+ T-cell lymphomas. U937 is a human macrophage line that does not produce LT (30). WEHI-164 is a murine fibrosarcoma line and L929 is a murine fibroblast line, both of which are highly sensitive to the cytotoxic effects of LT.

Cells were grown in RPMI with 8% fetal calf serum (H9) or 10% fetal calf serum alone (C81-66-45 and Jurkat E6-1) or supplemented with 50 μM beta-mercaptoethanol and 1 mM L-glutamine (MT-2 and HUT-102) or with 1 mM sodium pyruvate, 1 mM nonessential amino acids, and 0.5 mM essential amino acids (WEHI-164). L929 were cultured in minimal essential media supplemented with 5% fetal calf serum and 4% nonessential amino acids. Cell culture reagents were from GIBCO, Inc.

LT cytotoxicity assay. A total of 5×10^3 WEHI-164 cells (63) or 4×10^4 L929 cells in the presence of dactinomycin (21) were seeded into a 96-well plate that contained serial dilutions of the test samples. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye was used to evaluate cell death by comparing the A_{570}-A_{650} of test samples with that of control wells, which received culture media alone. LT units are defined as the lowest dilution that yields 50% cytotoxicity. Antibody neutralization was performed by preincubating test samples with polyclonal anti-LT antibody (Genentech, Inc.) at 4°C for 4 h (46).

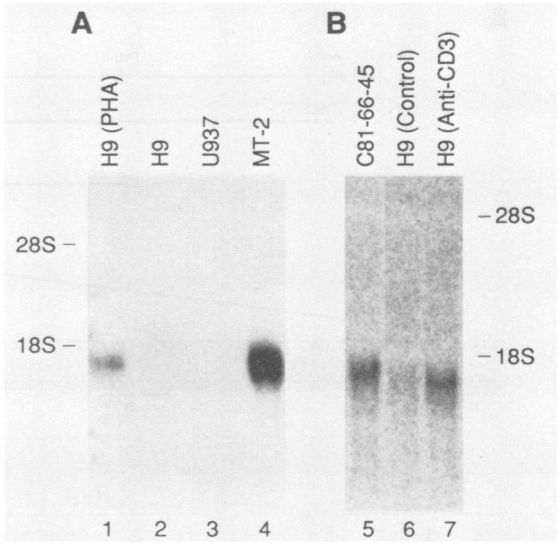
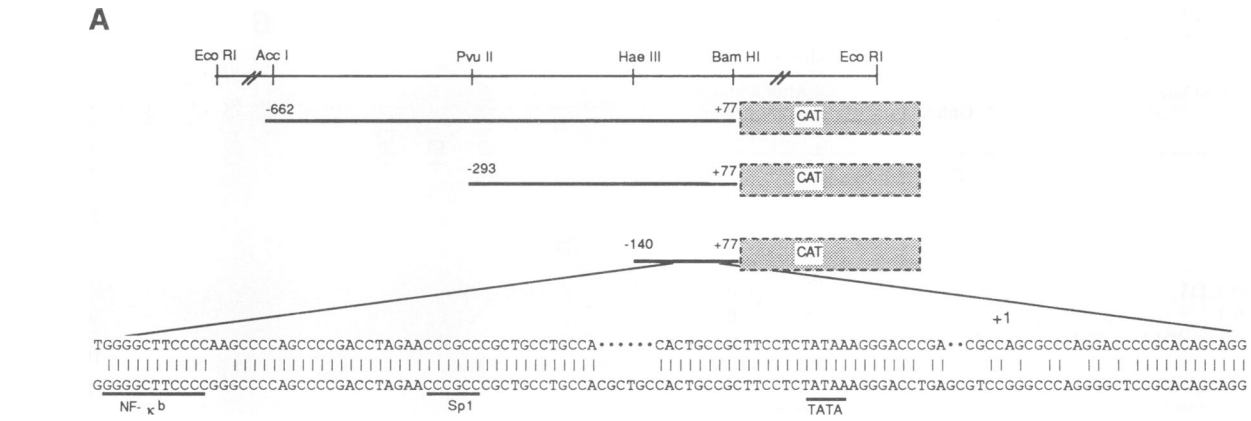


FIG. 1. Production of LT mRNA by human cell lines. Northern blot analysis was performed on (A) cytoplasmic RNA from PHA (10 μg/ml)-activated H9 T cells (lane 1), untreated H9 cells (lane 2), U937 macrophage cells (10 μg of RNA) (lane 3), and HTLV-I-infected MT-2 T cells (lane 4) and (B) total RNA from C81-66-45 cells (lane 5), H9 T cells treated with control antibody (2.5 μg of G10-1 per ml) (lane 6), and H9 T cells treated with antibody to CD3 (2.5 μg of G19-4 per ml) (lane 7). The GeneScreen Plus blot (A) was exposed to film for 24 h; the Nytran blot (B) was exposed to film for 8 days.

Northern (RNA) blot analysis. Cytoplasmic RNA was isolated by using Nonidet P-40 lysis and incubation with proteinase K and sodium dodecyl sulfate. Total RNA was isolated by guanidium thiocyanate lysis (6). RNA (20 μg) was electrophoresed on 1% formaldehyde-agarose gels (39) and transferred to a GeneScreen Plus (Dupont, NEN Research Products) or Nytran (Schleicher & Schuell) membrane in 10× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate). RNA was permanently fixed to the filters by use of baking at 80°C (GeneScreen Plus) or UV fixing (Nytran). Prehybridization, hybridization, and washing solutions were at high stringency, as recommended by the respective manufacturers. A *Kpn*I-*Hin*cII cDNA fragment from the fourth exon of the murine LT gene (37) was used to detect the highly homologous human LT mRNA after radioactive labeling by using the random primer method (14).

Transfection and chloramphenicol acetyltransferase (CAT) assay. Transfection of 20 μg of plasmid constructs for transient expression was facilitated by using DEAE-dextran and chloroquine as described by Cross et al. (8). Cells were lysed and heat treated (7), and 50 μg of protein was used in a 4-h enzyme assay as described by Gorman et al. (19) with 3 mM acetyl coenzyme A.

Plasmids. −662/+77, −293/+77, and −140/+77 upstream murine LT fragments were obtained by use of restriction enzymes and inserted into the *Hin*dIII site of pSV0CAT (13a). The κB mutant (M1) was prepared in the context of the −293/+77 LT-CAT construct by using gapped heteroduplex oligonucleotide mutagenesis (11, 33). One sample of −293/+77 LT-CAT was digested with *Hin*dIII, and another sample was digested with *Xmn*I, which cuts once in the vector; these fragments were annealed with the single-stranded



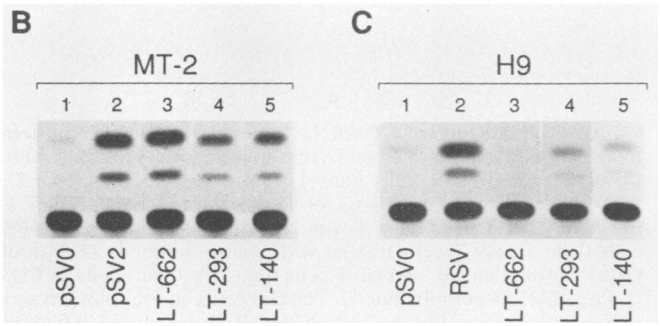


FIG. 2. Demonstration that LT promoter constructs are active in LT-producing cell lines. (A) The promoter constructs derived from the *Eco*RI fragment of the murine LT gene and the homologies between the mouse (top) and human (bottom) LT promoter sequences. Underlining designates that all three constructs contain NF-κB, Sp1, and TATA box sites near the transcription initiation sites. (B) CAT assay using 12.5 μg of cellular protein from transfected MT-2 cells, which gave the following quantitative results (percent acetylation): lane 1 (pSV0CAT), 0.3; lane 2 (pSV2CAT), 8.7; lane 3 (−662/+77 LT-CAT), 8.7; lane 4 (−293/+77 LT-CAT), 2.0; lane 5 (−140/+77 LT-CAT), 2.2. (C) CAT assay with 50 μg from transfected H9 cells, which gave the following quantitative results (percent acetylation): lane 1 (pSV0CAT), 0.3; lane 2 (RSVCAT), 10.7; lane 3 (−662/+77 LT-CAT), 0.7; lane 4 (−293/+77 LT-CAT), 1.2; lane 5 (−140/+77 LT-CAT), 1.1%. Values were derived from representative CAT assays that were repeated more than three times with DNA from different plasmid preparations.

oligonucleotide 5′-TCTTCTAAGCCTGTATCTTCCCCAA GCCCCAGC-3′. After selection with the mutant oligonucleotide, additional confirmation of mutant M1 was made by restriction analysis with *Bst*NI, one site of which is lost by the mutant, and by sequencing reactions with dideoxy nucleotides and T7 DNA polymerase (Sequenase; U.S. Biochemical). Plasmids were twice banded on CsCl and then extensively dialyzed before use in transfection assays.

Nuclear extract preparation and binding assay. Nuclei were isolated and extract was prepared as described previously (12, 35). The following double-stranded oligonucleotides were used for binding studies: human LT κB, 5′-TCGAC CCTGGGGGCTTCCCCGGGC-3′; Ig κB, 5′-TCGACAGA GGGGACTTTCCGAGAGGG-3′; or mutant Ig κB, 5′-TC GACAGAATTCACTTTCCGAGAGGG-3′. Binding assays were performed as described previously (35) with 8 μg of nuclear extract, 3 mM GTP, and 2 μg of poly(dI-dC) (unless noted otherwise).

RESULTS

Characterization of human cell lines for LT production. Previously, we demonstrated constitutive production of cytotoxic activity by HTLV-I-infected T-cell lines (53). We demonstrate here that LT is the major cytotoxic factor produced by HTLV-I-infected T-cell lines. An anti-LT antibody that had no effect on TNF-α completely neutralized the cytotoxic activity produced by the HTLV-I-positive MT-2, HUT-102, and C81-66-45 cell lines (Table 1). We also detected LT mRNA in MT-2 (Fig. 1, lane 4), HUT-102 (data not shown), and C81-66-45 (Fig. 1, lane 5) cells. The production of LT by these cell lines roughly correlated with their levels of HTLV-I expression. The C81-66-45 cell line produces functional *tax*-I gene product (32, 62), does not produce complete HTLV-I particles (55), and constitutively produces 4,000 U of LT per ml. The MT-2 cell line produces even higher levels of functional Tax-I protein than does C81-66-45 (62; N. Paul and N. Ruddle, unpublished data), produces complete HTLV-I virions (32, 44), and constitutively secretes 200,000 U of LT per ml. At the time this study was initiated, the HUT-102 cell line produced complete HTLV-I virions as described previously (32, 49), but with additional time in culture, production both of HTLV-I protein and of LT mRNA and protein have each decreased, though we have detected the HTLV-I provirus that is still present in an apparently latent form (Paul and Ruddle, unpublished observations). We therefore concentrated further study on the MT-2 and C81-66-45 cell lines.

To compare HTLV-I infection with other means of LT activation, we studied production of LT by T lymphocytes after mitogen and phorbol ester stimulation. We found that the CD4+ H9 T-cell line constitutively produced low levels of LT mRNA (Fig. 1) and biological activity as measured in the WEHI cytotoxicity assay (Table 1). Treatment of H9 cells with the T-cell mitogen phytohemagglutinin (PHA) or antibody to the CD3 component of the T-cell receptor each caused an increase in levels of biological activity (Table 1) and LT mRNA (Fig. 1, lanes 1, 2, 6, and 7). In contrast, the CD4+ Jurkat T-cell line produced no detectable LT cytotoxic activity even after PHA and phorbol-12-myristate-13-acetate (PMA) activation (Table 1). Activated Jurkat cells did, however, produce low levels of LT mRNA (data not shown; 30, 64). As expected for a nonlymphoid cell, the U937 macrophage line produced no LT biological activity (Table 1) or LT mRNA (Fig. 1, lane 3).

Activity of LT promoter constructs in human T cells. The activation of LT production could occur by an increase in transcription or a posttranscriptional mechanism such as mRNA stabilization. To determine whether HTLV-I has an

effect on transcription through the LT promoter, we performed studies with murine 5′ LT deletion constructs (Fig. 2A). These constructs were used because the degree of homology between murine and human sequences in these promoter regions was high (−662/+77, 62% homology; −293/+77, 75%; and −140/+77, 87% [20, 45]) and because the human sequences were unavailable for study. We assumed that the elevation of LT mRNA due to increased transcription would be reflected by the ability of LT promoter constructs to drive a reporter gene. In the MT-2 cells, plasmid pSV2CAT (19), which contains simian virus 40 early transcription regulatory sequences, was quite active in driving CAT gene transcription (Fig. 2B, lane 2). In contrast, pSV0CAT, from which the simian virus 40 regulatory regions have been deleted, was not active. The transfected CAT gene was activated when any of the three fragments of the LT promoter region (−662/+77, −293/+77, or −140/+77) was inserted into pSV0CAT (Fig. 2B, lanes 3 to 5). The largest LT promoter fragment (−662/+77) had approximately fourfold-greater activity than either smaller fragment. This result indicates that the LT promoter constructs are able to potently drive transcription of the heterologous CAT reporter gene in the MT-2 cell line. These results also revealed that substantial promoter function was retained in the smallest promoter fragment tested (−140/+77).

To further elucidate the nature of HTLV-I activation of the LT promoter constructs, we performed similar transfection studies in H9 T cells (Fig. 2C). pSV0CAT showed negligible activity in H9 cells, whereas RSVCAT, which contains Rous sarcoma virus regulatory elements (18), was very active in H9 cells (Fig. 2C, lane 2). In contrast with MT-2, the largest LT sequence tested (−662/+77) was not able to drive CAT activity in H9 cells (Fig. 2C, lane 3). However, the smaller LT promoter fragments, −293/+77 and −140/+77, were able to drive CAT activity in H9 cells, though to much lower levels than in MT-2 cells. The LT promoter constructs had similar patterns of activity when transfected into C81-66-45 cells (data not shown). The activities of these smaller constructs in these T-cell lines indicated the presence of functional regulatory elements that allowed constitutive transcription to occur through the LT promoter. To identify sequences that might be responsible for constitutive biological activity by these cells, we analyzed the smallest LT fragment able to direct CAT gene expression. By inspection, we discovered a sequence similar but not identical to the previously identified NF-κB-binding site on the immunoglobulin κ-chain gene (Fig. 2; 34). The finding of a putative NF-κB site was of interest because (i) HTLV-I-infected cells have been shown to have high levels of active NF-κB (9) and (ii) *tax*-I has been demonstrated to activate some cellular genes by causing an increase in NF-κB levels (1, 36, 54, 60).

The LT site specifically binds NF-κB-containing nuclear extract. To determine whether the putative κB site in the LT promoter region could be recognized by NF-κB, we carried out electrophoresis mobility shift assays. We first tested nuclear extract preparations from PHA-PMA-stimulated Jurkat cells, which are known to contain high levels of NF-κB (35). An oligonucleotide with the sequence of the putative LT κB (LT probe) formed a complex that resulted in a retarded band in mobility shift electrophoresis, which comigrated with the complex formed by the Ig κB probe (Fig. 3, lanes 1 and 10). Formation of this LT complex was prevented by addition of unlabeled Ig κB probe (Fig. 3, lanes 7 to 9). The parallel formation of an Ig κB probe complex with

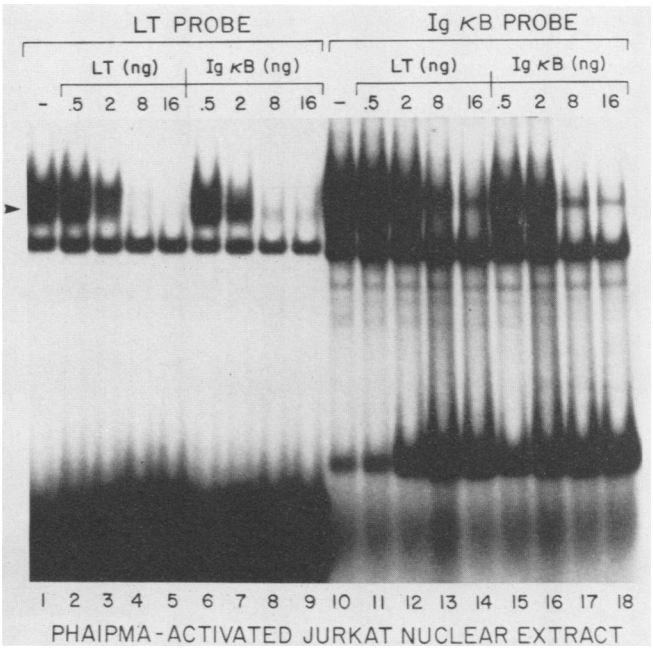


FIG. 3. Demonstration that the LT site specifically binds NF-κB produced from activated Jurkat cells. Mobility shift electrophoresis assay with 3 μg of nuclear extract from PHA-PMA-activated Jurkat cells (5 μg of PHA and 50 ng of PMA per ml; 3 h) shows binding of oligonucleotides LT (lane 1) and Ig κB (lane 10). Competition was performed by addition of 0.5, 2, 8, or 16 ng of unlabeled oligonucleotide LT or Ig κB to the nuclear extract. The arrowhead marks migration of the NF-κB complex.

nuclear extract from activated Jurkat cells was inhibited by addition of unlabeled LT probe (Fig. 3, lanes 11 to 14).

We next tested nuclear extract from LT-producing cells to determine whether they were also able to bind to the LT site. Nuclear extract from the HTLV-I-infected MT-2 cell line contains active NF-κB (9), and we found that this extract specifically bound to the LT probe (Fig. 4A). This binding was inhibited by addition of increasing amounts of unlabeled oligonucleotide LT or Ig κB but not by a mutated oligonucleotide Ig κB site. The nuclear extract from C81-66-45 T cells, which expressed the HTLV-I *tax* gene product and constitutively produced LT (Table 1), formed a complex with the probe Ig κB site in competition with the LT and Ig κB sites but not with a mutated Ig κB site (Fig. 4B). H9 cells constitutively produced low levels of LT, and H9 nuclear extract also retarded the migration of oligonucleotide LT, which was inhibited by addition of unlabeled probe LT or Ig κB (Fig. 4C). Thus, NF-κB from several cell lines, including those in which we have demonstrated LT production and promoter activity, recognizes the LT κB site.

Role of NF-κB in LT gene regulation by HTLV-I. To determine the functional role of NF-κB binding to the LT site, we prepared a mutation in the NF-κB-binding site of the −293/+77 LT-CAT construct. The design of this mutant (M1) was based on mutations that have been shown to abolish binding and activity of other κB sites (36). Mutant M1 was tested by transfection into MT-2 and H9 cells (Fig. 5), and the amount of CAT activity driven by the mutant M1 was compared with that of the wild-type −293/+77 LT-CAT construct. Mutation of the LT κB site in the context of the LT promoter reduced promoter activity in MT-2, H9 (Fig. 5), and C81-66-45 (data not shown) cells. This mutant LT did not bind or compete for NF-κB binding in mobility shift

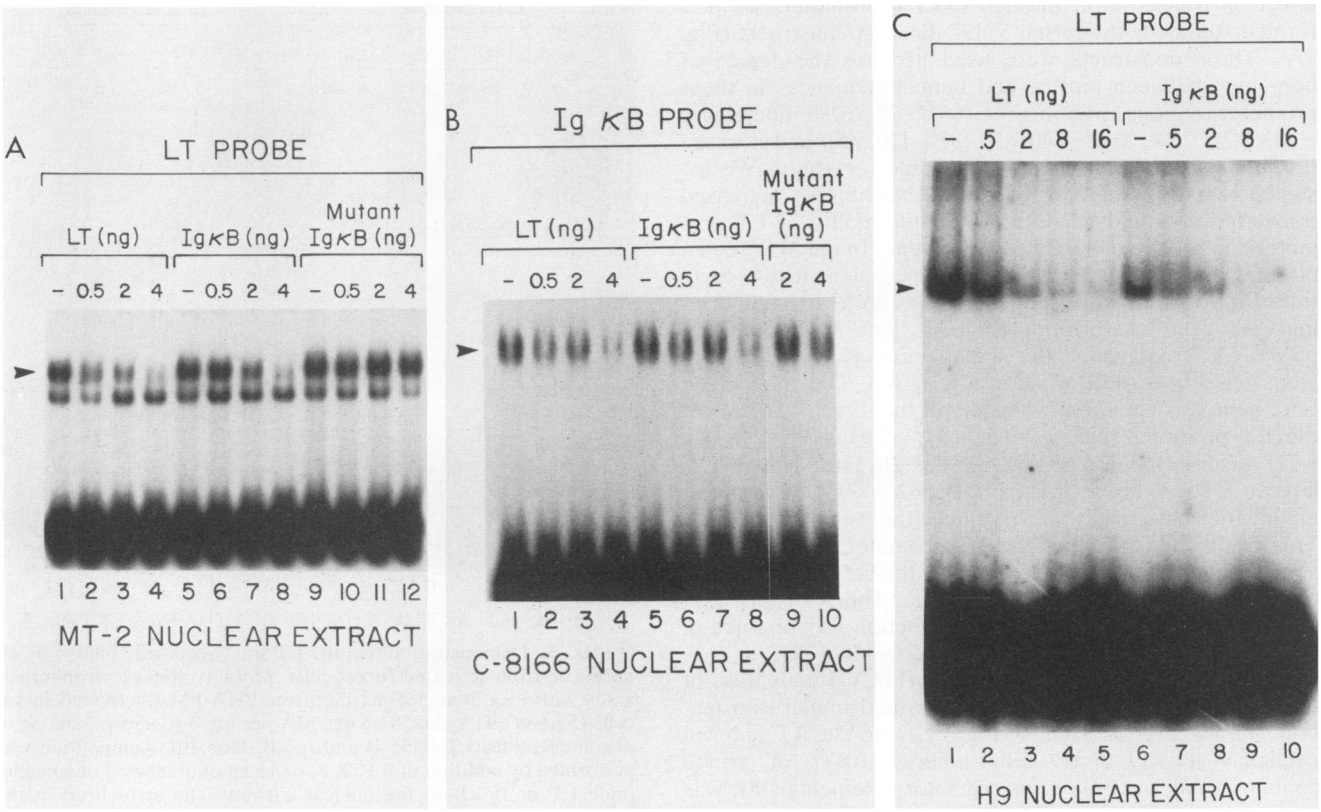


FIG. 4. NF-κB activity in HTLV-I-infected and noninfected human T cells that produce LT. (A) Nuclear extract from MT-2 HTLV-I-infected T cells forms a specific complex with oligonucleotide LT κB (lanes 1, 5, and 9) in competition with increasing amounts of added oligonucleotides LT κB and Ig κB (lanes 2 to 4 and 6 to 8, respectively). Formation of this complex is not inhibited by addition of a mutated oligonucleotide Ig κB (lanes 10 to 12). (B) The C81-66-45 (C-8166) nuclear extract produces a specific complex with oligonucleotide Ig κB (lanes 1 and 5) in competition with oligonucleotides LT and Ig κB (lanes 2 to 4 and 6 to 8, respectively) but not by a mutated oligonucleotide Ig κB (lanes 9 and 10). (C) Nuclear extract from H9 T cells specifically binds probe LT κB (lanes 1 and 6) in competition with unlabeled oligonucleotides LT κB (lanes 2 to 5) and Ig κB (lanes 7 to 10) in the mobility shift assay. The H9 binding assay was performed as described in Materials and Methods except that 3 μg of H9 nuclear extract was used in the absence of added GTP.

assays (Fig. 6). These data indicate that the LT κB site is important for activity of the −293/+77 LT promoter fragment.

DISCUSSION

We have presented an analysis of the mechanism of LT activation by concentrating on HTLV-I infection as a model system. We have confirmed and extended data that high levels of LT mRNA and protein are constitutively produced by HTLV-I-infected cells. We have also shown that T-cell lines that are not infected with HTLV-I vary in their ability to produce LT. H9 constitutively produces low levels of LT mRNA and protein and is induced to produce higher levels after activation with T-cell-specific stimuli. Jurkat cells produce no LT protein even after activation, though low levels of LT mRNA are observed. The activity of transfected LT promoter constructs in MT-2 and H9 cells correlates with their ability to constitutively produce LT. The minimal LT fragment that can drive the CAT gene in LT-producing cells contains a κB consensus sequence. The LT κB site competes

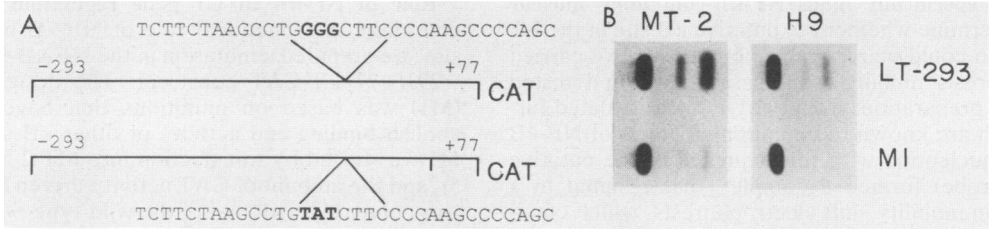


FIG. 5. Demonstration that mutation of the LT κB site reduces promoter activity. (A) The κB site of the −293/+77 LT promoter-CAT construct (top) was mutated (bottom) as shown and transfected into MT-2 and H9 cells; 50 μg of each was tested in the CAT assay (B). For MT-2, LT−293/+77 showed 9.9% acetylation and M1 showed 0.4% acetylation. For H9, LT−293/+62 showed 1.2% acetylation and M1 showed 0.0% acetylation. Transfection of plasmids from different preparations gave similar results.

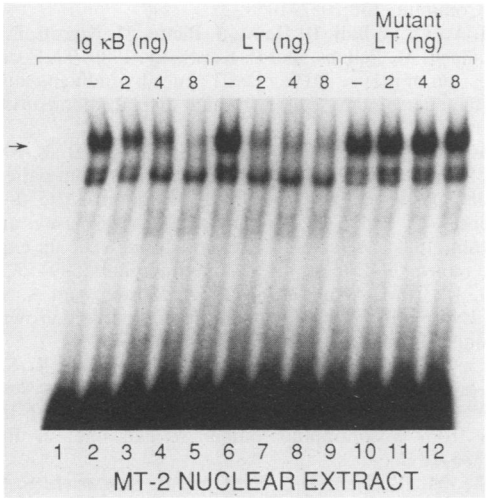


FIG. 6. Demonstration that mutated LT κB site does not bind NF-κB. Nuclear extract from MT-2 HTLV-I-infected T cells forms a complex with a radiolabeled oligonucleotide Ig κB (lanes 1, 5, and 9). Complex formation is inhibited by the addition of unlabeled oligonucleotides LT-κB and NF-κB (lanes 2 to 4 and 6 to 8, respectively) but is not inhibited by addition of the LT oligonucleotide used to generate the M1 mutant (Fig. 5) in double-stranded form (lanes 10 to 12).

with the canonical immunoglobulin κB enhancer sequence for binding to nuclear extracts from PHA-PMA-activated Jurkat T cells as well as extracts from MT-2, C81-66-45, and H9 T cells. A mutation of the LT κB site decreased promoter activity in MT-2 and H9 cells. These results indicate that NF-κB activity is an essential but not sufficient component in the induction of maximal production of LT.

The HTLV-I *tax* gene activates the transcription of several cellular genes through the NF-κB transcription factor (1, 36, 54, 60). Although we have not directly addressed the induction of LT by *tax*-I itself in this report, it is likely that *tax*-I activation is at least one mechanism by which HTLV-I activates LT production. Thus, Tax-I activation of the NF-κB transcription factor may contribute to LT activation. Additional means probably exist for HTLV-I activation of LT, including transcriptional activation through sequences in the −662/−293 5′ LT fragment, which is upstream of the κB site. We demonstrated high activity of a construct containing this LT fragment in the HTLV-I-positive MT-2 cells (Fig. 2B), in contrast to the absence of activity of this construct in H9 T cells (Fig. 2C). The inactivity of the −662/+77 LT-CAT construct has also been observed in non-LT-producing murine cells, which led to the suggestion that this region contains a negative regulatory element (13a). That the −662/+77 LT-CAT construct is highly active in MT-2 cells suggests that this HTLV-I-infected cell provides conditions in which this negative region does not function. Non-HTLV-I induction of LT production (antigen plus MHC, anti-CD3, mitogen, or IL-2 [45, 63]) may also utilize NF-κB activation as well as other LT regulatory elements.

The activation of the pleiotropic LT cytokine in HTLV-I-infected cells suggests a possible role for LT in HTLV-I-associated pathogenesis. At the molecular level, it is possible that LT itself contributes to NF-κB activation in HTLV-I-infected cells. This possibility is suggested because LT exhibits many of the same biological effects as the related TNF-α (15, 31, 52). TNF-α has been shown to activate the IL-2 receptor α chain, HIV long terminal repeat, and class I MHC by stimulation of NF-κB activity (25, 38, 47). In support of this hypothesis, LT has also been demonstrated to activate MHC class I expression (31) and HIV replication (15). LT can also activate TNF-α (22), which itself contains functional κB sites (58). At the level of HTLV-I-associated diseases, the activity of LT as an osteoclast-activating factor has been proposed to play a role in the hypercalcemia associated with another LT-producing lymphoid malignancy, multiple myeloma (17). LT may also contribute to the hypercalcemia that is a hallmark of HTLV-I-associated ATL (5). Another manifestation of HTLV-I infection is TSP (2, 26). Many cytokines are under investigation for their roles in neurological diseases, including LT and TNF-α, which are being studied for their possible roles in the demyelination observed in multiple sclerosis (4). We have found that an antibody that reacts with LT and TNF-α inhibits the transfer of murine experimental autoimmune encephalomyelitis, an animal model of both multiple sclerosis and TSP (54a). It will be interesting to determine whether such treatments that inhibit LT also affect hypercalcemia. Further study of HTLV-I activation of LT and the role of LT in HTLV-I-associated pathogenesis will enable better design of drugs to prevent and treat HTLV-I-associated diseases.

ACKNOWLEDGMENTS

We thank Mark Feinberg and Lee Ratner for cell lines; David Baltimore, in whose laboratory the binding analysis was initiated; Paula Kavathas and Jan Chalupny for collaboration on anti-CD3 activation; Katherine McGrath for initial contributions; Sarah Fashena for helpful comments; and Regina Turetskaya for critical review of the manuscript. We gratefully acknowledge Genentech, Inc., for the polyclonal antibody to human LT; Yuan DeVries and James Huang of E. I. duPont de Nemours & Co. for the recombinant human TNF-α; and Jeffrey Ledbetter of Oncogen for the G19-4 anti-CD3 and G10-1 anti-CD8 monoclonal antibodies.

This work was supported in part by Public Health Service grant RO1-CA-16885 from the National Cancer Institute to N.H.R. and an Investigator Award from the Cancer Research Institute to M.J.L. N.L.P. was supported by Public Health Service National Research Service Award training grant T32-AI-07019.

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